TOXICITY AND FATE OF PRISTINE AND AGED SILVER NANOMATERIALS FROM COMMERCIAL TEXTILES IN AGRICULTURAL SOIL-PLANT SYSTEMS

TOXICITÉ ET SORT DES NANOMATÉRIAUX D'ARGENT VIERGES ET VIEILLIS DE TEXTILES COMMERCIAUX DANS DES SOLS AGRICOLES PLANTÉS

A Thesis Submitted to the Division of Graduate Studies
of the Royal Military College of Canada
by

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In Partial Fulfillment of the Requirements for the Degree of Master of Applied Science

April 2018

Acknowledgements

I would first like to thank my supervisor, Dr. Kela Weber, for providing me with the opportunity to pursue this thesis. Thank you, Dr. Weber, for your continued support, guidance and cultivation of a challenging and enthusiastic environment for academics and personal growth.

Thank you to Dr. Vincent Gagnon for all your support over the course of my thesis project. I have greatly appreciated your advice, discussion and assistance in all respects throughout, including the many holidays spent helping me in the lab.

Thank you to all the current and former members of the Environmental Sciences Research Group for your support and assistance. To Dr. Mark Button, David Patch and Sarah Wallace thank you for your assistance with instrumentation and analysis.

To my friends and family, especially my parents, thank you for your endless support and encouragement.

Abstract

A healthy soil microbial community is important for agricultural productivity. Microbial processes play a key role in the bioavailability of nutrients and soil formation. The function of soil microbial communities is imperative to nutrient cycling; changes to the microbial populations or diversity can therefore directly impact plant productivity and health. Traditional agricultural practices result in nutrient losses that must be mediated through re-addition of supplemental nutrients which can be provided in the form of biosolids, organic matter recovered from waste treatment processes. Emerging substances of concern including silver nanomaterials, an antimicrobial agent increasingly common in consumer products, have been detected in biosolids and are currently unregulated with their risk potential being unknown.

The objective of this thesis was to determine the fate and toxicity of aged and pristine nanomaterials in soil ecosystems. Two three month exposure studies were conducted. The first using a clay loam soil, and the second using the same soil planted with *Triticum spp.* (wheat). In each, the microcosm systems were exposed to several types of pristine and aged nanomaterials including weathered nanomaterials originating from the washing of socks with silver-incorporated fibers.

Exposure to silver nanomaterials resulted in no significant changes to physical characteristics of the soil over the course of exposure. In the unplanted soil exposure, low concentrations of sulphidized nanoparticles decreased the microbial utilization of amino acids after three months. However, overall nanomaterial exposures did not seem to exhibit toxic effects to the soil microbial communities themselves over the same period. While silver in all forms was not mobile within the soil, changes to the morphology of sulphidized nanoparticles during scanning transmission electron microscopy prior to and after soil exposure indicated amorphous sulphur bonds. In soils planted with wheat, exposure to silver nanomaterials resulted in bioconcentratation and translocation into aboveground biomass, including grains, despite insignificant effects on the biomass and activity of heterotrophic soil bacteria. Yield of seeds produced by wheat plants was found to be greater than the control in all treatments except ionic silver ($AgNO_3$) at 67 mg/kg soil. In both the unplanted and planted soil studies a positive dose response in the abundance of *Frankia alni* (nitrogen-fixer) and *Xanthomonas oryzae* (phytopathogen) were seen in the ionic silver exposures.

Résumé

Une saine communauté microbienne dans le sol est importante pour la productivité agricole. Les processus microbiens jouent un rôle clé dans la biodisponibilité des nutriments et la formation du sol. La fonction des communautés microbiennes du sol est impérative pour le cycle nutritionnel et les changements apportés aux populations microbiennes ou leurs diversités peuvent donc influer directement la productivité et la santé des plantes. Les pratiques agricoles traditionnelles entraînent la perte des nutriments qui doivent être médiée par l'addition de nutriments supplémentaires qui peuvent être fournis sous forme de biosolides, qui sont des matières organiques récupérées lors du traitement eaux usées. Les nanomatériaux d'argent sont des substances chimiques émergeantes ayant des propriétés antimicrobiennes et sont de plus en plus répandu dans les produits de consommation. Les nanomatériaux d'argent ont été décelés dans les biosolides utilisés comme engrais agricole, mais leurs présences ne sont pas réglementées ce qui posent un risque potentiel.

L'objectif général de cette thèse est de déterminer le sort et la toxicité des nanomatériaux vierges et vieillis dans l'écosystème d'un sol agricole. Deux études de trois mois d'exposition ont été menées. Le premier utilisant sol agricole argileux non planté et le deuxième utilisant le même sol planté de *Triticum spp.* (tendre). Dans chacun, des systèmes de microcosmes ont été exposés à plusieurs types de nanomatériaux vierges et vieillis, dont des nanomatériaux altérés issus du lavages de chaussettes ayant des fibres recouvert d'argent.

Les nanomatériaux d'argent n'ont entraîné aucun changement significatif aux caractéristiques physiques du sol. L'exposition de nanomatériaux d'argent sulfurés à faibles concentrations dans les sols non plantés a eu un effet sur les microorganismes avec une diminution de l'utilisation des acides aminés après le troisième mois. Cependant, les expositions aux autres nanomatériaux n'ont pas eu d'effets toxiques sur les communautés microbiennes du sol sur la même période. L'argent sous toutes ses formes n'ont pas montré de mobilité dans le sol, des changements morphologique des nanoparticules sulfurées, avant et après l'exposition au sol, ont été démontrés par microscopie électronique et sont potentiellement indicatif de liaisons de soufre amorphe altérant le comportement des particules. Dans les sols plantés de blé, l'exposition aux nanomatériaux d'argent a entraîné la bioconcentration et la translocation de l'argent dans la partie aérienne, malgré un effet insignifiant sur la biomasse et l'activité des bactéries hétérotrophes du sol. Le rendement des graines produites par les plants de blé exposés à tous les traitements s'est avéré plus grand que le contrôle, sauf pour l'argent ionique ($AgNO3$) à 67 mg/kg sol. Dans les études du sol non plantés et plantés, une réponse positive à la dose de l'argentés ioniques a été montrée pour l'abondance du fixateur d'azote Frankia alni et du phytopathogène Xanthomonas oryzae.

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List of Acronyms

Ag	Silver
AgNM	Silver nanomaterial
AgNP	Silver nanoparticle
AMC	Amino methylcoumarin
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
AWCD	Average well colour development
CFU	Colony forming units
CLPP	Community level physiological profiling
CSUP	Carbon source utilization pattern
DLS	Dynamic light scattering
DNA	Deoxyribonucleic acid
DOF	Degrees of freedom
d.w.	Dry weight
EDS	Electron dispersive spectroscopy
GA-AgNP	Gum arabic coated silver nanoparticles
g/L	Grams per litre
GSH	Glutathione
ISO	International Organization for Standardization
ICP-MS	Inductively coupled plasma mass spectrometry
mg/kg	Milligram per kilogram
mg/L	Milligram per litre
MUB	Methylumbelliferone
NADH	Nicotinamide adenine dinucleotide dehydrogenase
PBS	Phosphate buffered saline
PCA	Principal component analysis
PCR	Polymerase chain reaction
PVP	Polyvinylpyrrolidone
PCR	Polymerase chain reaction
ROS	Reactive oxygen species
SAgNP	Sulphidized silver nanoparticle
S.D.	Standard deviation
SP-ICP-MS	Single particle inductively coupled plasma mass spectrometry
STEM	Scanning transmission electron microscopy
T-RFLP	Terminal restriction fragment length polymorphism
Wt%	Weight percent
µg/L	Microgram per litre

Glossary

Actinobacteria	Gram positive bacteria capable of organic matter decomposition
Ag^+	Ionic silver
Aged	Non-pristine nanomaterials, weathered or sulphidized
Biosolids	Organic matter recovered from waste treatment processes
Furrow depth	Depth at which a plow is used to till soil for planting or irrigation
Negative Control	Treatment in which no response is expected
Positive Control	Treatment in which a known response is expected
Pristine	Unaltered engineered nanomaterials
Weathered	Nanomaterials originating from a commercial product

1 Chapter 1: Introduction

1.1 Background

Engineered nanomaterials, increasingly prevalent within consumer products, have become known as an emerging substance of concern due to their detection in biosolids (waste treatment residuals) and relatively unestablished environmental risk potential (Monteith, et al. 2009). The scale of nanomaterials (1-100 nm) results in unique physicochemical properties which make them desirable for diverse applications but also entails potential for toxicity (Shahrokh and Emitiazi 2009; Choi, et al. 2009). Metallic nanomaterials, such as silver nanomaterials (AgNMs), which can act as an antimicrobial, are of particular concern due to their toxicity potential (Gatoo, et al. 2014).

Biosolids contain organic matter as well as many macro and micronutrients that are required for plant development and are readily available making them an affordable and effective alternative to traditional fertilizers in agricultural practices (Water Environment Association of Ontario 2009). This practice of amending agricultural soils with biosolids therefore provides a potential for toxic effects and bioaccumulation of nanomaterials within soil microbes and agricultural plants. Prior to use in agricultural amendments, household wastewaters which can contain nanomaterials released from consumer products (Benn and Westerhoff 2008) undergo treatment in wastewater treatment processes where high quantities of available sulphur and organic matter can transform metallic nanomaterials into insoluble or complexed forms (Hu 2010).

As much as 95% (Hu 2010) of some metallic nanomaterials can be sulphidized and bound to organic matter during wastewater treatment, mainly remaining in this form for durations in excess of 14 months (Wang, et al. 2016), however the fate and effects of these transformed or aged nanomaterials as well as those directly released from consumer products in agricultural soils remains largely unknown.

Due to the symbiotic relationship between plants, bacteria, and mychorrhizal fungi, and their fundamental role in mineral biotransformations vital to plant health, inhibition to any organisms as a result of nanomaterials could be detrimental to ecosystem health or agricultural productivity. Existing studies of nanomaterial toxicology and fate, which will be discussed in a literature review in Chapter 2, have primarily focused on pristine (untransformed engineered nanomaterials subjected to no process of wear or aging) in simplified media such as water and nutrient growth media. These studies may not provide a realistic representation of nanomaterials entering the terrestrial environment. To date no studies have examined toxicological effects or fate of nanomaterials directly released from consumer products representing a significant set of research gaps in the literature.

1.2 Objectives

To address these research gaps, the objectives of this thesis are to (1) determine the toxicity of AgNMs originating from commercial textiles on soil microbial communities, (2) determine the fate of AgNMs including those originating from commercial textiles in agricultural soils and (3) quantify the effect and uptake of pristine and aged AgNMs (sulphidized and weathered) in an agricultural crop (*Triticum spp.*).

This thesis provides an analysis of plant health and growth, changes to soil microbial communities when exposed to AgNMs and the effect of nanomaterials on soil physical and chemical properties. Experimentation also investigated the effect and extent of silver uptake in plants in relation to the type of AgNMs, pristine and aged. Novel aspects of this research include a full life-cycle analysis for common wheat kernel in nanomaterial exposures and the first analysis of the effects of weathered nanomaterials from consumer products to mixed microbial communities and wheat plants.

1.3 Organization

This thesis includes six chapters organized as follows:

Chapter 1 briefly introduces the topic of this thesis, objectives and organizational structure.

Chapter 2 provides a literature review of the relationships between microbial function, plant productivity and nutrient cycling, and silver nanomaterials and their toxicological effects.

Chapter 3 describes the methodologies and materials which were utilized in this thesis.

Chapter 4 provides a summary of fate and effects of silver nanomaterials in agricultural soil.

Chapter 5 provides an analysis of fate and effects of silver nanomaterials in agricultural soil planted with wheat kernel.

Chapter 6 concludes the findings of this study and provides recommendations for further research.

Appendix A summarizes supporting calculations and information for Chapter 2.

Appendix B summarizes supporting information and statistical analyses for Chapter 3.

Appendix C summarizes supporting information and statistical analyses for Chapter 4.

Appendix D summarizes supporting information and statistical analyses for Chapter 5.

2 Chapter 2: Literature Review

2.1 Soil Ecology

2.1.1 Microbial Function

A healthy soil microbial community is important for agricultural productivity since microbial processes have a key role in the bioavailability of nutrients (Jacoby, et al. 2017) and soil formation (Rashid, et al. 2016; Schulz, et al. 2013). Soil contains microbial populations of as many as 10^{10} bacteria/g soil (Torsvik, et al. 1996) comprising approximately 25,000-50,000 species (Roesch, et al. 2007). These microbial populations utilize both intra- and extracellular enzymes to break down organic matter, making nutrients bioavailable (Blagodatskaya, et al. 2016).

Diverse microorganism populations are present in the soil surrounding plant roots, known as the rhizosphere (Hiltner, 1904). Plant roots exude various compounds such as sugars, amino acids and enzymes which promote microbial populations that differ from those of the bulk soil (Gabreva, et al. 2004). The plant roots form mutualistic symbioses with soil fungi such as arbuscular mycorrhiza (Harley and Smith 1983), which aids in ecosystem nutrient cycling (Jeffries and Barea 1994). Rhizosphere bacteria can promote plant growth (Gray and Smith 2005) in addition to inhibiting pathogenic microorganisms through both synergistic and antagonistic mechanisms (Raaijmakers, et al. 2009). In the event of a perturbation to soil, diverse microbial communities are better able to compensate for changes to climate, nutrients or contaminants than soil microbial communities with less diversity. Since the function of soil microbial communities are imperative to nutrient cycling, changes to microbial populations or diversity can therefore directly impact plant productivity and health.

2.1.2 Plant Productivity

Plants require 16 essential elements for growth and development: nitrogen, carbon, sulfur, hydrogen, oxygen, phosphorus, potassium, calcium, magnesium, iron, zinc, manganese, copper, boron, molybdenum and chlorine (Uchida 2000). As a result, cycling of macronutrients has an important role in overall plant health and productivity (Hawkesford, et al. 2012).

2.1.2.1 Nitrogen Cycling

Nitrogen in the environment has several forms including ammonium (NH_4^+), nitrate (NO_3^-) and nitrogen rich polymeric compounds such as proteins, all of which are bioavailable to plants (Paungfoo-Lonhienne, et al. 2008). In order to produce nitrogen in these available forms, soil microbes utilize several processes as seen in Figure 2.1 which include nitrogen fixation, nitrogen mineralization and nitrification.

Biological nitrogen fixation refers to the process in which nitrogen gas is reduced to available forms of nitrogen such as ammonium by microorganisms (Hardy and Burns 1968). Nitrogen-fixing bacteria utilize a *nitrogenase* enzyme complex to catalyze the hydrolysis of adenosine triphosphate (ATP) and support fixation (McGill 2007; Berg, et al. 2002).

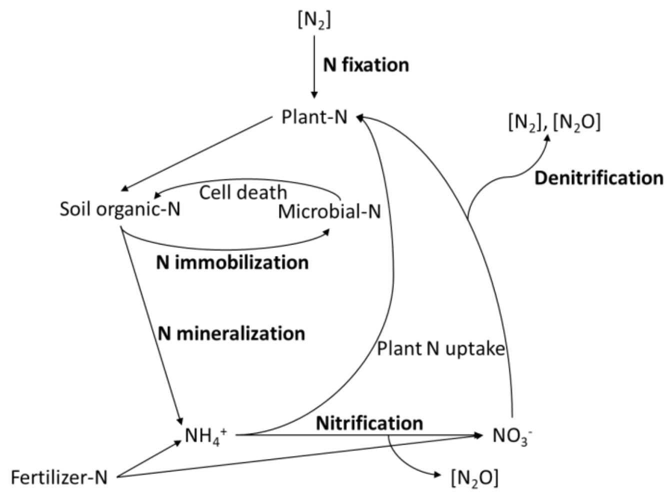

Figure 2.1: Schematic of the main processes involved in the terrestrial nitrogen (N) cycle adapted from Robertson and Groffman (2007). Processes mediated by soil microbes are in bold.

Nitrogen mineralization is the process through which organic nitrogen in soil is converted into ammonium. During decomposition of organic matter by soil microbes, inorganic nitrogen forms are produced as a by-product (Crohn 2004).

Nitrification is the process in which ammonium is transformed into nitrate. Generally, autotrophic bacteria such as *Nitromonas* and *Nitrobacter* oxidize ammonia to nitrite followed by a second oxidation to nitrate, respectively (United States Environmental Protection Agency 2002). Both nitrate and nitrite can also be reduced by facultative anaerobic bacteria in the absence of oxygen to nitrous oxide and nitrogen gas (Hofstra and Bouwman 2005). This process, known as denitrification, is particularly detrimental in agricultural soils where denitrification has been measured at mean rates of 13 kg N/ hectares per year due to increased soil nitrate availability from nitrogen in fertilizers (Barton, et al. 1999).

2.1.2.2 Carbon Cycling

Plants utilize atmospheric carbon in the form of carbon dioxide to undergo photosynthesis and produce sugars such as glucose and oxygen. Organisms can then use the products of photosynthesis to produce energy (ATP).

A large proportion of microbial soil communities are comprised of heterotrophs which derive their energy from organic carbon through cellular respiration (Bot and Benites 2005). Consequently, soil organic matter is an important aspect of carbon cycling within soil. Carbon metabolization by soil microbes is limited by its accessibility; whether it is in a form that the microbes can utilize (Six, et al. 2004). Enzymes in the soil, for example glucosidase, cellulose, phosphatase and amylase, catalyze biochemical reactions producing energy and substrates that microorganisms can use, decomposing organic wastes and maintaining soil structure (Kiss, et al. 1978; Sinsabaugh 1994).

2.1.2.3 Sulphur Cycling

Sulphur often enters into the soil environment in the form organic fertilizers however it is also present in minerals and can become available as weathering occurs. Plants assimilate sulphur via plant roots in the form of inorganic sulphate where it is subsequently reduced to form various proteins (Eriksen, et al. 1998). Sulphate, however, constitutes a low quantity of the total sulphur in soil where more than 95% is organically bound (Eriksen, et al. 1998).

Organic sulphur in soil is present primarily as ester sulphates and directly carbon-bonded S (Edwards 1998). In order to make the organic sulphur bioavailable for plants, mobilization and mineralization can be accomplished through both microbial and enzymatically-mediated depolymerization (Strickland and Fitzgerald 1984). Mineralized sulphur (S^0) can undergo microbial oxidation by sulphur oxidizing bacteria to form sulphate (Edwards 1998). Sulphate can also be reduced by sulphur reducing bacteria in anaerobic environments which can be volatilized as H_2S gas, however, this is not thermodynamically favourable in aerobic soils (Postgate 1979).

2.1.3 Nutrient Losses

Agricultural soils can often become deficient in nutrients as they are utilized by plants and harvested, therefore supplemental nutrients are required. Biosolids, dewatered solids from traditional waste treatment processes, have been used for this purpose in Canada since 1996 to supply organic matter, nitrogen, potassium, phosphorus, sulphur and other micronutrients (Ministry of Environment 1996). During waste treatment processes, sludge collected after settling is dewatered and treated with processes such as anaerobic digestion and composting to remove pathogens prior to its use as an agricultural amendment (Metcalf & Eddy, Inc. 2003).

Regulatory guidelines in Ontario require that biosolids meet specific requirements to be suitable for agricultural use. Non-agricultural source materials must be below specified metal concentrations for arsenic, cadmium, cobalt, chromium, copper, lead, mercury, molybdenum, nickel, selenium and zinc as well as meeting the requirement for maximum number of *Escherichia coli* colony forming units and odour detection threshold (Government of Ontario 2017). Emerging substances of concern, such as pharmaceutical compounds and personal care products, are identified as substances which have been detected in biosolids for which risk potential and regulations have not yet been established (Monteith, et al. 2009). Environment and Climate Change Canada has identified these domestic substances which need to be individually considered for risk assessment prioritization amongst which nanomaterials are currently being reviewed (Environment and Climate Change Canada 2016).

2.2 Engineered Nanomaterials

Nanomaterials are defined as a material with one or more dimension of 1-100 nm (Buzea, et al. 2007). Nanoparticles are therefore a subset of nanomaterials wherein all dimensions are on the nanoscale (ISO/TS 80004-2: 2015).

Engineered nanomaterials, nanomaterials intentionally produced through chemical and/or physical processes for their unique properties have been identified as emerging substances of concern (Monteith, et al. 2009). Prioritized nanomaterials will be assessed based on Canada's chemicals management plan to evaluate exposure risks to human and environmental health (Environment and Climate Change Canada 2016). As of 2010, the United States Environmental Protection Agency determined that there was insufficient data to determine if nanomaterials present a risk to human health in realistic exposures (United States Environmental Protection Agency 2010).

Engineered nanomaterials designed from metals or composites which demonstrate toxicity in bulk form can impart different mechanisms of toxicity due to their size-based interactions with biological systems (Gatoo, et al. 2014). Due to their scale, nanomaterials such as silver nanoparticles can interact with DNA, proteins and bacteria (Figure 2.2) (Mu, et al. 2014). These interactions at the microbial level provide the potential for adverse effects on microbial function and diversity which could impact ecosystem functionality and productivity (Fajardo, et al. 2012). It is therefore vital to understand the implications of these nanomaterials to entire ecosystems and determine under what conditions detrimental effects could occur.

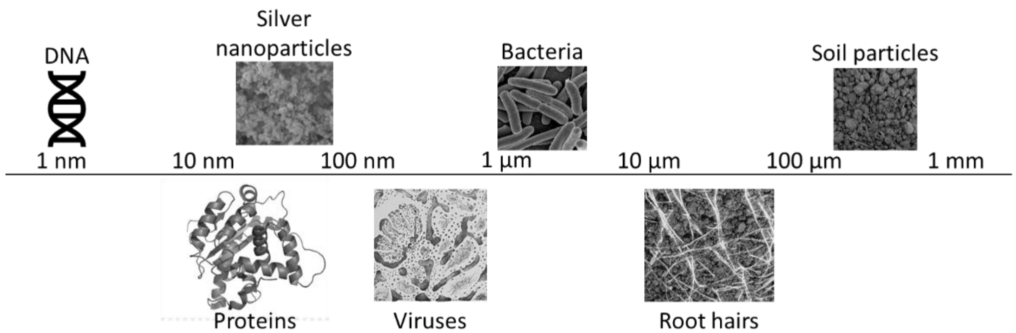

Figure 2.2: Scale of nanoparticles and biological and environmental structures

2.3 Nanoparticle Design and Synthesis

The design of nanomaterials is highly dependent upon the application. Additionally, these engineered nanomaterials having undergone no transformations after synthesis can be referred to as pristine. Nanomaterials can be synthesized in varied shapes most commonly rods, spheres and cubes using shape and/or size-controlled methodologies. Since the properties of nanomaterials are linked to their shape and size, these synthesis methods are imperative to achieving the desired properties for specific applications.

Size control of nanomaterials is conducted through the use of capping agents (Frens 1973). In reduction processes, surfactants, ligands, polymers or dendrimers can be used to confine the growth of nanoparticles (Jana, et al. 2001). Low surface energy of particles in these methods typically produce spherical particles, however, other shapes such as nanotubes, cubes and triangles can be generated through use of capping agents which have different interactions with the particle's growing faces (Jana, et al. 2001; Ahmadi, et al. 1996).

Synthesis methods for nanomaterials can use chemical, physical, photochemical and biological processes including methods such as laser ablation, gamma irradiation, electron irradiation, chemical reduction and microwave processing (Tran, et al. 2013; Iravani, et al. 2014). Physical methods for synthesis of nanomaterials include evaporation-condensation and laser ablation, wherein nanomaterials are generated using metallic bulk materials in solution (Iravani, et al. 2014). Chemical reduction using organic or inorganic reducing agents is commonly used in nanomaterial synthesis. Reducing agents for example sodium citrate, ascorbic acid, sodium borohydrate, and polyethylene glycol are used to reduce ionic salts to zero valent metallic nanomaterials (Khodashenas and Ghorbani 2015).

Metallic nanoparticles have been biologically synthesized through processes which include use of bacteria, yeasts, fungi, algae and plants (Senapati 2005; Debaditya and Rajinder 2005). Nanoparticles have been synthesized through bioreduction of ions in solution with culture supernatant bacterium such as *Bacillus licheniformis*, *Bacillus subtilis*, *Pseudomonas stutzeri* AG259, *Klebsilla pneumonia*, *E. coli*, *Enterobacter coacae*, through extracellular biosynthesis using *Fusarium oxysporum*, *Fusarium acuminatum*, *Phanerochaete chrysoporium*, *Aspergillus flavus*, C*oriolus verseicolor* and extracellular filtrate of *Cladosporiunm cladosporiodes* and *Penicillium fellutanum* (Iravani, et al. 2014). Bioreduction of ions to nanoparticles have also been conducted using marine algae *Chaetorceros calcitrans*, *Chlorella salina*, *Isochrysis galbana*, *Tetraselmis gracilis* (Sahverdi, et al. 2007), marine cyanobacterium *Oscillatoria wellie* NTDM01 and *Spirulina platensis*, and plant extracts from green tea, alfalfa, lemon grass and geranium (Iravani, et al. 2014).

Due to the size of nanoparticles, electrostatic forces between particles in solution can result in agglomeration and aggregation (Kim, et al. 2008). In order to reduce aggregates and agglomerates, stabilizing agents are added to the suspensions. Stabilizers added to suspensions alter the zeta potential (potential difference between the nanoparticle surface and the bulk solution); reducing particle attraction. Effects of stabilizing agents have also been studied to determine whether the stabilizing agent has a significant impact on the overall toxicity of the nanoparticles. Common stabilizing agents include polyvinylpyrrolidone, citrate, sodium borohydride and polyethylene glycol (Khodashenas and Ghorbani 2015).

2.4 Incorporation of Nanomaterials into Consumer Products and Commercial Applications

Nanomaterials are used in an increasing number of consumer products due to their desirable physicochemical characteristics which differ greatly from those of bulk materials (Shahrokh and Emitiazi 2009; Choi, et al. 2009). These include enhanced optical, electrical, catalytic and antimicrobial properties (Choi, et al. 2009; Anjum, et al. 2013).

Inorganic engineered nanomaterials consist of metal, metal oxide and metal complex nanomaterials which utilize the various enhanced properties of nanomaterials for consumer applications. Optical properties of nanomaterials are utilized in products such as paints, ceramics, sunscreens and cosmetics (Aitken, et al. 2006). Electronic properties of nanomaterials are utilized in batteries, solar cells and electronics (Suresh, et al. 2013). Nanomaterials such as CuO, ZnO, Ni, Pt and Pd are used for applications as catalysts (Wang and Gu 2015). Antimicrobial properties of nanomaterials, namely Ag and CuO, are used in consumer products for antimicrobial agents, medical devices, plastics, clothing, household appliances, biochemical assays and water filters (Suresh, et al. 2013).

Optical properties of nanoparticles which are exploited for consumer applications are largely the result of size-based light interactions. Nanoparticles below a metal-specific size range will be transparent as they interact with UV light rather than scattering light within the visible light spectrum. The varied response to light interaction in nanoparticles is derived from excitation of conductive electrons in metals which are referred to as plasmons (Kreibig and Vollmer 1995). When irradiated with light, an oscillating electric field causes conduction electrons to oscillate within the nanoparticle, displacing the electron cloud relative to the nucleus and creating a Coloumb attraction between the electrons and the nucleus which results in a restoring force (Kelly, et al. 2003). The oscillation frequency of the electron cloud is dependent upon the density of electrons, effective electron mass, shape and size of charge distribution which therefore varies with nanoparticle type, shape or size (Kelly, et al. 2003; Mock, et al. 2002). Electrical properties such as increased conductivity and resistivity of nanoparticles are utilized in electronic applications. Due to the size dependent interactions of nanoparticles with light energy, nanoparticles can produce differing electric fields which result in a range of dielectric permittivity and resistivity (Yurkov, et al. 2007). Nanoparticles are used in catalytic applications because their high surface area to volume ratio increases the rate of reaction. Antimicrobial applications of metallic nanoparticles are derived from the toxicity of the bulk metal; however, there are additional toxicity mechanisms that result from the reactive surface provided by the nanoparticles and potential cellular interactions due to their size (Gatoo, et al. 2014).

Silver nanoparticles (AgNPs) are one of the most commonly used types of nanomaterials which can be reportedly found in approximately 25% of the more than 1,300 nanomaterial-containing consumer products (Yu, et al. 2013). AgNPs are specifically desirable for diverse consumer applications due to their high electrical and thermal conductivity, surface-enhanced Raman scattering, catalytic activity, relative chemical stability, non-linear optical behaviour, bactericidal, fungicidal, antiviral and anti-inflammatory activity (Krutyakov, et al. 2008; Ahamed, et al. 2010; Ge, et al. 2014). These consumer products range from washing machines, refrigerators, vacuum cleaners, paints, textiles, medical devices, dressings, cosmetics, photocatalysts and food storage containers (Boxall, et al. 2007; Kim, et al. 2007; Blaser, et al. 2008; Klaine, et al. 2008; Ma, et al. 2010; Fauss 2008; Uchihara 2007; Sibbald, et al. 2007; Skirtach, et al. 2006; Galiano, et al. 2008). Nanosilver technology can be applied to consumer products in several forms including coatings, liquid colloids, filaments, and powders (Fauss 2008). Textile finishes can be applied as dispersions to various materials such as cotton, polyester, wool, nylon and rayon which are then woven into textile products (NanoHorizons, Inc. 2016). Dispersions and powders can also be used in solvent-based or melt processes for the formulation of foams, coatings, adhesives, sealants and elastomers (NanoHorizons, Inc. 2016).

2.5 Environmental Fate of Nanomaterials

In the case of accidental release, nanomaterials could enter the air, surface water, groundwater or soil. Engineered nanomaterials can also enter the environment through release associated with wear and weathering of consumer products and intentional release for environmental applications (Ray, et al. 2009). Environmental receptors consist of living organisms, the habitat supporting the organisms and natural resources which could be affected by environmental contaminations through release or migration (EUGRIS: portal for soil and water management in Europe 2016). The main environmental receptors of silver nanomaterials consist of sediment, soil, water, wastewater treatment residuals and the organisms within each of these compartments (Nyberg, et al. 2008). The risk posed to receptors is directly related to the transformations of the nanomaterials during and after consumer usage.

Textiles such as socks that use silver coated fibers as an antimicrobial have been proven to leach nanomaterials at concentrations up to 1.3 mg Ag/L after washing with distilled water four times (Benn and Westerhoff 2008). Metallic nanomaterials are not biodegraded during the wastewater treatment process (Figure 2.3), instead becoming bound to organic matter (Blaser, et al. 2008;

Mueller and Nowak 2008; VandeVoort and Arai 2012; Gottschalk, et al. 2009; Nicholson, et al. 2003). These nanomaterials having undergone some process of weathering, wear or transformation can be referred to as aged (Nowack and Mitrano 2018). The nanomaterials within waste treatment residuals can then be applied to agricultural soils in the form of biosolid amendments for which there are currently no regulatory guidelines. The presence of nanomaterials in biosolids is therefore of particular concern since soil-plant systems are important to ecosystem function, nutrient cycling, food production and could pose risks for bioaccumulation in crops (Coutris, et al. 2012).

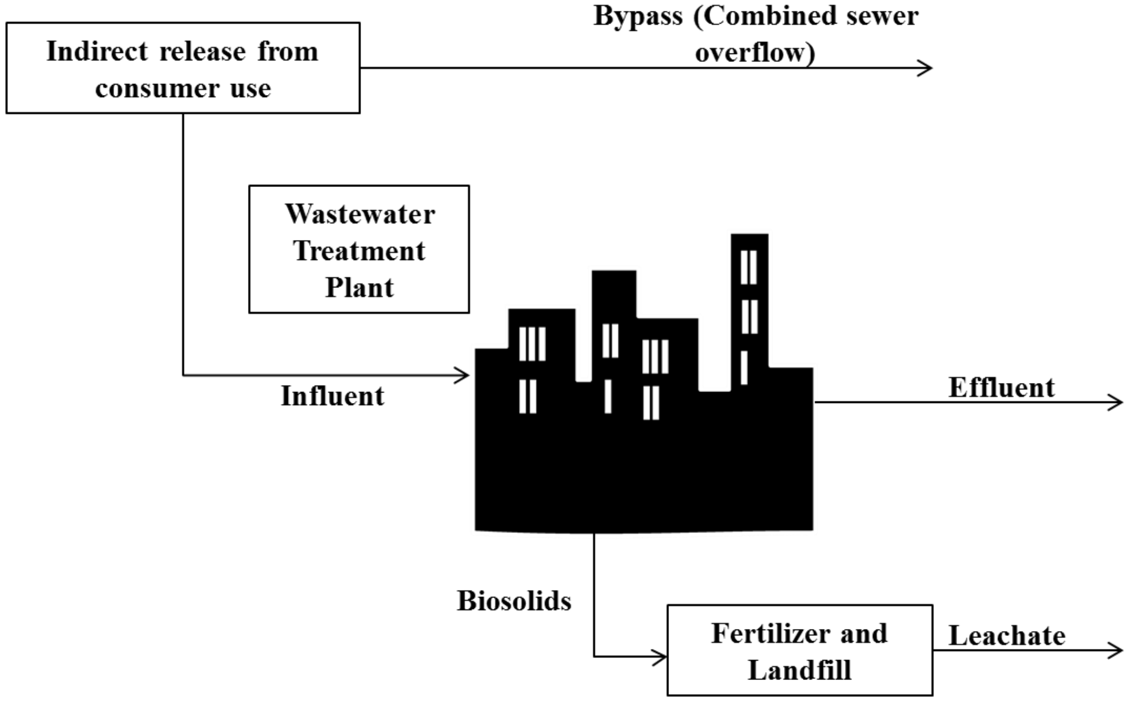

Figure 2.3: Environmental routes of exposure to nanomaterials

Nanomaterials could be expected to be released in a certain form during consumer product use, however, this form and the behaviour may be altered as the product use changes and weathering occurs (Ray, et al. 2009). Textiles utilizing nanofibers are at particular risk to this as they can be subjected to a range of conditions in use, washing and drying. Additionally, changes to detergents can alter the release rates of nanomaterials acting as a dispersant due to surface charge interactions (Hedberg, et al. 2014).

Ontario, Canada applies 120,000 tonnes of dry biosolids to approximately 150 km^2 of land annually (Lapen, et al. 2008). Gottschalk et al. (2009) modelled concentrations of AgNMs in environmental receptors for different geographic regions and predicted a sludge concentration of 1.29-5.86 mg AgNMs/kg sludge in the United States. Assuming similar usage rates of nanomaterials in Canada compared to the United States, a comparable AgNM sludge concentration should exist. Based on this concentration, application rate, an assumed furrow depth of 15 cm (Ontario Ministry of Agriculture, Food and Rural Affairs 2009) and an average soil bulk density of 1450 kg/m^3 (United States Department of Agriculture 2016), increases in AgNM soil concentrations in Canada could be estimated as high as 21.4 µg AgNMs/kg soil (0.0214 mg/kg) per biosolid application (Appendix

A). Background levels of silver in soils including Canadian garden soils have been reported between 0.06-0.4 mg Ag/kg meaning that any application of biosolids to soil will be cumulative to the existing background silver concentration (United States Environmental Protection Agency 1981; Rasmussen, et al. 2001). Silver nanoparticles added to sludge sequencing batch reactors, similar to those used in waste treatment, were 79% transformed to sulphidized silver nanoparticles (SAgNPs) with 87% remaining in this form in varied soils for over one year (Wang, et al. 2016). SAgNPs could therefore be a prominent AgNM species in environmental media due to silver's affinity for sulphur and the insolubility of silver sulphide.

In addition to the transformation of nanomaterials in soil to other complexes, the dissolution of the nanomaterials into their ionic form can also occur. Dissolution of silver nanomaterials has been found to increase with decreasing pH and decreasing levels of natural organic matter (Lui and Hurt 2010). Alternatively, nanomaterials can become sorbed to soil surfaces, where adsorption increases with increasing clay content (Cornelis, et al. 2010). Nanomaterials or their ionic form can be taken up into plants, bacteria or other organisms. Plant uptake has been demonstrated to be affected by metal concentration, pH, cation exchange capacity, organic matter, type and variety of plants and root age (Jung 2008). Concentration is generally accepted as a major factor affecting plant uptake while the main routes of uptake in plants are direct uptake and osmosis into root hairs (Alloway and Davies 1971; Adriano 1986). The fate of AgNPs in soil can therefore be summarized conceptually by Figure 2.4; additional transformations not shown include chlorination and complexation with organic ligands.

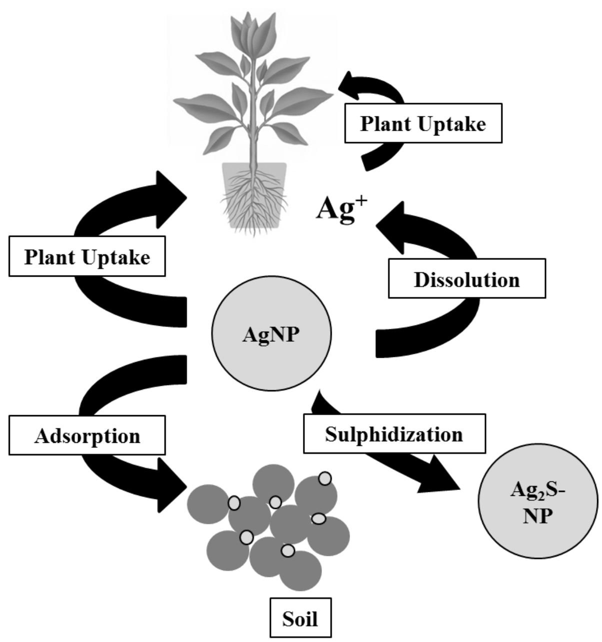

Figure 2.4: Conceptual model of soil-nanoparticle interactions

2.6 Engineered Nanomaterials and Nutrient Cycling

A limited number of studies have been conducted to determine the impact of metallic nanoparticles on microbial diversity and nutrient cycling in soil. Iron oxide nanoparticles were found to enhance the growth of actinobacteria in soil (He, et al. 2011). Contrastingly, pristine silver nanoparticles, titanium dioxide nanoparticles and zinc oxide nanoparticles have been found to reduce soil microbial biomass, potentially influencing the availability of nutrients for plant growth through nutrient cycling (Hansch and Emmerling 2010; Ge, et al. 2011; Ge, et al. 2012). Nitrogen-fixers have demonstrated susceptibility to Ag, TiO_2, CeO_2 and ZnO nanoparticles while microorganisms which aid in nitrogen cycle functionality have in general shown sensitivity to metallic nanoparticles (Ge, et al. 2011; Ge, et al. 2012; Priester, et al. 2012; Kumar, et al. 2011; Kumar, et al. 2012; Collins, et al. 2012). Silver nanomaterials have demonstrated toxicity to heterotrophic bacteria and chemolithotrophic bacteria responsible for denitrification and soil formation, respectively (Throback, et al. 2007). As a result, use of nanomaterials could have implications on soil microbial diversity, nutrient cycling, plant and ecosystem health which should be considered for the full lifecycle of nanomaterial-containing products and studied in environmental conditions.

2.7 Ecotoxicity of Nanomaterials

2.7.1 General mechanisms of toxicity

The differences between the physicochemical properties of nanomaterials and bulk materials affect the mechanisms of toxicity. It is suggested that the toxicity of nanomaterials is caused by their size, surface area and shape (Gatoo, et al. 2014). Decreases in the size of nanomaterials create a greater surface area relative to particle volume. This generates more reactive surfaces for interaction within biological systems (Gatoo, et al. 2014). Studies of a variety of nanomaterials have shown that nanomaterial size, surface chemistry, shape and aggregation can affect the generation of free radicals, reactive oxygen species (ROS) and oxidative stress which all influence toxicity (Aillon, et al. 2009).

Gene expression microarray analysis conducted with AgNPs and ionic silver (5 mg Ag/L growth media) in *Arabidopsis thaliana*, a terrestrial plant, demonstrated consistent expression levels after exposure for 446 and 405 genes, respectively (Kaveh, et al. 2013). 375 and 141 genes were expressed with either a less than 0.5 or greater than 2-fold change after exposure, respectively (Kaveh, et al. 2013). Among these, 15 genes were upregulated and 29 genes downregulated by both AgNPs and Ag^+, indicating that only some of the effects of AgNPs on gene expression originate from the release of ionic silver (Kaveh, et al. 2013).

2.7.2 Effect of pristine nanoparticles on pure cultures and single species

Proposed mechanisms of toxicity of nanomaterials on microorganisms include free silver ion uptake followed by disruption of ATP production and DNA replication, nanoparticle and ion generation of ROS and nanoparticle direct damage to cell membranes (Marambio-Jones and Hoek 2010) .

Silver nanoparticles may act as catalysts in reactions with oxygen leading to the production of excess free radicals which subsequently attack membrane lipids, break down the cellular membrane and mitochondrial function or cause DNA damage (Mendis, et al. 2005). Studies of eukaryotic cells imply that silver nanoparticles inhibit the antioxidant defense of cells, such as glutathione (GSH), by binding with GSH reductase or GSH maintenance enzymes, further increasing cellular ROS concentrations (Carlson, et al. 2008). In a study of the toxicity effect of Ag^+, AgCl and AgNPs on nitrifying bacteria, at the same level of intracellular ROS concentration, AgNPs appeared more toxic indicating that generation of ROS is not the only toxicity mechanism (Choi, et al. 2008).

Ag^+ interacts with respiratory chain reactants such as nicotinamide adenine dinucleotide dehydrogenase (NADH) and results in the uncoupling of respiration from ATP synthesis (Marambio-Jones and Hoek 2010). Silver ions bind with transport proteins, collapsing the proton motive force (Dibroc, et al. 2002; Holt and Bard 2005; Lok, et al. 2006), inhibiting phosphate uptake (Schreurs and Rosenberg 1982) and increasing DNA mutation during polymerase chain reactions through interactions with thiol groups in enzymes (Yang, et al. 2009). Bacterial cells exposed to Ag^+ undergo changes to their morphology such as cytoplasm shrinkage, detachment of the cell membrane, DNA condensation and cell membrane degradation resulting in intracellular content leakage (Feng, et al. 2000; Jung, et al. 2008). Additionally, physiological changes occur wherein bacterial cells enter a non-culturable state such that the bacteria are still active, however, they are unable to grow or replicate (Marambio-Jones and Hoek 2010).

Kim et al. (2007) observed no toxicity from silver nanoparticles or silver nitrate when in the presence of an antioxidant, suggesting that the antimicrobial mechanisms of silver nanoparticles against *Staphylococcus aureus* and *E. coli* are linked to free radical induced damage to the membrane. Silver nanoparticles have been observed to penetrate *E. Coli* cells and form pits in the cell membrane (Choi and Hu 2008; Raffi, et al. 2008; Sondi and Salopek-Sondi 2004). Similar accumulation on the cell membrane and cellular uptake has been demonstrated in other bacteria such as *Vibrio cholerae*, *Pseudomonas aeruginosa* and *Samonella typhi* (Marambio-Jones and Hoek 2010). Nanoparticles smaller than 10 nm have been observed inside some bacteria (Morones, et al. 2005) while nanoparticles up to 80 nm have been transported through the inner and outer membrane of *P. aeruginosa* (Xu, et al. 2004).

The mechanism which allows nanomaterials to penetrate cells is not fully understood, however, it is proposed, according to the theory of hard and soft acids and bases, that it results from silver's high affinity for reaction with phosphorus and sulfur compounds such as those found in proteins in the bacteria membrane (Hatchett and Henry 1996; Vitanov and Popov 1983; Ahrland, et al. 1958; Alcamo 1997; Liau, et al. 1997). Nanomaterials inside bacteria can then react with sulphur-containing proteins in the cell interior and phosphorus-containing compounds such as DNA (Kim, et al. 2007; Feng, et al. 2000; Morones, et al. 2005; Sondi and Salopek-Sondi 2004). Changes to morphology of the cell membrane and reactions between nanoparticles and DNA negatively affect processes such as cellular respiration and cell division, resulting in cell lysis (Alcamo 1997). Dissolution of nanomaterials can result in an increase in concentration of free ions which also contributes to the bactericidal effect of antimicrobial nanomaterials (Feng, et al. 2000; Morones, et al. 2005).

Choi et al. (2008) demonstrated that AgNPs (14 nm) at a concentration of 1 mg/L inhibited growth of 86% of autotrophic nitrifying bacteria in suspension using a batch extant respirometric assay. Among AgNPs, Ag^+ and AgCl colloids, AgNPs resulted in the greatest inhibition of nitrifying bacteria growth (Choi, et al. 2008). Choi and Hu (2008) found that AgNPs effect on bacterial growth was greater at sizes less than 5 nm and showed a size dependency.

In hydroponic studies, AgNPs have been demonstrated to reduce root elongation, seed germination and plant biomass production (Sillen, et al. 2015). Yin et al. (2012) studied the effect of silver nanoparticle exposure on germination and early growth of eleven wetland plants and determined that in pure culture PVP AgNPs (21 nm) had no effect on germination while at concentrations of 40 mg/L gum arabic coated AgNPs (GA-AgNPs, 6 nm) significantly reduced the hydroponic germination rate of three species, enhanced germination in one species and $AgNO_3$ enhanced the germination rate of five species. Primary root cells in maize were elongated in treatments with AgNPs and appeared thinner with more irregularities in $AgNO_3$ treatments as compared to controls (Pokhrel and Dubey 2013). Maize biouptake silver concentrations of 22 and 1.8 mg Ag/kg in seedlings were observed for $AgNO_3$ (127 mg/L) and citrate-AgNPs (73.4 mg/L), respectively, indicating a normalized Ag biouptake 7.06-fold greater in $AgNO_3$ compared to citrate-AgNPs (Pokhrel and Dubey 2013). These findings indicate that plants can be physiologically affected by both nanoparticles and ionic salts; however, it is unclear how these effects differ mechanistically.

Figure 2.5 displays inhibition as a function of exposure concentration for model organism type and general nanoparticle size (Table A.1) and demonstrates the lack of trend between inhibitory exposure concentrations across type of organism or nanoparticle size. These findings also indicate that microorganisms are more susceptible to AgNPs than plants in general. These ecotoxicity studies have been conducted with single organism species in growth media (Raffi, et al. 2008; Kim, et al. 2008; Arora, et al. 2009; Smetana, et al. 2008) or aqueous solutions (Yoo-iam, et al. 2014; Jiang, et al. 2012; Barrena, et al. 2009; Asghari, et al. 2012; Navarro, et al. 2008). While they provide a basis for further ecotoxicity studies and insight into toxicity mechanisms, the findings of Lee et al. (2012) indicate that toxicity of silver nanoparticles to crop plants in growth media (agar) differs from those in soil media due to reduced bioavailability in soil. For this reason, the toxicity of nanomaterials in soil cannot be inferred from ecotoxicity testing in less complex media.

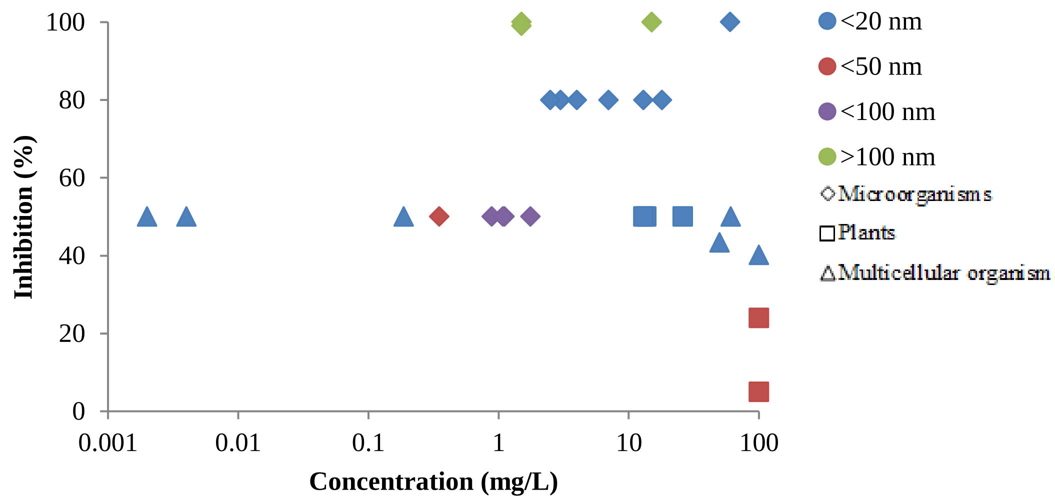

Figure 2.5: Inhibition as a function of exposure concentration of AgNPs with varied nanoparticle size and model organism species

2.7.3 Effect of nanoparticles in complex media

Concerns regarding use of AgNPs originate from their antimicrobial effects against both Gram-positive and Gram-negative bacteria (Morones, et al. 2005), and anti-fungal activity (Panacek, et al. 2009). Concentrations at which negative effects of AgNPs on plants have been observed in soil studies have been found to be higher than in hydroponic studies due to decreased bioavailability (Dimkpa 2014). The presence and importance of bacteria and fungi in plant rhizospheres and nutrient cycling mean that antimicrobial effects of AgNPs could impact plant health both indirectly or directly through uptake (Sillen, et al. 2015).

A study of sludge (3 hours of gravity settling) exposed to PVP AgNPs (68 nm) at concentrations of 1 mg/L showed approximately one log unit loss in heterotrophic culturability after 24 hours exposure as compared to the untreated control (Sun, et al. 2013). Planted biosolid amended soils with 0.14 mg AgNPs/kg (21 nm) were found to have significantly different microbial community composition compared to control treatments after one day through terminal restriction fragment length polymorphism (T-RFLP) analysis of 16S rRNA bacterial genes (Colman, et al. 2013). After 50 days, microbial community composition of the biosolid and AgNP treatment converged with the biosolid control despite containing 35% less microbial biomass and having decreased activity of extracellular enzymes leucine aminopeptidase (52%) and phosphatase (27%) (Colman, et al. 2013). Hansch and Emmerling (2010) determined that soil demonstrates a concentration-dependent decrease in microbial biomass as concentration of AgNPs (26 nm) increased. Contrastingly, decreased bacterial and archaeal abundance was observed with increasing soil concentration of Ag^+ (0.1-93 mg/kg) and Ag_2S-NPs (0.1-5590 mg/kg) and remained consistent for increasing concentrations of AgNPs (44 nm, 0-404 mg/kg) (Doolette, et al. 2016).

The maximum predicted concentration of AgNMs in biosolids would result in addition of 21.4 µg AgNMs/kg soil with each biosolids application, occurring every three years. As shown in Figure 2.6, AgNMs at this maximum exposure concentration from biosolids would equate to 100 mg AgNMs/kg soil after 14000 years of triennial biosolid applications. Kumar et al. (2011) used community level physiological profiling (CLPP) to examine soil which was treated with AgNPs (20 nm) at a concentration of 660 mg/kg soil (equivalent to over 90,000 years of biosolids loading), incubated for 176 days and determined that nanoparticle exposure resulted in a significant decrease in the culturable microbial population and diversity. AgNP treated soil was unable to utilize amines, phenolic compounds, polymers, and amino acids but could utilize carbohydrates and carboxylic acids (Kumar, et al. 2011). These findings indicate that silver nanoparticles impart toxicity effects on heterotrophic bacteria and potentially autotrophic bacteria as well; however, the impact on diversity is often concentration dependent.

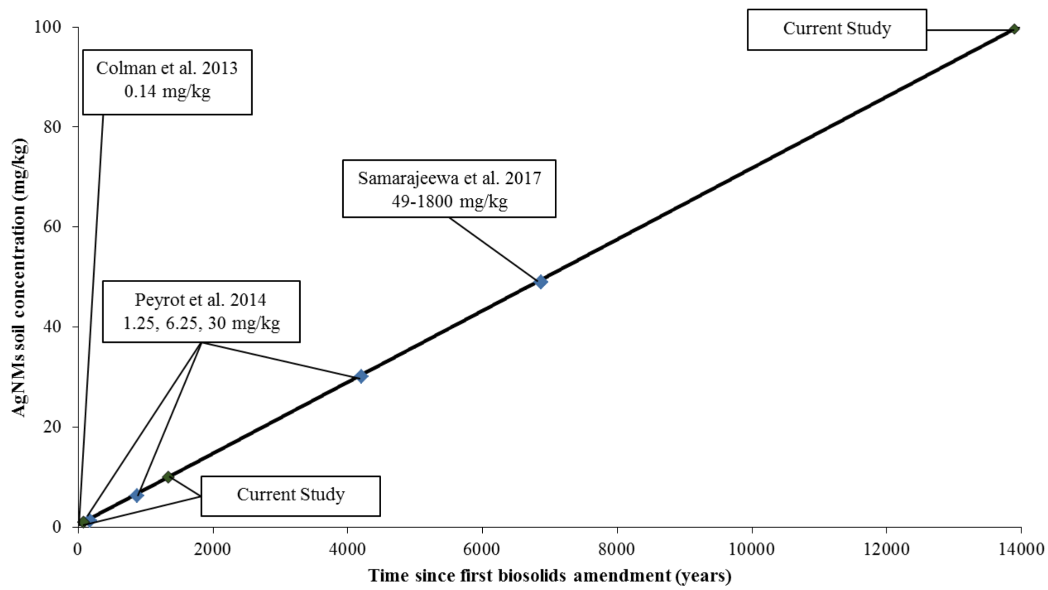

Figure 2.6: Maximum cumulative soil concentration of AgNMs resulting from biosolid amendments made every three years. Concentrations of AgNMs examined in this study as well several others from the literature are labelled.

Silver nanoparticles added to soil as a biosolid application at 0.14 mg/kg soil did not affect total root biomass of *Microstegium vimineum* (Japanese stiltgrass), however, AgNP treatments had 32% less aboveground biomass relative to controls (Colman, et al. 2013). In soil experiments, plant growth differed by species with *Lolium multiflorum* growing faster in both $AgNO_3$ and GA-AgNP (6 nm) exposures, ten other species having significantly reduced growth in GA-AgNP treatments and significantly reduced growth of *Phytolacca Americana* with PVP AgNPs (20 nm) (Yin, et al. 2012). Plant growth in soil studies has shown species dependent effects both negative and positive as a result of exposures to different types of silver nanomaterials and ionic silver (Colman, et al. 2013; Yin, et al. 2012). As such, to determine the effects of nanomaterials to any species of crop plant, considerations must be made as to the most relevant concentrations, complexes and particle size which will be present in a given environment.

3 Chapter 3: Materials and Methods

The following chapter discusses the materials, methods and instrumentation that have been used throughout this thesis.

3.1 Experimental Design

Two 84 day soil exposure studies were conducted in a greenhouse located at the Royal Military College of Canada, Kingston, ON to examine the effects of silver nanomaterials on soil microbial communities beginning on January 9th, 2017 (Chapter 4) and soil-plant systems beginning August 7th, 2017 (Chapter 5). The duration of 84 days was chosen to allow for a moderate-length analysis of soil microbial activity and to allow for the microbial community to become fully stabilized to its environment. Additionally, given the moderate temperature of the greenhouse and ideally minimal temperature fluctuations, 84 days was chosen as the approximate end of one full growth cycle of wheat since an average daily temperature of 21°C would equate to 1764 growing degree days within range of the 1768 growing degree days required for flowering completion (Haun 1973). Silver dispersions of ionic silver, uncoated AgNPs, PVP coated AgNPs, weathered nanomaterials or SAgNPs were individually added to soil. Three sample sets were generated for sacrificial sampling of one set per month over the course of three months (schematics shown in Table 4.1 and Table 5.1). Three replicates of each sample treatment type were generated by mixing 50 mL dispersions, as described in 3.5, into each of three 1.7 kg Ziploc bags of soil and subdividing 500 g into three pots for a total of nine pots per treatment. Control soils were treated with the equivalent volume of DI water. Sulphidized nanoparticle dispersions (150 mL) were added to 5.1 kg soil bags to avoid concentration differences between synthesized nanoparticle solutions. Planting cups (Dollarama, ON, CAN) with a depth of 13 cm, diameter of 8.5 cm at the top, 5.6 cm diameter at the bottom and two holes for drainage were filled with soil and placed on planting saucers (Canadian Tire, ON, CAN). In the planted experiment, Chapter 5, pots were each seeded with 30 wheat kernels (*Triticum spp.,* Bulk Barn, ON, CAN) one day following nanoparticle addition.

Soils were maintained for the duration of soil exposure studies through the addition of 30 mL of DI water three times weekly; this volume being comparable to the average summer rainfall in Kingston, ON (The Weather Network 2018). After one month of planted exposure (Chapter 5), watering was increased to 60 mL three times weekly due to the increased water requirement and suspended one week before the conclusion of the experiment to avoid over-saturation of the soil.

3.2 Soil preparation and analysis

Agricultural soil containing clay aggregates (0-20 cm depth) was collected from Harrowsmith, Ontario (N 44° 24.3' W 76° 38.8'). The soil structure was determined to be representative of a clay loam based on the behaviour of the soil (Thien 1979) and soil surveys of the region. A soil survey of Frontenac County in 1966 indicated that the Harrowsmith-Sydenham region consists of Bondhead soil, namely Bondhead loam and Bondhead sandy loam (Canada Department of Agriculture and Ontario Agricultural College 1966). In obtaining the soil, the top layer associated with plant roots was removed thus the lower soil layer was collected and is consistent with the B horizion of this soil type, clay loam (Canada Department of Agriculture and Ontario Agricultural College 1966). The soil was sieved (6 mm), homogenized using coning and quartering in a method adapted from Raab et al. (1990), sealed in Ziploc bags and stored at 4˚C until each experiment was conducted. Soils were analyzed for background silver concentrations. Total silver concentrations were assessed using a hot nitric acid digestion and inductively coupled plasma mass spectrometry (ICP-MS, Elan DRC II, Perkin Elmer, MA, USA) (Button, et al. 2012). A small quantity of each soil sample was dried overnight at 110˚C (Thermo Precision Econotherm Oven, Fisher Scientific, NH, USA). The dried soil was ground with a mortar and pestle. 0.3 g of dried, ground soil was weighed into 2 wt% nitric acid-washed digestion tubes in which 10 mL 15.7 M (70 wt%) HNO_3 acid (Trace element grade, Fisher Scientific, NH, USA) was added to each tube. The digestion tubes were then heated in a block heater (VWR, PA, USA) at 120˚C until dry. The samples were taken back into solution through addition of 2 mL of 50 wt% nitric acid and heated at 50˚C for 30 minutes. The samples were transferred to 15 mL centrifuge tubes (VWR, PA, USA) by vortexing (Fisher Vortex Genie 2, Fisher Scientific Analog Vortex Mixer, NH, USA) and rinsing with two additions of 5 mL deionized water (DI, 18 mΩ). After settling overnight, an additional 4x dilution was made. Digested samples were analyzed for silver content using ICP-MS in a final matrix of 2 wt% nitric acid with an internal rhodium standard. NIST SRM-2711a Montana soil (Sigma Aldrich, ON, CAN) was used as a standard reference material to quantify the silver recovery during each acid digestion and included with reagent blanks every 15 samples.

3.3 Nanomaterial Preparation

Uncoated and PVP coated (0.2 wt%) silver nanoparticles measuring 20-30 nm in diameter in powdered form were obtained from Skyspring Nanomaterials, Inc. (TX, USA). SAgNPs were synthesized from PVP coated nanoparticles and sodium sulfide (Sigma-Aldrich, ON, CAN) using a method adapted from Stegemeier et al. (2015). Nanopowders were dispersed in DI water using sonication at 50 Hz for 30 seconds (Fisher Scientific Model 505 Sonic Dismembrator, Fisher Scientific, NH, USA). Ionic silver in the form of $AgNO_3$ (Sigma-Aldrich, ON, CAN) was also used.

Sulphidation was completed using 10 or 100 mg of PVP AgNPs added to an Erlenmeyer flask containing 100 mL DI water and 90 or 900 mg $Na_2S{\cdot}9H_2O$, respectively. Two adjustable air pumps (National Geographic, Pacific Coast Distributing, Inc., AZ, USA) fitted with vinyl airline tubing (Python Products, Inc., WI, USA) and pipette tips were inserted into each aluminum foil wrapped flask, covered with parafilm and allowed to react for duration of one week. The water was kept at a constant level through addition of DI water daily. After one week, the resulting SAgNPs were obtained by pouring the solution into 50 mL centrifuge tubes (Fisher Scientific, NH, USA), washing three times by centrifugation at 4150 rpm for one hour (Sorvall Legend RT Centrifuge, Thermo Scientific, NH, USA), removing the supernatant and adding DI water followed by sonication at 50 Hz for 30 seconds.

Weathered nanomaterials were obtained from washings of “T.H.E Sock” (Lululemon Athletica, ON, CAN). These socks were chosen for further use in toxicological studies in an external product survey of 11 textile products advertised as containing silver nanomaterials (Gagnon, et al. 2017). X-ray fluorescence was conducted on these textile products amongst which six contained silver at concentrations above the detection limit of 37 mg/kg (Gagnon, et al. 2017). “T.H.E Sock” was chosen for further use due to its high quantity of silver per unit material mass and relatively low cost in comparison to these other products. Additionally, socks were chosen rather than other articles of silver-incorporated clothing because they undergo more natural wear than other garments. Finally, their size also makes them suitable for replicable laboratory-based methodologies. Socks were washed with Tide® Liquid Original detergent using a method adapted from ISO105-C06 in order to closely replicate household laundry washing. 300 mL of soap solution (4 g detergent/L tap water) was added to a cylindrical 2 L polypropylene container and heated to 40˚C. Two socks were placed inside the polypropylene container with 10 polypropylene balls (2.5 cm diameter), sealed and shaken at 40˚C and 150 rpm for 45 minutes (Innova 4320 Refrigerated Incubator Shaker, New Brunswick Scientific, CT, USA). The socks were then rolled and squeezed to remove the water. The resulting solution was transferred into smaller Nalgene containers with a subsample stored in a centrifuge tube and acid digested (250 µL HNO_3 to 250 µL sample at room temperature for one hour before dilution) to quantify total silver. 150 mL of sock wash solution was horizontally frozen in three 500 mL Nalgene bottles before being freeze dried (FreezeZone® Freeze Dry System 77520, Labconco, MO, USA). Freeze-drying of sock wash solution was required in order to reduce the overall solution volume and attain a final silver concentration which could be compared to pristine nanoparticles and quantified once added to soil. Freezing and freeze-drying immediately after washing also allowed for the nanomaterials to be removed from solution to prevent dissolution and allow for stable storage. This process was repeated until the three Nalgene bottles each contained 1.7 mg Ag as verified from cumulative measurement of silver concentrations in the wash water and solution volumes prior to freeze-drying. Soap controls with an equivalent volume of soap solution were also generated using this process. In the case of the planted study discussed in Chapter 5, sock washes were conducted without the use of soap, using DI water. Nanomaterials released from socks were characterized before and after freeze-drying as described in section 3.4 and had a similar size distribution and morphology indicating that freeze-drying did not alter the particle characteristics.

3.4 Nanomaterial Characterization

Nanomaterial characterization was conducted using single particle inductively coupled plasma mass spectrometry (SP-ICP-MS), scanning transmission electron microscopy (STEM) and dynamic light scattering (DLS). SP-ICP-MS determined the average particle size of nanoparticles in solution based on an assumed spherical shape. A sample of each nanomaterial solution was diluted in DI water by factors of 10 (10000-1000000x) and analyzed with SP-ICP-MS using an instrumental dwell time of 10 ms and standard 100 nm nanoparticle to verify the ionic silver calibration curve. The dilution in which the frequency of particle detection was below 5% of the sample volume analyzed was used to calculate the average particle size and size distribution to ensure that only a single silver particle was being measured at a time. Samples of each nanomaterial in solution were dispersed on a copper carbon type B TEM grid (Ted Pella, CA, USA) and dried to analyze the particle size using an environmental scanning and transmission electron microscope (S/TEM, Quanta FEG 250, ThermoFisher Scientific, OR, USA). A minimum of thirty images of each type of sample were collected to attain a representative measure, with electron dispersive x-ray spectroscopy (EDS) used periodically to confirm the particle composition. After collecting images of each sample, Fiji ImageJ software was used to quantify the size of nanomaterials. A minimum of 76 particle sizes were assessed in each sample, this being the lowest quantity due to fewer particles and larger aggregates present in a sulphidized nanoparticle sample. EDS was conducted using an Octane Elite Plus Silicon Drift Detector with a silicon nitride window (AMETEK, Inc., PA, USA) and analyzed using TEAM EDS Analyzer System (TEAM™ Software, AMETEK, Inc., PA, USA) to confirm the generation of sulphidized nanoparticles through comparison to elemental composition and mapping of PVP AgNPs (Appendix B). DLS (NanoBrook Omni, Brookhaven Instruments Corporation, NY, USA) was used to determine the number average hydrodynamic diameter of pristine nanoparticles in solution (DI) based on triplicate runs each 3 minutes in duration using autoslope normalization and a scattering angle of

90˚. Sizes of uncoated AgNPs, PVP AgNPs, SAgNPs with 10 mg of initial PVP AgNPs, SAgNPs with 100 mg of initial PVP AgNPs and weathered nanomaterials are summarized in Table 3.1 as the mean ± standard deviation (S.D.).

Table 3.1: Average nanomaterial size with standard deviation determined using single particle ICP-MS (SP-ICP-MS), dynamic light scattering and scanning transmission electron microscopy (STEM)

Nanomaterial Type	SP-ICP-MS Average Particle Size (nm)	Hydrodynamic Number Average Diameter (nm)	STEM Average Particle Size (nm)
Uncoated AgNPs	140 ± 78	36 ± 13	60 ± 92
PVP AgNPs	130 ± 74	24 ± 6.0	25 ± 52
SAgNPs-10 mg	120 ± 90	N/A	130 ± 340
SAgNPs-100 mg	160 ± 84	N/A	600 ± 260
Weathered	110 ± 54	N/A	210 ± 120

The size detection limit of the instrument for SP-ICP-MS is dependent upon the instrument ionic background and in this case results in a minimum quantifiable particle size of 50 nm. Consequently, pristine uncoated and PVP AgNPs wherein 73% and 97% of particles were less than 50 nm from STEM, respectively, indicates that DLS and STEM more accurately describe these particles whereas SP-ICP-MS measures the particle aggregates ≥50 nm. In the case of sulphidized particles, SP-ICP-MS was able to obtain a less variable measure of particle size as compared to STEM. The differences between the reported sizes of these particles in solution (SP-ICP-MS) and dried (STEM) could be the result of large aggregates being formed during drying. While pristine particles were spherical in shape with some particles forming aggregates, sulphidized particles were formed from aggregates of PVP coated particles and are therefore larger in size. STEM measures can also be biased towards larger, more visible particles as well as representing fewer particles than the alternative methods. STEM analysis of weathered nanomaterials generated a greater average particle size than that of SP-ICP-MS. Although this was the case, weathered nanomaterials were commonly found to consist of sheets comprised of smaller particles as seen in Figure 3.1. Rectangular sheets with the corresponding average mass measured from SP-ICP-MS and size from STEM would therefore have thicknesses on the nanoscale.

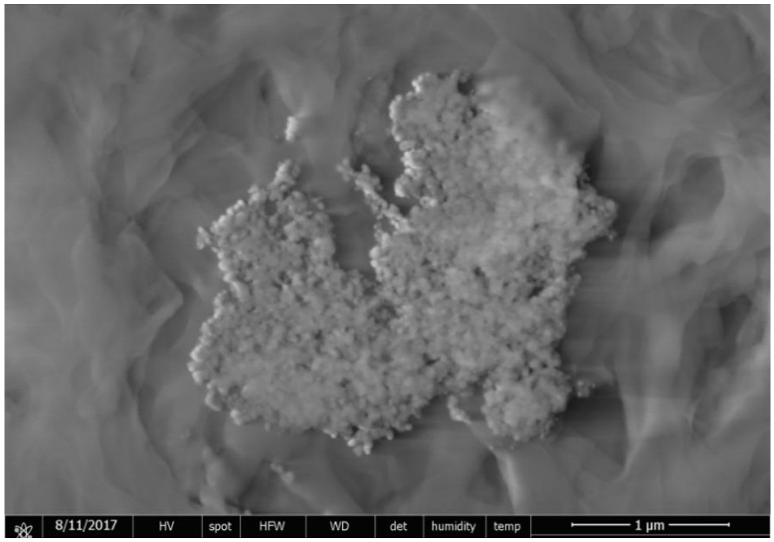

Figure 3.1: Weathered silver nanomaterial obtained from washing of T.H.E Sock filtered on a 0.45 µm millipore filter

3.5 Soil Nanomaterial Addition

Variability in nanoparticle dispersions is a prominent existing issue, as evidenced by reporting of concentration and nominal concentration in literature (Samarajeewa, et al. 2017). Benoit et al. (2013) spiked two agricultural soils with sodium polyacrylate stabilized silver nanoparticles (1-10 nm) and determined that analytical data for low concentrations 1-10 mg Ag/kg soil were inconsistent and only concentrations 25-100 mg Ag/kg were consistent. Due to heterogeneity of nanoparticle dispersion in soil media demonstrated at the desired concentration range of the following studies, initial experimentation was conducted to determine the most suitable method for introducing nanomaterials into soil. Stabilizing agents are commonly used in the dispersion of nanoparticles due to their tendency to aggregate from electrostatic interactions. To examine a range of nanomaterial types without the aid of an additional stabilizing agent it was important to find a spiking method capable of obtaining a more homogenous distribution in soil.

Uncoated AgNPs were chosen for preliminary analysis rather than the PVP coated, sulphidized or weathered since these nanomaterials have a coating or dispersant present which are thought to aid in a more homogeneous soil distribution. Uncoated AgNPs or ionic silver ($AgNO_3$) were added to each soil sample and mixed thoroughly to provide a homogenous dispersion in the soil. Ionic silver was chosen to be representative of a homogeneous distribution in soil since it is soluble and should therefore disperse well. Both dry and wet dispersion techniques were tested to determine which was best suited for nanomaterial application to soil. Dry dispersant applications were made in approximately 20 mg of inert talc powder (Fisher Scientific, NH, USA) whereas wet dispersion applications were made in 50 mL of DI water and sonicated for 5 minutes in a sonicating bath (Fisher Scientific FS140H, NH, USA). Solutions of $AgNO_3$ and AgNPs were prepared at 300 mg Ag/L and 30 mg Ag/L through a 10 times dilution before being added to 1.5 kg of soil in plastic resealable Ziploc bags (33 x 45.7 cm) by pipetting 26 mL in 2 mL increments, mixing manually by rotation of the soil followed by shaking and repeating for the remaining 24 mL. Both ionic silver and uncoated AgNPs were mixed in agricultural soil via talc or solution to obtain a final silver concentration of 1 mg/kg or 10 mg/kg in soil. Each of these eight soil treatments was then subsampled for five replicates which were nitric acid digested and examined using ICP-MS as in 3.2. Mean silver concentrations with S.D. of treatments are reported in Table 3.2.

Table 3.2: Average soil silver concentration with standard deviation in ionic silver and uncoated AgNPs treatments prepared in talc or solution

Dispersion Type	Treatment	Average Soil Silver Concentration (mg/kg)
Talc	Ionic 1 mg/kg	1.5±0.33
	Ionic 10 mg/kg	7.8±1.8
	Uncoated AgNPs 1 mg/kg	2.3±0.82
	Uncoated AgNPs 10 mg/kg	6.5±1.2
Solution	Ionic 1 mg/kg	1.8±0.28
	Ionic 10 mg/kg	9.9±3.4
	Uncoated AgNPs 1 mg/kg	0.71±0.48
	Uncoated AgNPs 10 mg/kg	9.4±4.4

Statistical analyses indicated significant differences between mean concentrations of uncoated AgNPs and ionic silver treatments applied in solution for objective soil concentrations of 1 mg Ag/kg as well as between uncoated AgNPs 1 mg Ag/kg applied in talc or solution ($p<0.05$). Talc and solution treatments are therefore interchangeable at the higher concentration of 10 mg Ag/kg soil but need to be more carefully considered for their application of different treatments at 1 mg Ag/kg soil. Solution treatments had less variation at 1 mg/kg as compared to talc; however, greater variation was present at 10 mg/kg. It was determined that spiking soil with aqueous solutions rather than mixing nanomaterials into talc attained concentrations closer to the desired mean concentration in most cases. This method of addition is also viewed as advantageous for future studies as it does not add any additional constituents to the soil that need to be considered when conducting ecotoxicology studies.

Due to the heterogeneity of nanomaterial dispersions before the nanomaterials were added to soil, treatments in both Chapters 4 and 5 showed differences between the nominal concentrations of aged and pristine nanomaterials, as well as the ionic control at both objective concentrations. In both Chapters 4 and 5 pristine nanomaterials at each concentration level were however statistically similar ($p<0.05$).

3.6 Nanomaterial Fate Analysis

To further investigate the fate of nanoparticles during soil exposures, uncoated AgNPs, PVP AgNPs and freeze-dried SAgNPs were adhered to carbon conductive tabs (PELCO Tabs™, Ted Pella Inc, CA, USA) on a wooden stick submerged in untreated soil. Each planting cup (Dollarama, ON, CAN) approximately 10 cm in depth and 5 cm in diameter was filled with 200 g of soil. Carbon tabs were analyzed using STEM and EDS before being placed in the soil at the start of the experiment, immediately after soil exposure and after three months exposure. Soils were maintained through addition of 12 mL DI water thrice weekly, an equivalent volume of water per unit mass of soil as the unplanted soil exposure study.

3.7 Chemical Analysis Methods

Chemical analysis of soil and plants in this thesis was conducted using ICP-MS to analyze the concentration of silver in addition to the elemental composition of soil after nitric acid digestions described in section 3.2.

3.7.1 Silver Uptake by Plants

Plant silver uptake was measured according to the section of the plant, which included roots, shoots and grains when possible. Plant sections were digested in nitric acid prior to being analyzed using ICP-MS. Approximately 1 g of plant sample was dried overnight at 110˚C in a glass digestion tube to obtain a dried sample of approximately 0.3 g (exact masses were measured and used for calculations). 10 mL of concentrated nitric acid (70 wt%) was added to each digestion tube and the sample was heated in a block heater at 120˚C until almost dried (Button, et al. 2012). The samples were taken back into solution in 0.5 mL of 50 wt% nitric acid and heated on the block heater at 50˚C for an additional 30 minutes. The samples were transferred to 15 mL centrifuge tubes by two rinses of 4.75 mL DI water, vortex mixing after each addition. The supernatant of the sample after settling was then transferred to a second centrifuge tube and analyzed using ICP-MS. In the case of harvested seeds, due to their low mass, all seeds from replicated treatments (3) were combined, weighed, digested, taken back into solution using 0.5 mL of 50 wt% nitric acid, heated at 50˚C for 30 minutes and diluted to a final volume of 6 mL using two additions of 2.75 mL DI water. Certified reference materials GBW 07604 GSV-2 (bush, twigs and leaves), IAEA 140 (sea plant homogenate) and reagent blanks were included every 15 samples for quality control.

3.7.2 Soil Silver Analysis

An upper soil layer (homogenized upper half) and lower soil layer sample (homogenized lower half) of each potted soil (0.3 g) was dried, ground using a mortar and pestle and quantified for total silver after digestion in 10 mL concentrated nitric acid as described previously in 3.2.

3.7.3 Elemental Analysis

Elemental analysis was conducted using ICP-MS on soil samples prepared using nitric acid digestion. ICP-MS allows for quantification of the concentration of elements and potential ions in the soil using multi-element calibration standards (High-Purity Standards, ICP-MS-68A, SC, USA). Samples were analyzed for silver and element analysis simultaneously; however, more dilute samples (1600-16000x dilution factors) were required to quantify more prominent elements. The following elemental concentrations in Table 3.3 were obtained. Concentrations have been reported as the average of 6 replicate samples of control soil alongside the recovery of standard reference soil SRM 2711a. A wide range of SRM recovery was obtained; this is most likely due to the wide range of elements targeted within a single scan. The particularly low recovery of potassium and calcium is likely due to interference with the argon gas used in ICP-MS and hydrogen which can equate to masses of 39-42 atomic mass units (Murphy, et al. 2002), coinciding with the masses of these elements. These elements have however still been included to provide an estimate of quantities of plant macro and micronutrients within the soil.

Table 3.3: Average elemental composition of experimental agricultural soil and standard reference material (SRM 2711a Montana II Soil) recovery

Element	Concentration (mg/kg)	SRM Recovery (%)
Magnesium	5570±1740	64.2
Phosphorus	816±76.5	79.7
Potassium	2130±306	13.0
Calcium	2120±269	37.8
Manganese	430±68.4	62.5
Iron	13800±3930	63.9
Nickel	17.8±2.74	90.9
Cobalt	9.30 ±1.06	87.3
Copper	15.9±2.77	88.5
Zinc	89.0±10.6	90.7

3.8 Soil Characterization

Physical properties of the soil were characterized at the conclusion of each exposure study based on the following measures.

3.8.1 Particle Size Distribution

Particle size of soil samples was adapted from ASTM Standard D422-64. 50 g of dry soil was progressively sieved using seven standard pre-weighed sieves on a sieve shaker (Meinzer II, CSC Scientific, VA, USA) for five minutes. The mass of each sieve was then weighed to determine the mass of soil greater than 2 mm, 1 mm, 500 µm, 425 µm, 63 µm and less than 63 µm.

3.8.2 Moisture Content

Moisture content was assessed by measuring the difference in mass between a 50 g moist soil sample and a dried soil sample according to ASTM Standard D2216-10. The moist soil was heated overnight at 110˚C before the mass of the dry soil was determined. Moisture content can therefore be expressed as the ratio of water mass lost (wet soil less dry soil mass) to dry soil mass.

3.8.3 Organic Content

Organic matter content was assessed using a method adapted from Hoogsteen et al. (2015). 25 g of dry soil in a crucible was burned at 550°C in a muffle furnace for three hours (Thermolyne 1300 Furnace, Fisher Scientific, NH, USA). After allowing the sample to cool enough to be removed from the oven, the sample was placed in a desiccator and allowed to cool to room temperature. The mass of the remaining ash was weighed and the organic content expressed as the ratio of lost mass (dry soil mass less mass of ash) to dry soil mass.

3.8.4 Water Holding Capacity

Water holding capacity of the soil was assessed after 24 and 48 hours for a given soil sample by saturating soil, contained in cheesecloth, with water and measuring the mass of the saturated soil. 50 g of moist soil was placed in a piece of cheesecloth (approximately 10 cm x 10 cm), an elastic band was used to secure the cheesecloth which was then placed within a beaker of water for 24 hours. The soil bag was removed from the water and weighed before being placed in a pre-weighed beaker, suspended on a skewer and covered with parafilm pierced for ventilation. The water was allowed to drip from the soil bag which was removed and the mass of the beaker recorded after 24 hours before the water was emptied and this process repeated after 48 hours. After the 48 hour water measurement, the contents of the soil bag were emptied into a pre-weighed aluminum dish and the mass of the cheesecloth and elastic band were recorded. The soil was dried overnight at 110˚C and the dry mass recorded. The water holding capacity can be expressed as the ratio of water mass contained within the soil bag at each time point to the dry soil mass.

3.8.5 pH and Conductivity

Measurements of pH and conductivity were conducted on a solution of soil and DI water (10 g: 50 mL) that was mixed and allowed to settle for one hour. Measurements were made using a multimeter (Hanna Instruments HI5522, RI, USA) with conductivity and pH probes as in the ASTM Standard D4972-1.

3.9 Biological Characteristics

Biological activity and functionality of soil microbial communities and plant health were assessed using the measures described hereafter. Functional diversity, enzymatic activity and plant health were assessed monthly while all remaining measures were made at the conclusion of the three month exposures.

3.9.1 Functional Diversity of the Microbial Community

Functional diversity of microbial communities was examined using BIOLOG Ecoplates (BIOLOG Inc., CA, USA) to assess community level physiological profiles. 1 g of soil was added to an autoclaved Erlenmeyer flask with 100 mL of autoclaved 10 mM phosphate buffered saline (PBS) adjusted to a pH of 7.4 using NaOH (Anachemia Canada, Inc., QC, CAN) or HCl (Fisher Scientific, NH, USA). PBS was made from 1.22 g Na_2HPO_4 (Sigma-Aldrich, ON, CAN), 0.20 g NaH_2PO_4 (Sigma-Aldrich, ON, CAN) and 8.5 g NaCl (Fisher Scientific, NH, USA) per litre of DI water in an autoclavable Schott bottle. The Erlenmeyer flasks were then placed on an orbital shaker (Innova 2000 Platform Shaker, New Brunswick Scientific, CT, USA or Mini Shaker, VWR, PA, USA) for 3 hours at 100 rpm (Legge and Weber 2010). After this duration, some of the sample supernatant was poured into a sterile petri dish and 100 µL of sample was added to each well of a BIOLOG Ecoplate using a multichannel pipette (Eppendorf, DEU) and autoclaved pipette tips within a sterile hood (Class II A/B3 Biological Safety Cabinet, Forma Scientific, NH, USA).

BIOLOG Ecoplates contain 31 carbon source substrates and a blank in triplicate with a tetrazolium violet redox dye indicator. The production of NADH through cell respiration reduces the tetrazolium to formazan which results in a change in colour that can be used to quantify the extent of reaction photometrically (Weber and Legge 2010). Absorbance was measured at 4 hour intervals for duration of 96 hours using a microplate reader, stacker (Eon Microplate Spectrophotometer, BioTek Instruments, Inc., VT, USA) and Gen5 All-in-One Microplate Reader Software (Version 2.05.5, BioTek Instruments, Inc.) at a wavelength of 590 nm after 3 seconds of shaking.

The average well colour development (AWCD) of the BIOLOG Ecoplate is used as a metric of average microbial metabolic activity based on the substrate utilization of a given sample. This metric is used to analyze the data at a specified time point in the incubation chosen to obtain the most variation within the data set while minimizing the number of over-saturated wells (absorbance units greater than 2.0) (Weber and Legge 2010). The microbial activity of each sample expressed as AWCD can be calculated according to the following:

$$AWCD = \sum_{i=1}^{31} (A_i - A_0)/31 \quad (1)$$

where A_i is the absorbance in a given well and A_0 is the absorbance of the blank well.

Since the soil activity tended to be slow to develop, 96 hours was chosen to represent the greatest colour development and variation as the latest time point examined. In the case of the planted soil study, Chapter 5, Biolog plates were incubated, enclosed in the dark on an orbital shaker at 100 rpm for 24 hours before being placed on the microplate stacker for the remaining 72 hours to avoid evaporation.

Another metric used to express the sample functionality is richness. Richness represents the number of utilized carbon substrates on each microplate and can be expressed as the number of substrates in which the blank-corrected absorbance (A_i-A_0) is greater than 0.25 (Garland 1997).

3.9.1.1 Guild Analysis and Groupings

Substrates within the BIOLOG Ecoplate can be classified according to several guilds based on chemical composition including polymers, carbohydrates, carboxylic and acetic acids, amino acids, amines/amides (Table 3.4) (Weber and Legge 2009). Classification of substrates into these guilds allows for analysis of sample biodiversity with greater ease in identifying significant trends in microbial function and activity by compressing the data from 31 dimensions into 5 dimensions. In addition to these guilds, constituents of root exudates have also been grouped for analysis; these substrates being D-xylose, D-mannitol, 2-hydroxy benzoic acid, 4-hydroxy benzoic acid, D-malic acid, L-arginine, L-asparagine, L-phenylaline, L-serine, L-threonine (Ogilvie 2017). Interpretation of community shifts resulting from silver treatments can be made from observed changes to the community's ability to utilize carbon sources within each grouping. This is represented by the average AWCD contribution of each grouping based on treatment.

Table 3.4: Biolog Ecoplate carbon source guild groupings (Weber and Legge 2009) with root exudates denoted

Well number	Label	Substrate	Guild Grouping
Well 1	C0	Water (blank)	
Well 2	C1	Pyruvic acid methyl ester	Carbohydrate
Well 3	C2	Tween 40	Polymers
Well 4	C3	Tween 80	Polymers
Well 5	C4	Alpha-cyclodextrin	Polymers

Well number	Label	Substrate	Guild Grouping
Well 6	C5	Glycogen	Polymers
Well 7	C6	D-cellobiose	Carbohydrates
Well 8	C7	Alpha-D-lactose	Carbohydrates
Well 9	C8	Beta-methyl-D-glucoside	Carbohydrates
Well 10	C9	D-xylose	Carbohydrates/ Root Exudate
Well 11	C10	i-erythritol	Carbohydrates
Well 12	C11	D-mannitol	Carbohydrates/ Root Exudate
Well 13	C12	N-acetyl-D-glucosamine	Carbohydrates
Well 14	C13	D-glucosaminic acid	Carboxylic & acetic acids
Well 15	C14	Glucose-1-phosphate	Carbohydrate
Well 16	C15	D,L-alpha-glycerol phosphate	Carbohydrate
Well 17	C16	D-galactonic acid-gamma-lactone	Carboxylic & acetic acids
Well 18	C17	D-galacturonic acid	Carboxylic & acetic acids
Well 19	C18	2-Hydroxy benzoic acid	Carboxylic & acetic acids/ Root Exudate
Well 20	C19	4-Hydroxy benzoic acid	Carboxylic & acetic acids/ Root Exudate
Well 21	C20	Gamma-hydroxybutyric acid	Carboxylic & acetic acids
Well 22	C21	Itaconic acid	Carboxylic & acetic acids
Well 23	C22	Alpha-ketobutyric acid	Carboxylic & acetic acids
Well 24	C23	D-malic acid	Carboxylic & acetic acids/ Root Exudate
Well 25	C24	L-arginine	Amino acids/ Root Exudate
Well 26	C25	L-asparagine	Amino acids/ Root Exudate
Well 27	C26	L-phenylalanine	Amino acids/ Root Exudate
Well 28	C27	L-serine	Amino acids/ Root Exudate
Well 29	C28	L-threonine	Amino acids/ Root Exudate
Well 30	C29	Glycyl-L-glutamic acid	Amino acids
Well 31	C30	Phenylethylamine	Amines/amides
Well 32	C31	Putrescine	Amines/amides

3.9.1.2 Relative Community Divergence

To reduce the complexity of CLPP data sets and to allow comparison between both treatment and time, a single metric of Euclidean distance was used. An analysis method adapted from Weber and Legge (2009) was used to present Euclidean distance as a dissimilarity measure of the microbial communities within each type of treatment over the three month period after treatment.

Carbon source utilization patterns (CSUPs) from CLPP were used in this analysis where negative values (responses less than the blank) are coded as zeroes to represent no response and CSUP have been standardized using AWCD. Absorbance of a given well, k, was standardized according to the following equation:

$$\bar{A}_k = \frac{A_k - A_0}{\frac{1}{31}\sum_{i=1}^{31}(A_i - A_0)} \qquad (2)$$

where A_i represents the absorbance of well *i* and A_0 is the absorbance of the blank (Weber and Legge 2010).

Since the same agricultural soil is used in all treatments, the averaged CSUP of control soil at time zero represents the initial community of all treatments and can therefore be used as the origin to calculate Euclidean distance. Euclidean distance (ED) has been calculated according to the following:

$$ED = \sqrt{\sum_{i=1}^{31}\left(\bar{A}_{TC_i} - \bar{A}_{CC_i}\right)^2} \qquad (3)$$

where $\bar{A}_T$ represents the standardized absorbance of a treatment, T, at the *i*-th carbon source (C_i), and $\bar{A}_C$ is the standardized absorbance of the initial average control soil at the *i*-th carbon source. Relative community divergence can therefore be represented as the average Euclidean distance for a given treatment at a specific time (month 1, 2 or 3).

3.9.1.3 Principal Component Analysis

Soil microbial communities were characterized from CSUPs every four weeks for the duration of exposures. CSUPs as previously described provide information on the utilization of 31 carbon sources for the 11 treatments examined, equating to 2046 data points at each measure. Principal component analysis (PCA) was performed on standardized CSUPs obtained from Biolog Ecoplate data to visualize differences between objects based on carbon source utilization (Legendre and Legendre 1998). PCA is a multivariate statistical technique which can be used to compress the 32-dimensional data set obtained from CLPP onto a 2-dimensional plane while maintaining maximal variance of the data (Weber, et al. 2008).

Data for PCA was first transformed using a Taylor transform according to Weber et al. (2007). Although PCA is robust such that analyses are valid without meeting assumptions of normality and homoscedasticity, analysis can be improved if these assumptions are met (Weber et al. 2007). The Taylor transformation, commonly used for the transformation of ecological data, was therefore used to stabilize variance and normality (Legendre and Legendre 1998). The Taylor power law transformation assumes:

$$S^2 = a\bar{y}^2 \qquad (4)$$

where S is the standard deviation of the sample, $\bar{y}$ is the sample mean and a is the sampling factor. The linearization of equation 4 leads to equation 5,

$$\log S^2 = \log a + b \log \bar{y}^2 \qquad (5)$$

where b is the slope which can be obtained from linear regression of the data. The transformation of the variables is therefore obtained from the following equation:

$$y_i' = y_i^{(1-b/2)}, b \neq 2 \quad (6)$$

where y_i' is the value of the transformed variable.

Each Biolog Ecoplate used to extract the CSUPs contains analytical replicates resulting in a total of 99 objects to be ordinated. In order to allow for the PCA to be visually interpretable, physical and analytical replicates were averaged for each treatment to reduce the objects to be ordinated to 11. A covariance matrix (n-1) was generated from the transformed CSUPs allowing the 11 objects to be ordinated on factor plane of the two principal components. Using a covariance matrix as the basis of the PCA was chosen to preserve scale (Weber, et al. 2007). The PCA ordinations can then be used to interpret ecological shifts and qualitatively group samples from the CSUPs.

3.9.1.4 Hierarchal Cluster Analysis

Clustering analysis was performed in addition to PCA for standardized CSUP data after each month. This analysis method was chosen as it was previously deemed to provide similar results to PCA for CSUPs while presenting all treatment replicates without affecting interpretability (Legendre and Legendre 1998, Weber, et al. 2008). An unweighted pair-group arithmetic average method was used based on the squared Euclidean distance dissimilarity matrix of CSUPs for each treatment replicate. This analysis was conducted individually on the data from months 1, 2 and 3 to compare the treatments at each time.

3.9.2 Enzyme Assays

While CLPP provides a measure of the active microbial community, enzyme assays can measure microbial activity in the soil and more generally, the activity of specific enzymes which can also occur extracellularly in soil. These enzymes can provide an overview of nutrient cycling in the soil, in this case carbon, nitrogen and phosphorus cycling. Analyses were made according to the methods developed by Bell et al. (2013) and Weintraub et al. (2007). This was conducted by adding the supernatant of a soil slurry in sodium acetate buffer, extracted through three hours on an oscillating shaker at 100 rpm, to a microtiter plate (Grenier Cellstar 96 well Black microplates, Sigma-Aldrich, ON, CAN) prepared with fluorometric enzyme substrates (4-MUB-β-D-glucosidase, 4-MUB-α-D-glucosidase, 4-MUB-β-D-xylosidase, 4-MUB-β-D-cellobiosidase, 4-MUB-N-acetyl-β-D-glucosaminidase, 4-MUB-phosphatase, and L-Leucine-7-amino-4-methylcoumarin) and measuring the fluorescence using a microplate reader (Tecan Infinite M1000 Pro, CHE) and Tecan i-control software (Version 1.10.4.0). Standards for the fluorometric assays were prepared from 4-methylumbelliferone (MUB) and 7-amino-4-methylcoumarin (AMC) and plated with sample soil slurries. All fluorescent enzymes and standards were obtained from Sigma-Aldrich. 1 g of each soil sample was added to an autoclaved Erlenmeyer flask with 100 mL of 50 mM sodium acetate buffer autoclaved and pH adjusted to 5 using glacial acetic acid (Trace metal grade, Fisher Scientific, NH, USA). The soil slurry was shaken on an orbital shaker for three hours before a portion of the sample was poured into a sterile petri dish and 200 µL was pipetted into the appropriate columns of the sample plate and row of the standard plate. Microplates were pre-prepared with 50 µL of buffer, fluorescent standards (0 µM to 50 µM) and enzyme substrates (200 µM) and refrigerated at 4˚C for up to 24 hours before being removed, stored in the dark and allowed to reach room temperature before sample inoculation. Assay microplates were read using a microplate photometer at an excitation wavelength of 360 nm and emission wavelength of 450 nm after 3 hours of dark incubation and subsequent quenching with 10 µL of 1M NaOH in each well. Standards and enzyme substrates were prepared just prior to each three month exposure study and stored at -20˚C. Enzyme substrates (Table 3.5) were plated in standard plates according to Table 3.6.

Table 3.5: Enzyme substrates used in enzymatic assays

Enzyme	Enzyme Substrate
β-1,4-Glucosidase	4-MUB-β-D-glucoside
α-1,4-Glucosidase	4-MUB-α-D-glucoside
β-1,4-Xylosidase	4-MUB-β-D-xyloside
β-D-1,4-Cellobiosidase	4-MUB-β-D-cellobioside
β-1,4-N-Acetylglucosaminidase	4-MUB-N-acetyl-β-D-glucosaminide
Acid phosphatase	4-MUB-phosphate
Leucine aminopeptidase	L-Leucine-7-amino-4-methylcoumarin

Table 3.6: Standard Plate for AMC and MUB with samples, buffer blanks and substrate controls

	1	2	3	4	5	6	7	8	9	10	11	12
A	Standard MUB and Sample 1					Standard AMC and Sample 1					Buffer	
	0 μM	0.5 μM	5 μM	25 μM	50 μM	0 μM	0.5 μM	5 μM	25 μM	50 μM		
B	Standard MUB and Sample 2					Standard AMC and Sample 2					Buffer	
	0 μM	0.5 μM	5 μM	25 μM	50 μM	0 μM	0.5 μM	5 μM	25 μM	50 μM		
C	Standard MUB and Sample 3					Standard AMC and Sample 3					Buffer	
	0 μM	0.5 μM	5 μM	25 μM	50 μM	0 μM	0.5 μM	5 μM	25 μM	50 μM		
D	Standard MUB and Sample 4					Standard AMC and Sample 4					Buffer	
	0 μM	0.5 μM	5 μM	25 μM	50 μM	0 μM	0.5 μM	5 μM	25 μM	50 μM		
E	Standard MUB and Sample 5					Standard AMC and Sample 5					Buffer	
	0 μM	0.5 μM	5 μM	25 μM	50 μM	0 μM	0.5 μM	5 μM	25 μM	50 μM		
F	Standard MUB and Sample 6					Standard AMC and Sample 6					Buffer	
	0 μM	0.5 μM	5 μM	25 μM	50 μM	0 μM	0.5 μM	5 μM	25 μM	50 μM		
G	Standard MUB and Sample 7					Standard AMC and Sample 7					Buffer	
	0 μM	0.5 μM	5 μM	25 μM	50 μM	0 μM	0.5 μM	5 μM	25 μM	50 μM		
H	Standard MUB and Sample 8					Standard AMC and Sample 8					Buffer	
	0 μM	0.5 μM	5 μM	25 μM	50 μM	0 μM	0.5 μM	5 μM	25 μM	50 μM		

3.9.3 Heterotrophic Plate Count

Heterotrophic plate count was assessed as a metric of heterotrophic microbial activity according to a protocol adapted from Csuros and Csuros (1999). 1 g of each soil sample was added to an individual autoclaved Erlenmeyer flask with 99 mL of autoclaved 7.4 pH PBS. The flasks were placed on an oscillating shaker for 1h at 180 rpm. 1 mL of the suspension was added to an autoclaved glass test tube containing 9 mL of buffer. The test tubes were sealed using aluminum foil and mixed using a vortex mixer. 100 µL of soil extract dilutions up to 10^{-5} in PBS were spread in triplicate on petri dishes of R2A agar media (Thermo Scientific Oxoid R2A Agar, Thermo Scientific, NH, USA) using a sterilized glass rod (American Public Health Association 1999; Bevivino, et al. 2014). After inoculation, the media was incubated before the colonies were counted after 5 days at 20°C using an Interscience Scan®300 automatic colony counter (Interscience Laboratories Inc., MA, USA) in Chapter 4 or alternatively imaged using the automatic colony counter after two days at 20°C then counted using OpenCFU software (Geissmann 2013) in Chapter 5. Colony forming units are represented as the number of colonies counted per g dry weight (d.w.) soil mass (CFU/g d.w. soil).

3.9.4 Substrate-Induced Respiration

Substrate-induced respiration was used as a measure of microbial activity wherein respiration can infer the ability of a soil to biodegrade organic matter. Concentration of CO_2 was measured using a LI-COR 820 CO_2 analyzer (LI-COR, NE, USA) to determine the quantity of CO_2 respired by 10 g of soil upon addition of 40 mg dextrose (Anachemia Canada Inc., QC, CAN) dispersed in 50 mg talc as an inert dispersant at room temperature (Nakamoto and Wakahara 2004; Forster, et al. 2006). Dextrose and talc were each added to a 15 mL centrifuge tube and mixed through shaking. The mixture was then added to an adapted 50 mL centrifuge tube containing the 10 g soil sample and mixed. The 50 mL centrifuge tube was adapted by drilling two holes in the lid of the tube and mounting two 4.76 mm aquarium airline micro ball valves (Pets & Ponds, ON, CAN) secured and sealed using LePage Plastic Super Glue Adhesive (Canadian Tire, ON, CAN). The sample was sealed using the ball valves and incubated for one hour before an air pump (National Geographic, Pacific Coast Distributing, Inc., AZ, USA) at a flow rate of 0.5 L/min was attached to one of the two valves via vinyl tubing while the other valve was attached to tubing connected to the CO_2 analyzer. Within the centrifuge tube, a small length of tubing was attached to the valve connected to the pump to allow for the air to flush out the CO_2 from the bottom of the tube. After incubation, the tubing was attached to the pump and analyzer, the valves opened, and the peak CO_2 recorded using LI-COR software (V1.0.6). Once the CO_2 reading returned to the basal level of the room, both valves were closed. This process was repeated hourly for a duration of three hours and the respiration rate calculated from the third hour measurement when the respiration had stabilized (Nakamoto and Wakahara 2004). The respiration rate can be expressed as the volume of CO_2 respired per gram of dry soil per hour. The design of the apparatuses used for this measurement is shown in Figure 3.2.

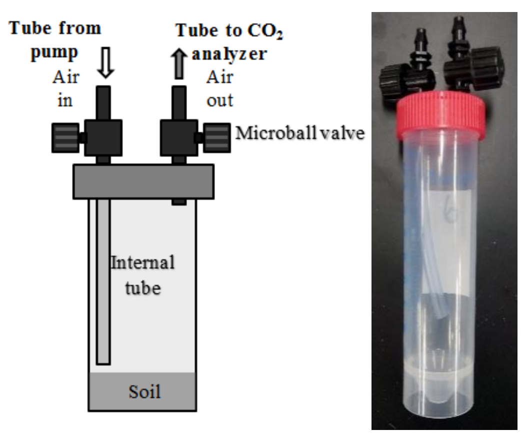

Figure 3.2: Apparatus for measurement of substrate-induced respiration. Two 4.76 mm microball valves are attached to the lid of a 50 mL centrifuge tube with one valve's internal stem having a short vinyl tube attached.

3.10 Metagenomic Analysis

DNA from soil samples was extracted using the FastDNA™ SPIN Kit for soil according to the protocol revision #116560200-201203 with some adaptations. Briefly, 500 mg of soil sample was added to a Lysing Matrix E tube with 978 µL sodium phosphate buffer and 122 µL MT buffer. The contents of the tube were then homogenized using the FastPrep® Instrument for 40 seconds with a speed setting of 6.0 before being centrifuged for 15 minutes at 14,000 x g to pellet debris. The supernatant after centrifugation was added to a 2.0 mL centrifuge tube followed by 250 µL of protein precipitation solution and mixed by hand through shaking 10 times. The tube was then centrifuged at 14,000 g for 5 minutes to pellet the precipitate. The supernatant (~ 900 µL) was transferred to a 2 mL tube and resuspended in 750 µL binding matrix, inverted by hand for 2 minutes and allowed to settle for 3 minutes. After settling, 500 µL of the supernatant was removed and discarded. The settled binding matrix was resuspended in the remaining supernatant by repeated pipetting. 500 µL was then transferred to a SPIN™ filter tube and centrifuged at 14,000 x g for 1 minute. The catch tube was emptied and 700 µL of the resuspended binding matrix was added to the SPIN™ filter and centrifuged. The catch tube was again emptied and 500 µL SEWS-M added to the filter and pipetted to resuspend the pellet. The tube was then centrifuged for 14,000 x g for 1 minute, the catch tube emptied and the SPIN tube centrifuged again at 14,000 x g for 2 minutes to dry the matrix. The filter was placed in a new catch tube and the previous catch tube discarded. The SPIN filter was air dried for 5 minutes before the matrix was resuspended in 75 µL of DNase/ Pyrogen-Free Water, centrifuged at 14,000 x g for 1 minute to bring the eluted DNA into the catch tube. The SPIN filter was discarded and the DNA sample stored at -20˚C.

Quantification of DNA was performed using a Qubit® Fluorometer (Invitrogen, ON, CAN) and Qubit® dsDNA BR assay kit. DNA samples previously stored at -20°C were removed from the freezer and placed on ice to thaw. A working solution of Qubit® dsDNA BR reagent and BR buffer (1:200) was prepared. 0.5 mL Qubit tubes were used for all samples and standards. Two standards containing 0 ng/µL and 100 ng/µL in TE buffer were prepared with each containing 10 µL standard concentrate and 190 µL Qubit working solution. Samples were prepared using 1 µL of DNA sample and 199 µL of Qubit working solution. After addition of the working solution, tubes were vortexed for 2-3 seconds and allowed to incubate at room temperature for two minutes. The Qubit® 2.0 fluorometer was calibrated using the two standards and each sample's DNA concentration was calculated after incubation as the value given by the fluorometer multiplied by 200 since the initial sample was diluted in a 1:200 dilution.

Metagenomic sequencing of 16S ribosomal RNA gene amplicons was performed using a 16S sequencing library prepared for the Illumina MiSeq System (Protocol Part #150442223 Revision B, Illumina Canada, BC, CAN). Using this method, variable V3 and V4 regions of 16S rRNA gene were sequenced using the recommended primer pair sequences to create a single amplicon. Firstly, 2.5 µL of 5 ng/µL microbial genomic DNA was combined with 5 µL of each 1 µM amplicon PCR forward and reverse primers and 12.5 µL of NEBNext Ultra II Q5 Master Mix (New England BioLabs Ltd., ON, CAN) in a 96 well polymerase chain reaction (PCR) plate. PCR was then performed using a T100 Thermal Cycler (Bio-Rad Laboratories (Canada) Ltd., ON, CAN) with a program of 95°C for 3 minutes, 25 cycles of 95°C for 30 seconds, 55°C for 30 seconds, 72°C for 30 seconds followed by an extension step of 72°C for 5 minutes and holding at 4°C. The 16S amplicon was then purified using Agencourt AMPure XP beads (Beckman Coulter Genomics, ON, CAN) and 80% ethanol (Sigma-Aldrich, ON, CAN). Dual indices and Illumina sequencing adapters were then attached to the amplicon using 25 µL of NEBNext Ultra II Q5 Master Mix, and 5 µL or each Nextera XT Index 1 Primer Set A and Nextera XT Index 2 Primer (Illumina Canada) using 8 cycles of the previously mentioned PCR thermal cycle and washing again with AMPure beads. Finally, the concentration of the sample library was measured using the Qubit dsDNA HS assay kit (Invitrogen, ON, CAN) and Qubit fluorometer and sequenced using the MiSeq (Illumina Canada) after normalizing the samples to 4 nM, pooling the normalized DNA and denaturing with 0.2 M NaOH. The denatured DNA was diluted to 2 pM using HT1 Hybridization Buffer and spiked with 10% PhiX Control Kit v3 similarly denatured and diluted to the same concentration as the amplicon library. The Miseq Reagent Kit v3 for 600 cycles (Illumina Canada) was used to sequence paired 300-bp reads and resulted in 24.58 million total reads after a 65 hour run time. These results were then analyzed using the BaseSpace 16S metagenomics application (Illumina Canada) to perform taxonomic classification. The Shannon diversity index (H) was used to assess the species diversity of each treatment and was calculated according to equation 7,

$$H = -\sum_{i=1}^{S} p_i \ln p_i \qquad (7)$$

where p_i is the proportion of a species, i, to the total population present and S is the number of species present.

The species evenness (E) can also be used to assess how evenly the bacterial population is distributed within the present species and was calculated according to equation 8,

$$E = H/\ln(S) \qquad (8)$$

where H is the Shannon diversity index and S is the number of species present.

3.11 Plant Health

Plant health was assessed based on one week germination rates, final number of plants reaching the heading stage and yield. Plant growth was assessed using the mass of aboveground and belowground biomass. At the conclusion of each month, aboveground biomass was cut from the roots and weighed while roots were removed from the soil to assess belowground biomass. The total dry weight of aboveground and belowground biomass was determined from the total fresh weight and the moisture content of segments of plants and roots that were dried for total silver analysis. After three months of exposure, roots from this time point were dried to quantify total belowground biomass after removing the roots from moist soil by immersing in DI water, manually removing soil followed by rinsing in a beaker of DI water.

3.12 Statistical analysis

Preliminary data analysis and dose-response curves were generated using Microsoft Excel (Microsoft Excel 2016, Microsoft Corporation, WA, USA). Repeated measure analysis of variance (ANOVA) using SPSS (SPSS Inc. version 23, IL, USA) was conducted to test for treatment effects versus time for measurements that were made on monthly bases. Mauchly’s sphericity test was used to verify equal variances in the data for repeated measures. One-way ANOVA was used to identify differences between treatment means in individual measurements while post-hoc Tukey’s tests were used to identify significantly different treatment groups. Post-hoc Dunnett’s tests were used to identify treatments which significantly differed from the control. In cases where the data was not normally distributed (Shapiro-Wilk’s test) and variance was homogenous (Levene’s test), analysis of variance was conducted using a Kruskal-Wallis test. A student t-test was used when sample mean comparisons were made between only two treatments. SPSS was also used to conduct hierarchical cluster analysis of carbon substrate utilization patterns from CLPP while XLSTAT (XLSTAT 2014.6, Addinsoft, FRA) was used for PCA analyses. All statistical comparisons were made at the 95% confidence level and data is summarized as the mean $\pm$ S.D.

4 Chapter 4: Fate and effects of pristine and aged silver nanomaterials in agricultural soil

4.1 Introduction

As of 2014, 24% of 1800 products identified in a consumer product inventory of nanomaterial-incorporated products contained silver nanomaterials, representing one of the most commercially available nanomaterials (Vance, et al. 2015). AgNMs are commonly used in consumer products for their antimicrobial properties (Nowack, et al. 2011). Due to this known toxicity, increasing use of these products and their unknown long-term impacts on environmental receptors, there has been an increasing precedence to study their toxicological effects in the environment.

AgNMs used in textile products provide a source of nanomaterials in household wastewaters due to leaching during washing. Button et al. (2016) estimated the concentration of nanomaterials leached from X-static nanofiber-containing textiles to be as much as 1.5 mg/L, which can subsequently enter surface waters or waste treatment processes. Silver's affinity for sulphur and organic matter present in waste treatment processes results in binding of the silver to treatment residuals (Wang, et al. 2016). Approximately 95% of silver becomes bound to these residuals (Hu 2010) resulting in modeled concentrations of 1.29-5.86 mg AgNMs/kg biosolids (Gottschalk, et al. 2009), mainly in the form of silver sulfide and SAgNPs (Wang, et al. 2016).

Plants have a symbiotic relationship with soil microbial populations wherein plants aid in maintaining soil structure and health and microbes break down organic matter and transform macronutrients into bioavailable forms (Blagodatskaya, et al. 2016; Hawkesford, et al. 2012). Due to this relationship, toxic effects of nanomaterials to microbes could extend to entire ecosystems. Concentrations and transformations of silver nanomaterials in biosolids are therefore of concern due to the use of biosolid amendments in agriculture. Biosolids contain organic matter as well as many macro and micronutrients that are required for plant development and are readily available making them an affordable and effective alternative to traditional fertilizers (Water Environment Association of Ontario 2009). Since plants are harvested from fields, nutrient losses are extensive in agricultural practices and require regular re-addition of nutrients. In Canada, biosolids can be applied to agricultural land every three years (Canadian Council of Ministers of the Environment 2010).

Based on the current maximum concentration of silver nanomaterials in biosolids (5.86 mg/kg), Canadian application rates of 0.8 kg/m^2, a tilling depth of 15 cm and an assumed average soil bulk density this equates to an increase of 21.4 µg AgNMs/kg in soil every three years. Previous studies on amended soil mainly used pristine manufactured AgNMs with final concentrations ranging from 1.25-1800 mg/kg soil (Peyrot, et al. 2014; Kumar, et al. 2012; Samarajeewa, et al. 2017) corresponding to cumulative biosolid applications of 175 to 252,000 years. Results of these studies indicated that AgNMs negatively affected soil enzyme activity (Peyrot, et al. 2014; Samarajeewa, et al. 2017), altered microbial activity and community composition (Kumar, et al. 2012; Samarajeewa, et al. 2017). Studies such as those conducted by Colman et al. (2013) and Wang et al. (2016) have investigated the effects of AgNMs aged in sludge on soil microbial communities and plants at concentrations of 0.14 mg/kg and 1 or 10 mg/kg, respectively. Colman et al. (2013) found that community composition of AgNM treatments converged with the control slurry after 50 days; however, enzymatic activity and microbial biomass were reduced. Wang et al. (2016) determined that sulphidation of AgNPs after waste treatment (aged 14 months) posed low risk to wheat plants despite uptake and suggested that elevated chlorine concentrations in soil may increase bioavailability of silver due to soluble or colloidal $AgCl_x$ complexes. The long-term fate of AgNM in soil is of specific importance since their toxicity is largely dependent on bioavailability. As previously discussed, AgNMs applied to soil in biosolids will be mainly in the form of Ag_2S or AgCl which are insoluble (Kaegi, et al. 2011; Wang, et al. 2016; Kim, et al. 2010; Brunetti, et al. 2015). Once added to soil, factors such as pH and organic matter can influence dissolution of nanomaterials wherein low pH and low NOM increase dissolution into ionic form, increasing toxicity (Lui and Hurt 2010). Sorption of nanomaterials to soil surfaces and organic matter can also decrease their bioavailability as increasing soil clay content increases adsorption (Cornelis, et al. 2010). To examine the effects of AgNMs to soil it is therefore important to consider concentrations, transformations such as SAgNPs and released nanomaterials which are most representative of those present in terrestrial environments. To date, no toxicology studies have been conducted using nanomaterials released from consumer products. This study will therefore examine the fate and effects of pristine, sulphidized and weathered nanomaterials from a consumer product to agricultural soil at environmentally relevant concentrations.

4.2 Materials and Methods

Materials and methods used in this chapter are previously described in Chapter 3.

4.2.1 Experimental Design

A three month study of agricultural soil exposed to silver nanomaterials was conducted through the use of three randomized sample sets of 11 treatment types (Table 4.1) in triplicate. This equated to a total of 99 potted soil samples and allowed for destructive sampling of one sample set after each month of exposure conducted in greenhouse conditions.

Aqueous solutions of uncoated AgNPs, PVP AgNPs, ionic silver, SAgNPs, weathered nanomaterials from sock wash water were added to the soil to obtain final silver concentrations of 1 and 10 mg Ag/kg as well as controls with soap or negative control (DI water); however, differences between nanomaterial dispersions caused by aggregation and adhesion to preparation vessels, as discussed in 3.5, resulted in final concentrations summarized in Table 4.2 where significantly different treatments within low or high concentrations are denoted by different letters. Recovery of the standard reference material SRM 2711a was 77±29%. Due to the existing background concentration of silver in the soil, total silver concentrations of treatments represent the sum of the background silver concentration and silver added in the treatment. An ionic maximum treatment was also subsequently added for comparative purposes. This treatment has been excluded from statistical analyses, with the exception of bacterial community composition, as it was not conducted at the same time and therefore may have some differences in experimental exposure conditions such as greenhouse temperature, humidity and hours of daylight. Total silver concentrations, enzyme analysis and functional diversity (CLPP) were assessed at four week intervals while all methods described in Chapter 3 were analyzed at the conclusion of the three month exposure.

Table 4.1: Schematic of a sample set of soil including PVP AgNPs, uncoated AgNPs, weathered nanomaterials from sock wash water, sulphidized AgNPs, ionic silver (positive control), soap control and no AgNPs (negative control). *Maximum ionic treatment added at a later date for comparative purposes.

	Concentration Level of Ag Added to Soil					
	Low	High	Low	High	Low	0
Replicate #1	PVP	PVP	Uncoated	Uncoated	Weathered	Soap Control
Replicate #2	PVP	PVP	Uncoated	Uncoated	Weathered	Soap Control
Replicate #3	PVP	PVP	Uncoated	Uncoated	Weathered	Soap Control
	Low	High	Low	High	Maximum*	0
Replicate #1	Sulphidized	Sulphidized	Ionic	Ionic	Ionic	Control
Replicate #2	Sulphidized	Sulphidized	Ionic	Ionic	Ionic	Control
Replicate #3	Sulphidized	Sulphidized	Ionic	Ionic	Ionic	Control

Table 4.2: Total concentrations of silver in soil after each treatment (n=18). Significantly different treatment groups within low or high concentration treatments from post-hoc Tukey tests are denoted using different letters.

Concentration Level of Ag Added to Soil	Treatment Type	Soil Concentration (mg Ag/kg)
0	Control	0.33±0.17
	Soap Control	0.39±0.18
Low	Sulphidized	0.82±0.30[bc]
	Weathered	1.2±0.29[a]
	Uncoated	0.54±0.20[b]
	PVP	0.64±0.27[b]
	Ionic	1.0±0.20[ac]
High	Sulphidized	3.4±0.80[a]
	Uncoated	5.5±2.1[ab]
	PVP	6.2±4.4[b]
	Ionic	9.1±2.4[c]
Maximum	Ionic	76±14

Sulphidized, uncoated and PVP AgNPs on carbon conductive tabs were examined in duplicate without soil, with soil and after three months of soil exposure to observe nanomaterial fate through potential transformations or changes to morphology using STEM and EDS. A total of eight measurements were made for each nanomaterial type and condition. The atomic percent composition of sulphur (S) and silver were averaged for each treatment at a given exposure condition and compared to the other conditions for the same nanomaterial. This allowed for determination of firstly, whether the addition of soil alters the composition with respect to these elements and if extended soil exposure results in changes in association between sulfur and silver phases. These specific elements were chosen for analysis since silver preferentially binds to sulphur in waste treatment processes and the behaviour and effects of these particles over time are not well known.

4.3 Results and Discussion

4.3.1 Fate of silver nanomaterials in agricultural soil

Metallic nanomaterials, specifically AgNMs, are not biodegraded during wastewater treatment processes, instead becoming bound to organic matter or transformed primarily into SAgNPs; which can then be applied to agricultural soils in the form of biosolid amendments (Blaser, et al. 2008; Mueller and Nowak 2008; VandeVoort and Arai 2012; Gottschalk, et al. 2009; Nicholson, et al. 2003). After application to soil, AgNMs can become sorbed to organic matter, dissolve into ionic form, undergo chemical transformations or remain as insoluble complexes such as SAgNP and AgCl which can have variable bioavailability and mobility (Wang, et al. 2016; Kaegi, et al. 2011; Lui and Hurt 2010). Depending upon their bioavailability and mobility, silver species including ionic silver which potentially represents the most mobile form, could be readily available to microbes and plants in addition to being a potential groundwater contaminant. Therefore, it is important to understand how AgNMs behave in soil with respect to their form and transport.

Repeated measure ANOVA of silver concentrations in soil treatments for the three months of the study indicated that there was no effect of time and no interaction effect between treatment and time ($p>0.05$). Meaning AgNMs were not leaching from the systems over the course of the experiment. A student t-test indicated no significant difference between silver concentrations in the upper and lower regions of the soil ($p>0.05$). Silver concentrations were therefore consistent each month between and within individual treatment replicates. Insignificant change between concentrations in the upper and lower soil regions means that the silver is relatively immobile in the soil such that transport has not affected the fate of the ionic or nanosilver over the three month duration. The results of this experiment generally agree with previous findings in literature. Sorption of the silver to the soil and organic matter is relatively high due to the clay content and high organic matter content (Lui and Hurt 2010; Cornelis, et al. 2010). In saturated soil column experiments, two soils with high clay content and moderate organic matter content spiked with 0.7 mg AgNPs/L showed infiltration to depths of approximately 7 cm with the greatest silver concentrations being present within 4 cm depth after circulation of at least 9 pore volumes (Cornelis, et al. 2013). This indicates that AgNPs become strongly bound to the soil and organic matter such that their transport into lower soil layers as well as pore water is relatively limited.

Surface transformations of uncoated, PVP coated and SAgNPs were assessed using STEM and EDS for unexposed samples, after initial soil exposure and three months of soil exposure. The silver and sulfur compositions based on atomic percentage for each exposure condition are shown in Figure 4.1. ANOVA analysis of the composition of each type of nanoparticle under the examined conditions revealed statistically significant differences between PVP AgNPs before and after initial soil exposure (6.4% difference) and between SAgNP before soil exposure and after three months of exposure to soil (6.3% difference) ($p<0.05$). Despite these differences, no treatments showed significantly different compositions between initial soil exposure and three months of soil exposure. This indicates that over the duration of the soil study, the association of sulphur with silver in these nanoparticles did not change. Although this result was determined based on the mean composition under each condition, some sulphur losses 1-2% could be occurring in SAgNP treatments after three months of exposure, however, due to the variation present in this method this decrease is not considered significant. These findings are in agreement with previous studies wherein addition of SAgNP to soil either directly or within sludge were stable with $\geq$87% in this form regardless of soil pH for up to 14 months (Wang, et al. 2016; Pradas del Real, et al. 2016; Sekine, et al. 2015). AgNPs or $AgNO_3$ added to soil in the absence of sludge were found to favour AgCl in acidic conditions and Ag_2S under alkaline or neutral conditions such as those present in this study (Sekine, et al. 2015). Contrastingly, Settimio et al. (2014) found that $AgNO_3$ was reduced to metallic silver in alkaline soils, indicating that transformations of Ag within soil are dependent upon soil characteristics such as redox potential, pH and organic matter. The lack of mobility of silver regardless of its exposure form is also unsurprising given that AgNPs or $AgNO_3$ transformation to SAgNPs has been found to occur within 1 day in sludge or soil with extractable silver being equivalent to only 0.0029% of the total silver concentration (10 mg Ag/kg soil) after 400 days (Wang, et al. 2016).

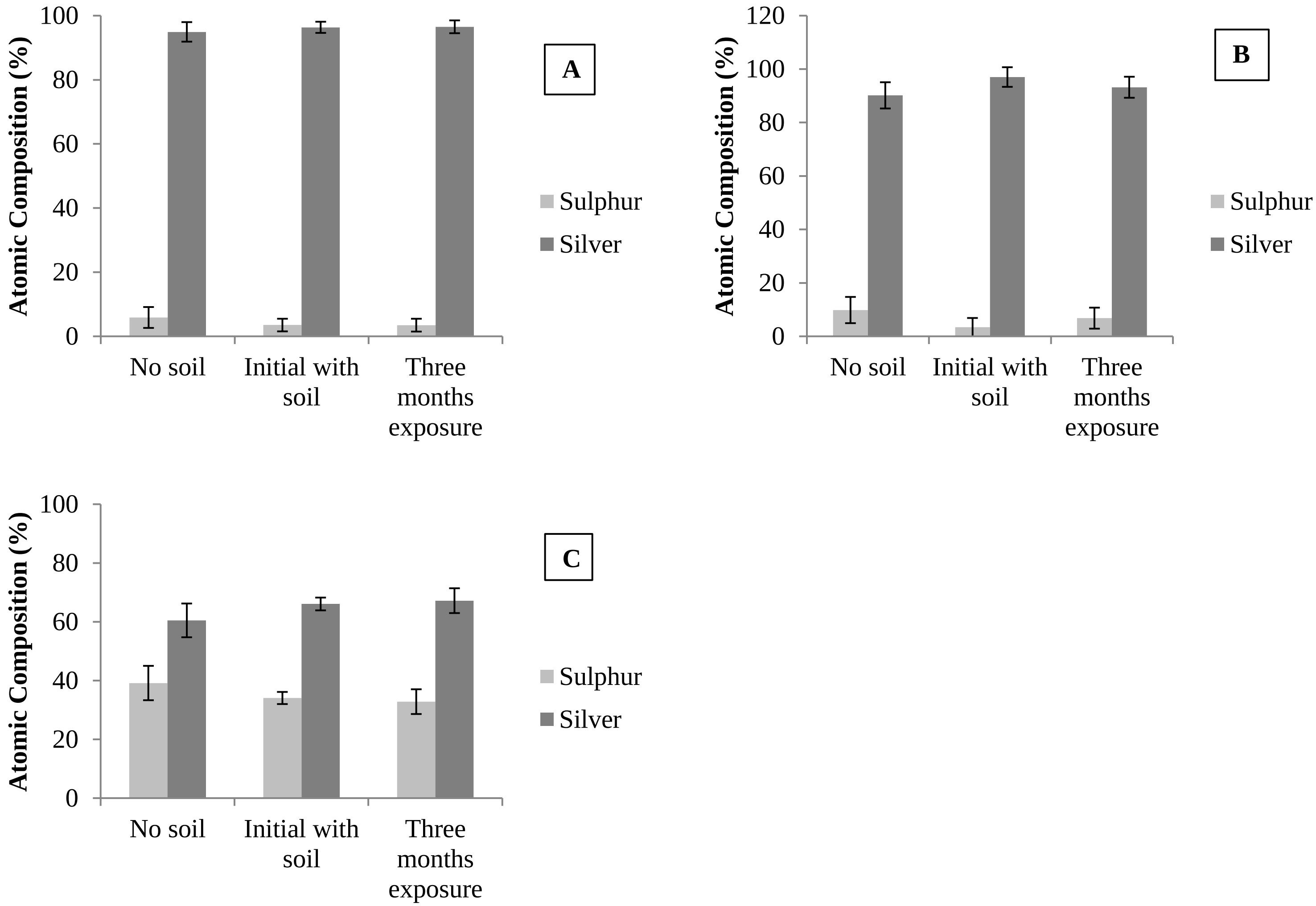

Figure 4.1: Sulphur and silver composition of uncoated (A), PVP (B) and sulphidized (C) silver nanoparticles without soil, with soil and after three months of soil exposure as determined from EDS.

4.3.2 Effects of silver nanomaterials on soil properties

Soil characteristics including soil particle size distribution, moisture content, organic matter content, water holding capacity, pH and conductivity were assessed to determine if exposure to AgNMs impacted these characteristics and could therefore influence microbial community structure or activity through altered physical properties. Previous studies have indicated that toxicity of AgNMs to soil microbes decreased with increasing pH and clay content, however overall toxicity is dependent upon the combination of soil properties (Schlich and Hund-Rinke 2015; Shoults-Wilson, et al. 2010). Significant differences between treatments did not occur for pH and water holding capacity while moisture content and conductivity showed significant differences between treatments as summarized in Table 4.3. Organic matter of sulphidized-low, uncoated-low, ionic-low, sulphidized-high, PVP-high and ionic-high were also significantly less than the control (Dunnett's test), however, this could be due to an outlying control replicate. The average organic matter content of all treatments ranged from 9-12%, water holding capacity was approximately 0.5 g water/ g d.w. soil and pH ranged from 6.69-6.93.

Table 4.3 summarizes the physical properties of soil treatments after three months of exposure with different letters denoting significantly different treatment groups as determined from post-hoc Tukey tests. Correlations exist between soil properties including moisture content, soil texture (particle size), organic matter content, and water holding capacity, thus variations in any of these properties can be linked to others (Tarboton 2003). Weathered-low treatment moisture content was significantly greater than that of the PVP-low and sulphidized-high treatments. Weathered-low treatments did not differ from any additional treatments indicating that the differentiation is likely the result of the natural variance between these samples. Since treatments were found to have no negative effects on moisture content and water holding capacity, it can be concluded that induced moisture stress which could impact soil microbial activity did not occur. While pH was not significantly different between any treatments, weathered-low and ionic-high treatments indicated the lowest average pHs as well as being among treatments with the greatest variance between replicates. This is the result of higher ionic content in these treatments which is further supported by their conductivity. Soap and weathered-low treatment conductivities were greater than all other treatments with the exception of weathered-low with respect to ionic-high. Soap and weathered treatments had an increased conductivity due to the conductivity provided by the ions present in the soap. Similarly, the ionic high treatment had an increased conductivity relative to other treatments due to the higher ionic salt content within the treatment.

Soil particle size distribution was determined using progressive sieving as shown in Figure 4.2. Variation between treatments is the result of heterogeneities in the soil from the large aggregates present.

Table 4.3: Physical properties of soil treatments measured after three months exposure. Significantly different treatment groups from a post-hoc Tukey test are identified using different letters (p<0.05).

Treatment	Moisture Content (%)	Organic Matter (%)	Soil Water Holding Capacity- 24 h (g water/ g d.w. soil)	Soil Water Holding Capacity- 48 h (g water/ g d.w. soil)	pH	Conductivity (µS/cm)
Control	22.7 ± 2.0^{ab}	12.1±2.3	0.48±0.02	0.99±0.05	6.81±0.13	108.0 ± 13.7^{a}
Soap Control	23.9 ± 2.1^{ab}	9.9±0.4	0.48±0.02	0.99±0.01	6.90±0.05	171.0 ± 5.2^{b}
Weathered Low	26.8 ± 1.1^{b}	9.7±0.2	0.48±0.02	0.97±0.04	6.69±0.28	160.8 ± 17.3^{bc}
Sulphidized Low	22.0 ± 1.8^{ab}	9.2±0.4	0.48±0.02	0.97±0.02	6.88±0.11	112.1 ± 9.5^{a}
Uncoated Low	21.9 ± 0.1^{ab}	8.9±0.2	0.45±0.01	0.89±0.03	6.92±0.12	109.0 ± 0.8^{a}
PVP Low	21.4 ± 0.7^{a}	9.5±0.2	0.45±0.02	0.91±0.04	6.91±0.13	101.1 ± 0.6^{a}
Ionic Low	23.0 ± 1.2^{ab}	9.1±1.0	0.47±0.02	0.95±0.05	6.89±0.03	104.5 ± 10.9^{a}
Sulphidized High	20.4 ± 2.2^{a}	9.3±0.5	0.46±0.10	0.93±0.21	6.91±0.16	107.6 ± 6.1^{a}
Uncoated High	23.2 ± 0.2^{ab}	9.7±0.2	0.48±0.01	0.97±0.02	6.80±0.22	112.3 ± 7.1^{a}
PVP High	22.1 ± 0.6^{ab}	9.2±0.6	0.47±0.03	0.93±0.03	6.93±0.05	101.5 ± 9.2^{a}
Ionic High	23.8 ± 0.9^{ab}	9.4±0.4	0.43±0.07	0.86±0.13	6.74±0.16	130.0 ± 13.9^{ac}
Ionic Maximum	30.0±1.2	9.6±0.5	0.54±0.01	1.1±0.03	6.88±0.29	130.1±18.5

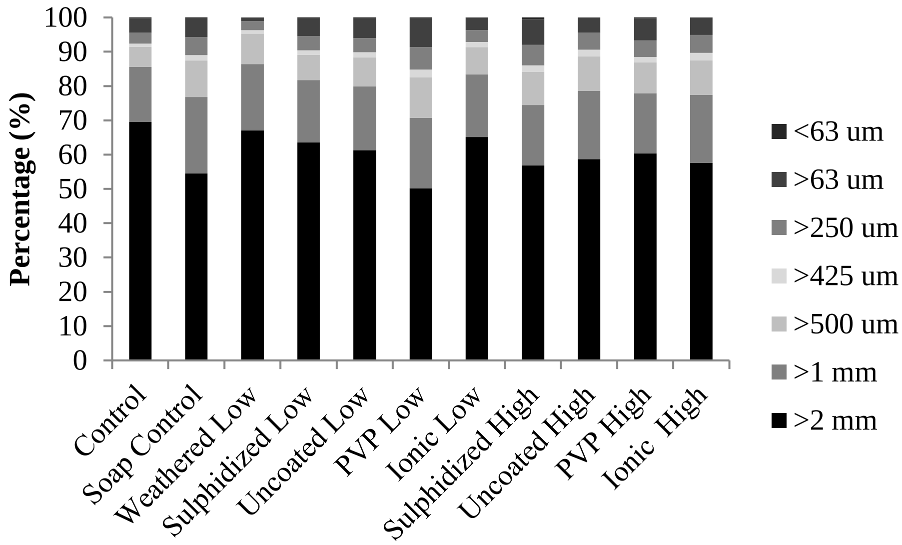

Figure 4.2: Average soil particle size distribution of treatments (n=3)

4.3.3 Effects of silver nanomaterials on soil microbial activity, function and diversity

The function, activity and diversity of soil microbial communities exposed to silver nanomaterials was assessed using several methods including community level physiological profiling, heterotrophic plate counts, substrate-induced respiration and metagenomic sequencing to determine potential effects. Since the soil microbial community has a vital role in nutrient cycling and ecosystem functionality, it is important to determine if AgNMs have detrimental effects on microbial health.

Functional diversity of treatments was assessed using community level physiological profiling. The average well colour development of each treatment was assessed 96 hours after inoculation on BIOLOG Ecoplates and is presented in Figure 4.3. A repeated measure ANOVA was conducted to identify statistically significant differences between all months. Each month was examined individually to determine what differences exist between treatments at each time point.

Statistically significant differences were found to exist between treatments in only month 2 while months 1 and 3 had no statistically significant differences between treatments due to treatment means being within the variation of the method. A post-hoc Tukey test identified similar treatment groups denoted in Figure 4.3. Month 2 demonstrated higher activity than both months 1 and 3, likely due to the environmental conditions at this time since all treatments vary relatively consistently between months. A Dunnett test indicated that the ionic-high treatment had significantly less activity than the control. Soap control and weathered-low treatments were significantly different from the ionic-low and ionic-high treatments indicating somewhat increased activity in these treatments relative to the ionic treatments. Soap provides additional carbon sources within the soap control and weathered-low treatments that likely increased microbial activity due to availability of additional substrates. Ionic-low and ionic-high treatments resulted in decreased activity due to the toxicity effect of ionic silver affecting the activity and diversity of the microorganisms present. Inhibition of the microbial community was most evident with the ionic-maximum treatment. After three months of exposure, sulphidized-low activity also decreased and diverged from other treatments as well as its activity in previous months.

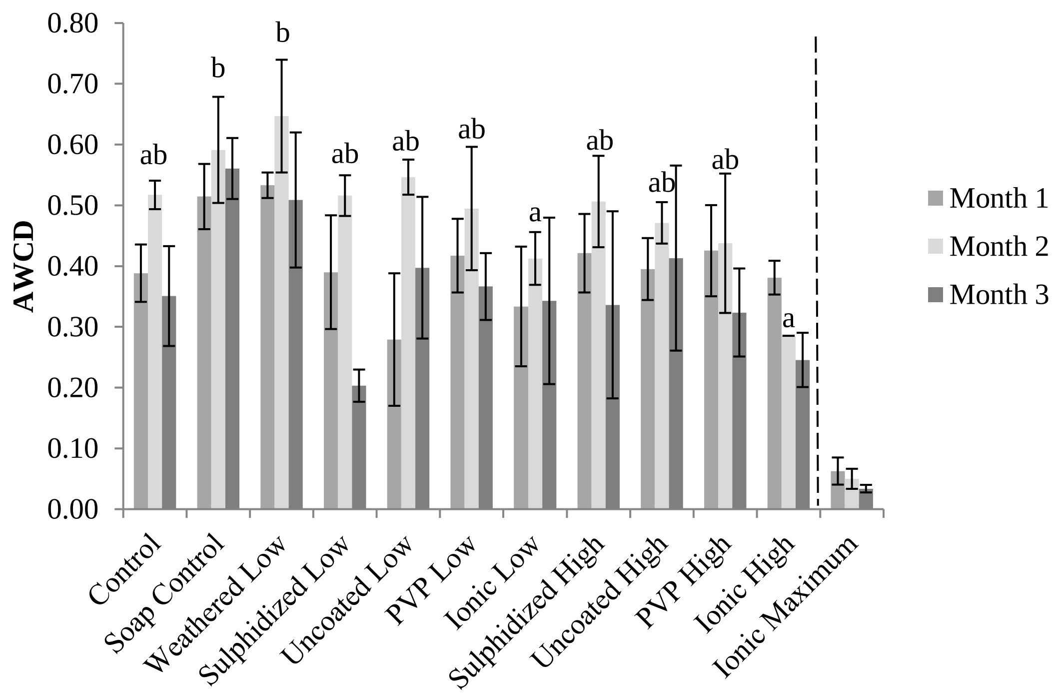

Figure 4.3: Average well colour development of treatments assessed each month. Significantly different treatment groups in month 2 from a post-hoc Tukey test are identified using different letters (p<0.05).

At the second month of exposure, the only time when any treatments differed significantly from the control, uncoated-high and ionic-high treatments reduced AWCD by 10% and 45% relative to the control, respectively. These findings compared to the literature are summarized in Table 4.4 for uncoated and ionic silver treatments relative to controls for the metric of AWCD. In the literature, exposures of soil extracts (1 g soil extracted in 100 mL buffer and subsequently treated with aqueous Ag solutions) to uncoated AgNPs and ionic silver had IC50s indicating a 50% reduction in AWCD at concentrations of 0.535 and 0.064 mg Ag/L, respectively (Zhai, et al. 2016). The orders of magnitude difference between the finding of this study and those of Zhai et al. (2016) are likely the result of the direct contact in the soil extracts, and the lack of an acclimation of the microbial community over the short exposure period. The soil extract is immediately analyzed after direct exposure to a solution containing silver. This prevents the microbial community from adapting to the addition of the silver. Silver nitrate in both cases however resulted in greater toxicity than the AgNPs. The weathered-low treatment, for which no literature comparison currently exists, increased the AWCD by 25% compared to the control and 18% relative to the soap control as seen in Figure 4.3. This increase in AWCD upon exposure to nanomaterials released from a commercial textile indicates that these nanomaterials when released in a soap solution were not toxic to soil microbial communities.

Table 4.4: Summary and literature comparison of AgNMs and $AgNO_3$ effects on AWCD relative to controls

	Particle Type	Particle Size (nm)		Concentration	Media	Effect on AWCD
		Supplier	Average Measured			
Uncoated High	Uncoated AgNPs	20-30	140	5.5 mg Ag/kg	Soil	Insignificant decrease of 10%
Ionic High	$AgNO_3$	N/A	N/A	9.1mg Ag/kg	Soil	Significant decrease of 45%
Zhai et al. (2016)	Uncoated AgNPs	20-40	187	0.535 mg Ag/L	Soil Extract*	Significant decrease of 50%
	$AgNO_3$	N/A	N/A	0.064 mg Ag/L	Soil Extract*	Significant decrease of 50%
Weathered Low	Released from textile	N/A	110	1.2 mg Ag/kg	Soil	Insignificant increase of 25%

*Ex-situ: 1 g of soil in 100 mL of buffer directly exposed to solution with Ag

Significant differences between treatments with respect to richness were also found to exist only in month 2 (p<0.05). A Tukey post-hoc test was conducted and indicated similar treatment groups overlaid on treatment average richness (Figure 4.4). Increased richness values present in month 2 correspond to the increase in activity relative to both months 1 and 3. Similarly to the AWCD, soap control and weathered-low treatments had significantly increased richness relative to the ionic-high treatment at the month 2 timepoint. Ionic-high was capable of utilizing sufficiently less substrates than soap-containing treatments, 9 substrates as opposed to 17. A Dunnett test additionally indicated that the ionic-high treatment utilized significantly less substrates than the control, indicating the toxicity of the ionic silver. Sulphidized-low treatments also decreased richness, potentially indicating increasing bioavailability over time.

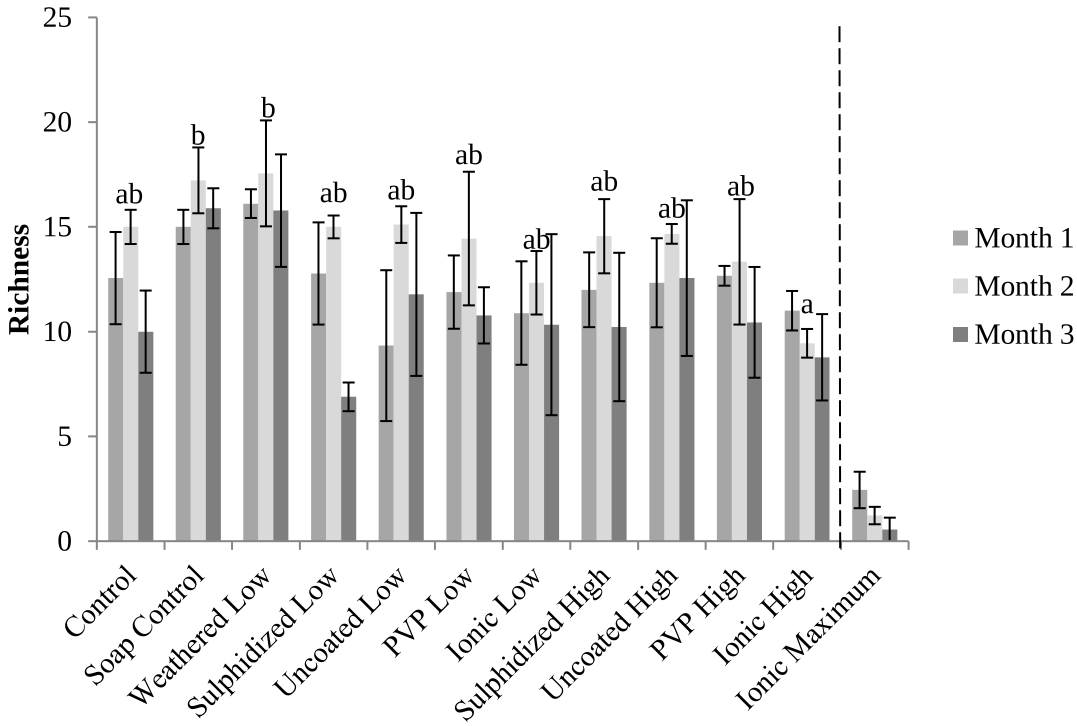

Figure 4.4: Average richness of treatments assessed each month. Different treatment groups in month 2 from a post-hoc Tukey test are identified using different letters (p<0.05).

A comparison of results to those found in the literature for the effects of ionic silver and AgNPs on diversity or substrate utilization of soil is summarized in Table 4.5 relative to their respective controls. In this current study, increasing concentrations of ionic silver decreased the substrate utilization or richness of the microbial community. Similar to the findings of Zhai et al. (2016), both ionic silver and AgNPs resulted in inhibition of the microbial community with ionic silver resulting in greater inhibition than AgNPs. Contrastingly, weathered AgNMs in a soap solution increased substrate utilization.

Table 4.5: Summary and literature comparison of AgNMs and $AgNO_3$ effects on substrate utilization in Biolog Ecoplates relative to controls

	Particle Type	Particle Size (nm)		Concentration	Media	Effect on Substrate Utilization
		Supplier	Average Measured			
Uncoated High	Uncoated AgNPs	20-30	140	5.5 mg Ag/kg	Soil	Insignificant Decrease
Ionic High	$AgNO_3$	N/A	N/A	9.1 mg Ag/kg	Soil	Insignificant Decrease
Zhai et al. (2016)	Uncoated AgNPs	20-40	187	0.598 mg Ag/L	Soil Extract*	Significant Decrease
	$AgNO_3$	N/A	N/A	0.316 mg Ag/L	Soil Extract*	Complete inhibition
Weathered Low	Released from textile	N/A	110	1.2 mg Ag/kg	Soil	Insignificant Increase

*Ex-situ: 1 g of soil in 100 mL of buffer directly exposed to solution with Ag

Guilds/ groupings in the Biolog Ecoplates consisting of carbohydrates, polymers, carboxylic and acetic acids, amino acids, amines/amides and root exudates were examined with one-way ANOVAs of each treatment guild's contribution to the AWCD to determine how treatments affected CSUPs. Statistically significant differences between treatment means were present in month 1 (carboxylic and acetic acids, amino acids and root exudates), month 2 (carbohydrates, carboxylic and acetic acids, amino acids and root exudates) and month 3 (amino acids, amines/amides and root exudates) ($p<0.05$). A post-hoc Tukey test identified similar treatment groups within each guild in cases where ANOVA yielded significant results. Similar treatment groups are denoted using the same letter in Table 4.6- Table 4.8 which show the AWCD contribution of specified guilds after each month.

In month 1, no treatments differed significantly from the control treatment. The weathered-low treatment had significantly greater contribution to AWCD with respect to carboxylic and acetic acids, amino acids and root exudates when compared to several other treatments. The higher activity in the weathered-low treatment compared to the soap control potentially indicates a combined positive effect of weathered nanomaterials and soap.

Table 4.6: Average well colour development contribution of guilds in Month 1. Significantly different treatment groups from a post-hoc Tukey test are identified using different letters ($p<0.05$).

	AWCD Contribution		
Treatment	Carboxylic and Acetic Acids	Amino Acids	Root Exudates
Control	0.11±0.03[ab]	0.07±0.01[ab]	0.110±0.009[ab]
Soap Control	0.16±0.02[ab]	0.110±0.001[ab]	0.160±0.003[ab]
Weathered Low	0.20±0.02[b]	0.13±0.02[b]	0.20±0.01[b]
Sulphidized Low	0.09±0.03[ab]	0.07±0.02[ab]	0.11±0.03[a]
Uncoated Low	0.09±0.05[a]	0.06±0.02[a]	0.09±0.03[a]
PVP Low	0.09±0.02[a]	0.07±0.01[ab]	0.110±0.007[a]
Ionic Low	0.08±0.03[a]	0.07±0.02[ab]	0.09±0.03[a]
Sulphidized High	0.12±0.04[ab]	0.07±0.02[ab]	0.12±0.02[ab]
Uncoated High	0.07±0.02[a]	0.09±0.01[ab]	0.11±0.02[a]
PVP High	0.11±0.03[ab]	0.11±0.04[ab]	0.15±0.05[ab]
Ionic High	0.11±0.02[ab]	0.09±0.02[ab]	0.12±0.01[ab]
Ionic Maximum	0.006±0.003	0.007±0.001	0.010±0.002

In month 2, an increase in activity in all treatments relative to month 1 indicates a change potentially due to environmental conditions since treatments tended to increase proportionally to one another. A post-hoc Dunnett test indicated significantly less contribution to AWCD of the ionic-high treatment in carboxylic and acetic acids, amino acids and root exudates when compared to the control. Statistically different treatments from post-hoc Tukey tests are summarized in Table 4.7. Ionic treatments demonstrated a concentration dependent decrease in activity, as expected due to the known antibacterial effect of silver ions (Doolette, et al. 2016; Zhai, et al. 2016). The soap control and weathered-low treatments again had increased activity relative to the control.

Table 4.7: Average well colour development contribution of guilds in Month 2. Significantly different treatment groups from a post-hoc Tukey test are identified using different letters ($p<0.05$).

	AWCD Contribution			
Treatment	Carbohydrates	Carboxylic and Acetic Acids	Amino Acids	Root Exudates
Control	0.150±0.001[abc]	0.17±0.02[ab]	0.09±0.003[ab]	0.18±0.01[ab]
Soap Control	0.18±0.02[bc]	0.17±0.03[ab]	0.100±0.006[ab]	0.20±0.03[b]
Weathered Low	0.20±0.05[c]	0.210±0.004[b]	0.10±0.02[ab]	0.21±0.06[b]
Sulphidized Low	0.14±0.02[abc]	0.16±0.03[ab]	0.10±0.01[b]	0.17±0.01[ab]
Uncoated Low	0.15±0.01[abc]	0.18±0.01[ab]	0.100±0.002[b]	0.19±0.01[ab]
PVP Low	0.14±0.02[abc]	0.17±0.06[ab]	0.09±0.01[ab]	0.16±0.03[ab]
Ionic Low	0.11±0.01[ab]	0.13±0.02[ab]	0.08±0.02[ab]	0.13±0.02[ab]
Sulphidized High	0.14±0.03[abc]	0.16±0.04[ab]	0.10±0.01[ab]	0.17±0.01[ab]
Uncoated High	0.14±0.01[abc]	0.14±0.02[ab]	0.09±0.01[ab]	0.14±0.02[ab]
PVP High	0.11±0.02[ab]	0.14±0.05[ab]	0.09±0.03[b]	0.15±0.03[ab]
Ionic High	0.09±0.01[a]	0.07±0.03[a]	0.05±0.02[a]	0.10±0.02[a]
Ionic Maximum	0.020±0.003	0.010±0.001	0.010±0.002	0.010±0.003

In month 3, decreased activity was again evident in the ionic-high treatment indicating that ionic silver had the most consistently detrimental effect to microbial populations. Differences in activity between sulphidized-low and sulphidized-high treatments were evident in month 3, including low activity of sulphidized-low treatment relative to the control in carboxylic and acetic acids, amino acids and root exudates while the sulphidized-high treatment demonstrated increased activity compared to the control in amino acids and root exudates. Although none of these differences were significant (p>0.05) when compared to the control, the contrast between these treatments indicates that the behaviour of sulphidized treatments has diverged from month 2 to month 3 and correlates with amino acid utilization where it did not before. Contribution of amines to the AWCD was also significantly higher in the soap control relative to the control and was slightly increased in the weathered-low treatment.

Table 4.8: Average well colour development contribution of guilds in Month 3. Significantly different treatment groups from a post-hoc Tukey test are identified using different letters (p<0.05).

	AWCD Contribution			
Treatment	Carboxylic and Acetic Acids	Amino Acids	Amines	Root Exudates
Control	0.09±0.04	$0.06±0.02^{ab}$	$0.006±0.003^{a}$	$0.12±0.03^{ab}$
Soap Control	0.16±0.03	$0.12±0.01^{b}$	$0.043±0.016^{b}$	$0.20±0.02^{b}$
Weathered Low	0.14±0.05	$0.09±0.03^{ab}$	$0.031±0.018^{ab}$	$0.17±0.05^{ab}$
Sulphidized Low	0.06±0.01	$0.02±0.01^{a}$	$0.008±0.002^{ab}$	$0.05±0.01^{a}$
Uncoated Low	0.12±0.06	$0.08±0.02^{ab}$	$0.014±0.009^{ab}$	$0.14±0.04^{ab}$
PVP Low	0.11±0.02	$0.07±0.02^{ab}$	$0.012±0.011^{ab}$	$0.11±0.02^{ab}$
Ionic Low	0.09±0.06	$0.06±0.02^{ab}$	$0.011±0.007^{ab}$	$0.10±0.04^{ab}$
Sulphidized High	0.08±0.06	$0.09±0.04^{ab}$	$0.005±0.002^{a}$	$0.14±0.07^{ab}$
Uncoated High	0.11±0.06	$0.07±0.03^{ab}$	$0.025±0.018^{ab}$	$0.13±0.05^{ab}$
PVP High	0.07±0.04	$0.06±0.02^{ab}$	$0.004±0.003^{a}$	$0.12±0.04^{ab}$
Ionic High	0.03±0.02	$0.04±0.03^{ab}$	$0.002±0.001^{a}$	$0.07±0.03^{ab}$
Ionic Maximum	0.005±0.001	0.003±0.001	0.0011 ± 0.0004	0.006±0.002

Available sulphur in soil promotes the synthesis of sulphur-containing amino acids through a biosynthesis pathway (Noji and Saito 2003). Sulphur must be transformed into sulphate before it is accessible (Noji and Saito 2003; Zhao, et al. 2014). This transformation therefore requires sulphur oxidation where oxidation rates are dependent on factors including number and species of sulphur-oxidizing bacteria, pH, moisture content, organic content, oxidation potential and temperature (Zhao, et al. 2014). Sulphur oxidation has been demonstrated for elemental sulphur within a range of soils as 21-100% oxidized after 96 days with oxidized sulphur increasing as a function of time (Zhao, et al. 2014). This lag time has been demonstrated in multiple soil studies (Lettl, et al. 1981, Wainwright, et al. 1986) and has been attributed to the time required to accrue a population capable of oxidizing elemental sulphur as well as colonization on the surface of the sulphur (Chapman 1989; Lawrence and Germida 1991). Bioavailability of sulphur in soil has been previously found to be related to successional changes in functional diversity (Lupwayi, et al. 2001). As such, after approximately three months, the available sulphur in SAgNPs could become sufficiently oxidized for synthesis of sulphur-containing amino acids to occur thus promoting an increase in the activity of microbial populations which can utilize amino acids. While this could be demonstrated in the sulphidized-high treatment, the inverse is seen in the sulphidized-low. The increased surface area of the low treatment (120 nm aggregates opposed to 160 nm aggregates) and a similar ratio of sulphur to silver could result in sulphur oxidation on the nanoparticle surface occurring and subsequently allowing for a greater quantity of ionic silver to be dissolved, contributing to the decrease in activity through inhibition. Although EDS analysis of sulphidized nanoparticles over this time does not indicate that sulphur and silver become disassociated, it cannot be determined to what extent the sulphur could be oxidized on the surface of the particle.

A morphological change was observed when analyzing samples from both SAgNP treatments using STEM prior to and after soil exposure. Exposure to the electron beam during STEM caused the smooth surfaces of SAgNPs to deform and elongate. This can be seen from STEM images of sulphidized particle aggregates as seen in Figure 4.5 where morphological changes were visually observed within the areas circled. Decreased microbial activity of SAgNPs-low treatments suggest that SAgNPs are biologically available and could have a low toxicity effect after three months possibly as a result of amorphous sulphur content.

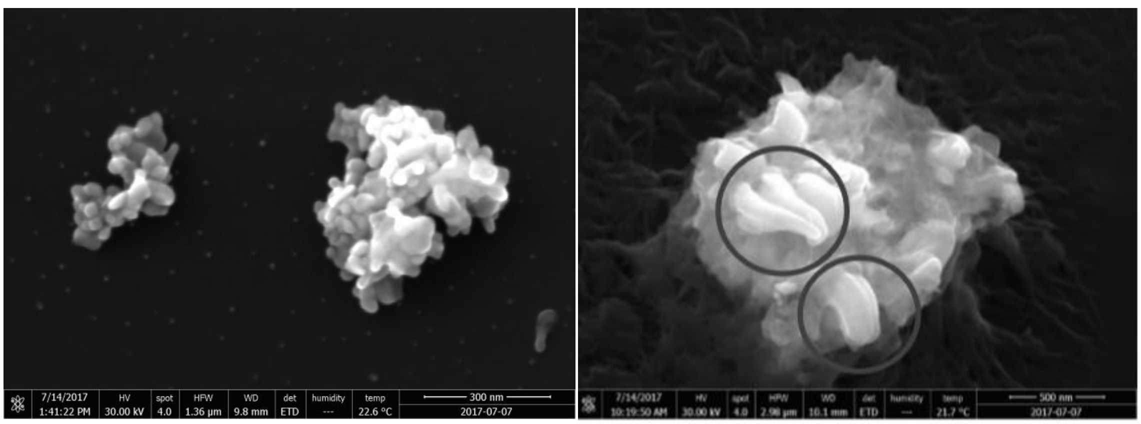

Figure 4.5: STEM images of monodisperse and aggregates of PVP AgNPs on a TEM grid (left) and sulphidized AgNPs on a 0.45 µm filter (right). Visible morphological changes to the surface of the particle aggregate are encircled and occurred during electron microscopy indicating potential amorphous sulphur bonds in the image on the right.

While silver sulfide in its mineral form is insoluble and biologically unavailable, several studies suggest that SAgNPs can be formed from both amorphous and crystalline Ag-S bonds wherein amorphous sulphur has differing properties (Pradas del Real, et al. 2016; Levard, et al. 2011; Kraas, et al. 2017). Pradas del Real et al. (2016) identified the presence of amorphous sulfur species (24-46% of silver-sulphur species) in sludge treated with PVP AgNPs through x-ray absorption spectroscopy analysis. Transformations varied between amorphous and crystalline Ag_2S over the course of one month in soil when compared to initial sludge, indicating instability (Pradas del Real, et al. 2016). Kraas et al. (2017) observed changes to the morphology of sulphidized particles occurring under an electron beam during electron microscopy which they proposed as indicative of weakly-crystalline or amorphous Ag-S phases aside from crystalline Ag_2S particles.

PCA was performed using Taylor transformed CSUPs extracted from CLPP measurements made at month 1, month 2 and month 3. PCA ordinations are shown in Figure 4.6- Figure 4.8 and constitute 55-60% of the variation in CSUPs. In month 1 treatments are well dispersed within the PCA ordination indicating that CSUPs were variable between treatments. Month 2 CSUPs PCA ordination indicates that the ionic-high treatment does not group with the rest of the treatments. This means that the CSUP of the ionic-high treatment differs from the other treatments due to a significant inhibition of the microbial community at month 2. At month 2 and month 3, PCA ordinations indicated that the soap control and weathered-low treatment CSUPs could be grouped. This means that the microbial populations present in these treatments utilized substrates in the Biolog Ecoplate similarly, therefore the increase in activity and richness of the weathered-low treatment is likely caused by the presence of the soap. The increasing convergence of weathered-low and soap control treatments over time suggests that the presence of soap was able to stabilize the microbial community, reducing any effects of the weathered nanomaterials. In general, treatment CSUPs began to separate further within the PCA after three months when compared to the previous month. The behaviour of treatments may therefore be beginning to diverge from one another and could demonstrate more inhibitory effects over a longer exposure time.

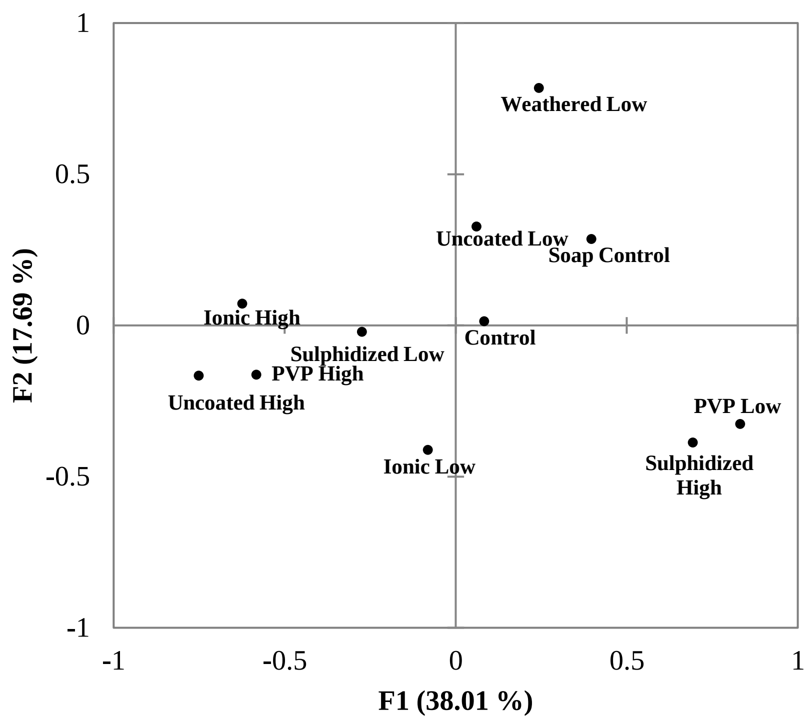

Figure 4.6: Principal component analysis ordinations for carbon source utilization patterns obtained from Biolog Ecoplates for average treatments at Month 1.

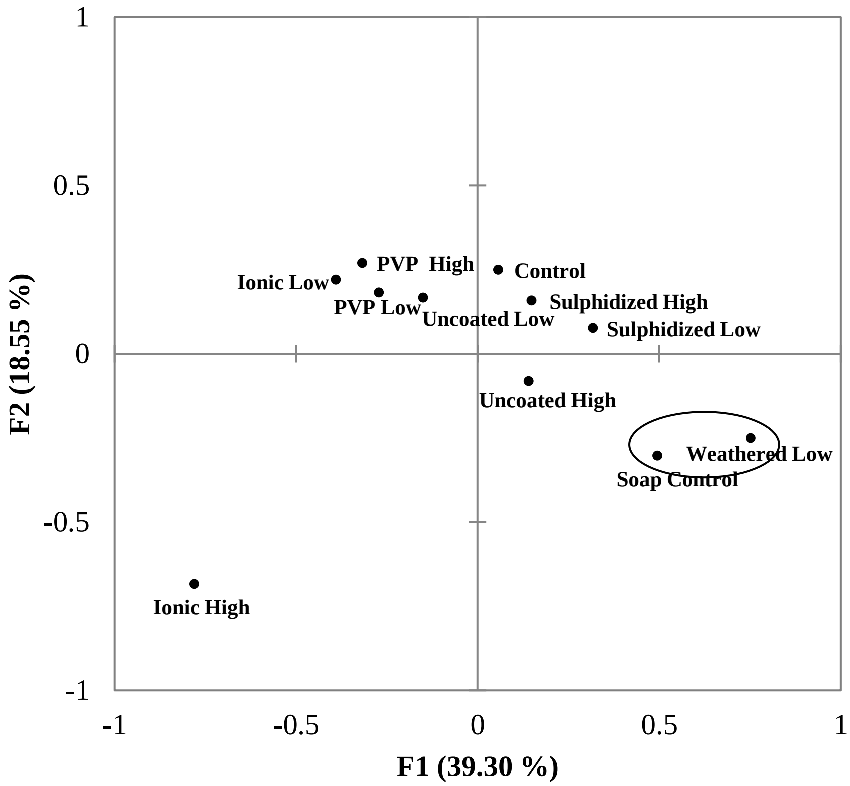

Figure 4.7: Principal component analysis ordinations for carbon source utilization patterns obtained from Biolog Ecoplates for average treatments at Month 2.

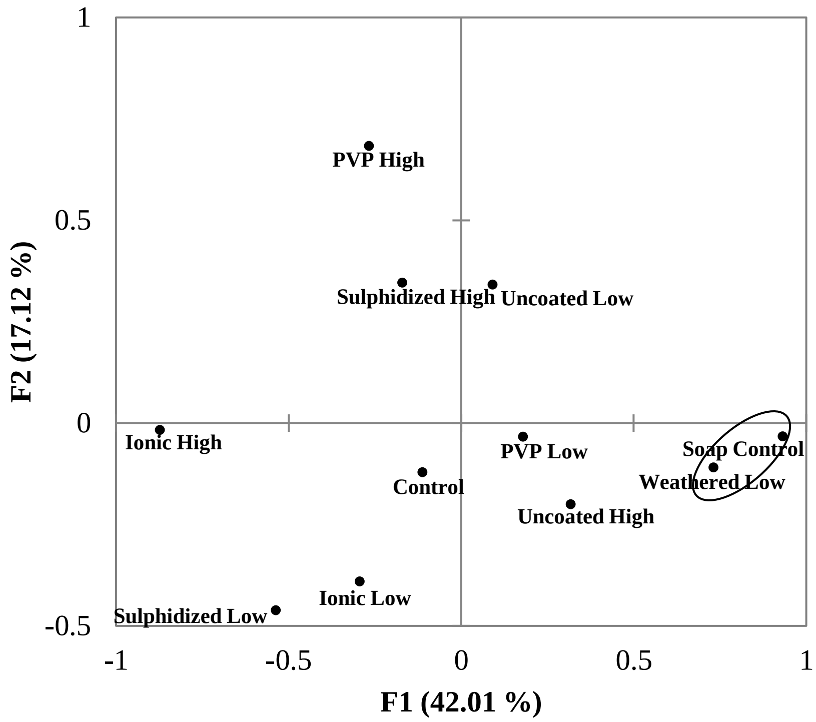

Figure 4.8: Principal component analysis ordinations for carbon source utilization patterns obtained from Biolog Ecoplates for average treatments at Month 3.

Figure 4.9 represents the relative community divergence of CSUPs of treatments from the initial microbial community over three months. At month 1, sulphidized-high, uncoated-high and soap control treatments showed the greatest relative community divergence while this value decreased at subsequent timepoints, indicating that the microbial community of the soil may have been impacted by these treatments over the first month before re-establishing a similar community to the control by the next measure at month 2. This does not indicate that the changes are the same in all cases, rather that the magnitude of divergence is similar. At two months after treatment, PVP-low and ionic-high treatments began to diverge more from the initial microbial community. While some treatments generally converged with the control community at month 3, treatments which exhibited the greatest divergence at this endpoint were weathered-low, soap control, sulphidized-low and ionic-high. Since the soap control and weathered-low treatments exhibited similar trends in divergence it can be inferred that this results from substrates within the soap. The divergence seen in sulphidized-low treatments correlates to the decreased activity that this treatment exhibited at the end of the experiment, potentially as a result of sulphur-oxidation related toxicity and subsequent ionic silver release. Ionic-high treatment divergence was also expected due to the toxicity of ionic silver to heterotrophic bacteria which was most defined after two months. At month 3, relative community divergence between treatments had an increasing range compared to month 2 indicating that treatment CSUPs could be beginning to diverge in behaviour as also suggested by the principal component analysis (Figure 4.8).

Relative community divergence of the control indicated that the control diverged from the initial community with the greatest divergence being at the first timepoint, month 1. Divergence of the control initially increased over the first month, decreased at month 2 before increasing again by month 3. This could be indicative of a thermal acclimation period of the microbial community as it adjusted from storage temperatures to the greenhouse temperature during the first month before a steady-state is reached. In previous soil studies, thermal acclimation has resulted in shifts in the microbial community in favour of propagation of gram positive bacteria thus changes to CSUPs could result (Wei, et al. 2014).

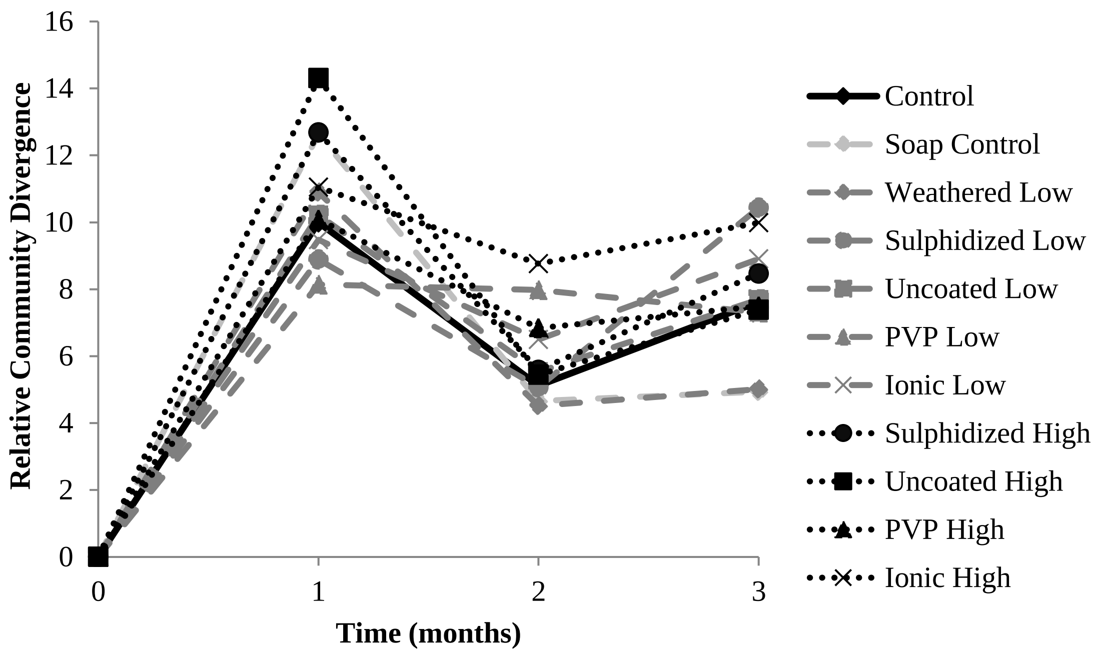

Figure 4.9: Relative community divergence of treatments over three months of exposure

Figure 4.10 shows homogenous groups generated from clustering analysis using the squared Euclidean distance between CSUPs measured for treatments after 1, 2 and 3 months. Hierarchal cluster analysis at month 1 indicated that all treatments linked together at a linkage distance of 1 with the exception of several sulphidized-high replicates, an ionic-high and a soap control replicate. At month 2 the linkage distance between clusters increased while most treatment clusters linked at a distance of 5, excluding ionic-high treatments, an ionic-low and PVP-low treatments. Ionic-high and PVP-low treatments were last to become linked to the other treatments indicating the most difference from other treatment group’s utilization patterns. After month 3, linkage distances between clusters increased further indicating the increasing variance between treatments. Clusters linked by a linkage distance of 10 with the exception of an uncoated-low, ionic-low, sulphidized-high and ionic-high replicate. These results are generally consistent with those of the AWCD, PCA and relative community divergence where the ionic-high treatment was the least similar to other treatment CSUPs across time and variation tended to increase at the last timepoint. Some discrepancies between relative community divergence, PCA and cluster analysis may result from the PCA and relative community divergence being used to describe average treatments while cluster analysis was conducted using the CSUPs of all treatment replicates, however, all findings indicate that treatments group relatively homogenously with generally small shifts in the microbial community between the majority of treatments.

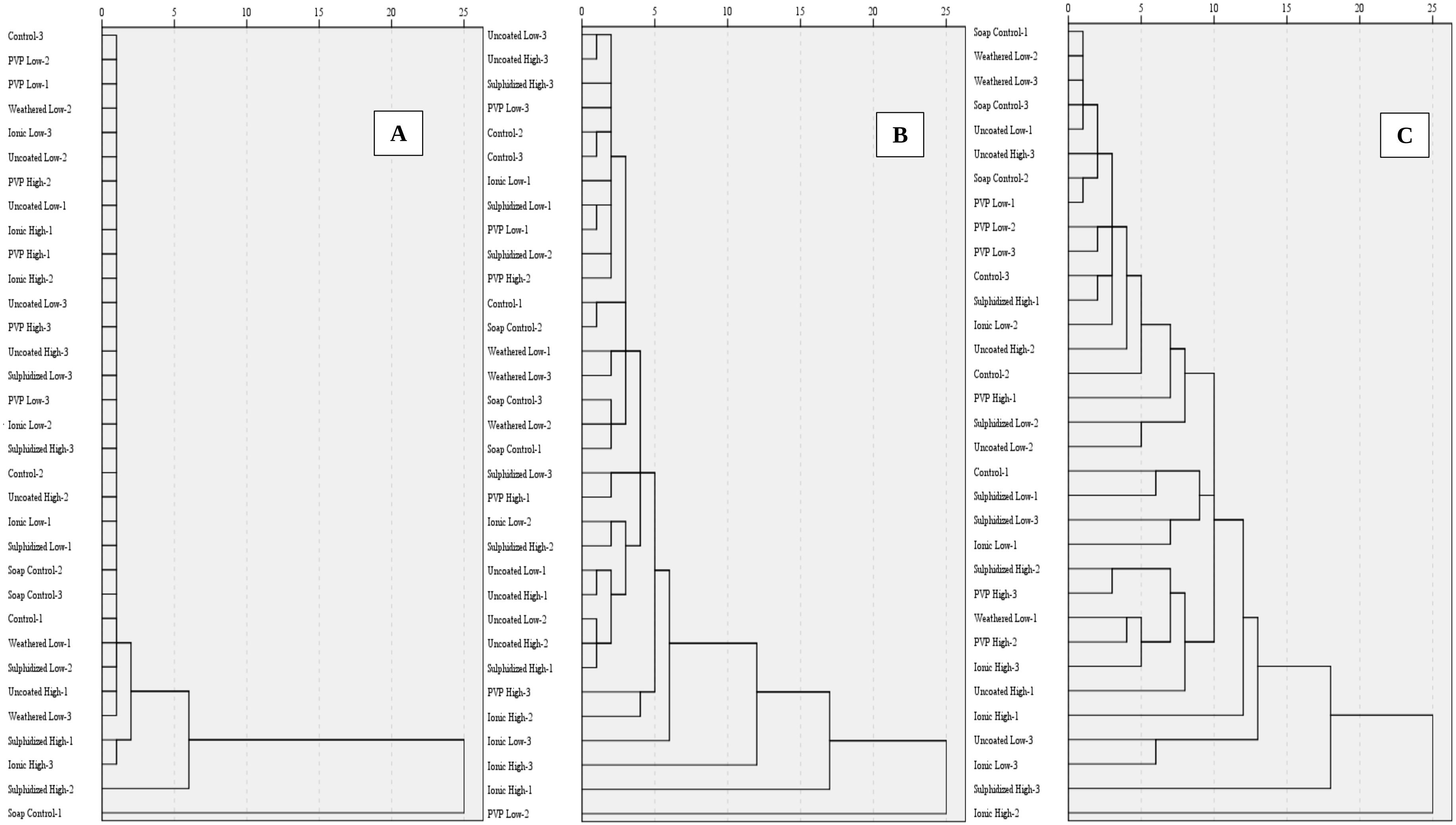

Figure 4.10: Hierarchal cluster analysis of carbon source utilization patterns based on squared Euclidian distances at Month 1 (A), Month 2 (B) and Month 3 (C) of exposure.

Enzyme activity of soil enzymes including β-glucosidase, α-glucosidase, xylosidase, cellobiosidase, n-acetylglucosaminadase, phosphatase and leucine amino peptidase were assessed monthly to determine if treatments affected extracellular enzyme activity. Average enzyme activities for each treatment in each month of analysis are shown in Appendix C Table C.59. No significant differences between treatments with respect to enzyme assays or specific nutrient cycles could be determined due to the variation present in this measure. Enzymatic activity of soil can be variable due to a large range of extractable fraction in soil which can range from 0.01-72.6% depending upon the specific enzymes and factors such as pH and organic matter content (Stursova and Baldrian 2011). No significant relationship between enzyme activity and soil treated with AgNPs after 1 day, 7 days, 14 days and 4 months incubation was found by Hansch and Emmerling (2010) due to high standard deviation between treatment replicates though slightly decreased leucine aminopeptidase activity was present until 14 days. Decreases in leucine aminopeptidase and phosphatase were found after 50 days incubation of AgNPs treated biosolid/soil slurry (0.14 mg Ag/kg slurry) relative to the control slurry; however, the difference was not significant (Colman, et al. 2013). Previous studies have determined significant inhibition to soil extracellular enzymes such as β-glucosidase, phosphatase and leucine aminopeptidase after addition of silver nanoparticles or silver ions (1.25-1815 mg Ag/kg) (Peyrot, et al. 2014; Samarajeewa, et al. 2017).

The frequent inhibition of leucine aminopeptidase activity suggests an effect of AgNPs on soil nitrogen cycling. Specific genera of bacteria, particularly nitrifying bacteria, have demonstrated inhibition to metal contaminants including AgNPs (Doolette, et al. 2016; Choi and Hu 2008). EC_{50}s for nitrate production after 28 days of exposure to Ag^+, AgNPs and SAgNPs were 19, 42 and 619 mg Ag/kg soil (Doolette, et al. 2016), respectively, indicating that ionic silver is the most inhibitory form of silver in this respect.

Heterotrophic plate counts, substrate-induced respiration values and extracted DNA quantities after three months are summarized in Table 4.9. Heterotrophic plate counts did not differ significantly between treatments. In general, higher concentration silver treatments had lower mean CFU than those of lower concentration treatments and controls. While treatment means for substrate-induced respiration were not significantly different, the lowest mean substrate-induced respiration was present with the ionic-high treatment. Lower respiration values were again generally present in treatments with higher silver concentrations indicating inhibition as a result of silver exposure. The average quantity of DNA extracted from soil treatments after three months was consistently between 383-598 ng/ g d.w. soil indicating that exposure to ionic and silver nanoparticles did not affect the overall size of the microbial population present ($p>0.05$).

Table 4.9: Heterotrophic plate counts, substrate-induced respiration measures and extracted DNA quantities of treatments after three months of exposure.

Treatment	Heterotrophic Plate Count (CFU/ g d.w. soil)	Substrate-Induced Respiration (µl CO_2/ g d.w. soil h)	Extracted DNA Quantity (ng/g d.w. soil)
Control	$4.98x10^8 \pm 1.42x10^8$	10.54±0.73	566±76.0
Soap Control	$4.55x10^8 \pm 2.13x10^8$	10.65±0.45	383±36.0
Weathered Low	$3.11x10^8 \pm 1.83x10^7$	10.54±0.57	491±81.0
Sulphidized Low	$3.06x10^8 \pm 4.68x10^7$	10.71±0.45	544±94.0
Uncoated Low	$4.22x10^8 \pm 1.01x10^8$	10.86±0.86	471±88.0
PVP Low	$3.76x10^8 \pm 6.41x10^7$	11.58±0.24	491±21.0
Ionic Low	$3.58x10^8 \pm 1.01x10^8$	9.84±1.13	550±106
Sulphidized High	$5.57x10^8 \pm 1.55x10^8$	11.66±1.74	598±141
Uncoated High	$2.72x10^8 \pm 2.58x10^7$	10.81±0.59	510±76.0
PVP High	$3.28x10^8 \pm 1.05x10^8$	9.53±0.64	505±79.0
Ionic High	$3.67x10^8 \pm 1.28x10^8$	8.54±0.28	459±100
Ionic Maximum		4.99±0.34	530±77.0

Table 4.10 summarizes and compares the effects of AgNPs and $AgNO_3$ on heterotrophic plate count and substrate-induced respiration with respect to controls in this study as well as results found in the literature. In this study, neither PVP AgNPs (6.2 mg/kg) or $AgNO_3$ (9.1 mg/kg) significantly impacted heterotrophic plate counts or substrate-induced respiration rates though both demonstrated decreases. The weathered-low treatment also had a slight decrease in heterotrophic plate counts but had no effect on the substrate-induced respiration rate. Samarajeewa et al. (2017) found that soil treated with AgNPs ($\geq$49 mg/kg) incubated for up to 50 days had a negative concentration dependent effect on both heterotrophic plate counts and substrate-induced respiration and also found no observable toxicity at concentrations of 10 mg/kg. In substrate-induced respiration measurements made for various soils exposed to AgNPs or Ag^+, minimal dose-response

effects were found with inhibition from Ag^+ being significant in three arable soils at 5 mg/kg, and from AgNPs in one soil at the same concentration (Schlich and Hund-Rinke 2015). Arable soils, suitable for agricultural crops, were therefore generally subject to greater inhibition from ionic silver than AgNPs. Additionally, concentrations representing hundreds of years equivalent of biosolids loadings were often required to illicit a significant inhibition to the microbial community based on these metrics of heterotrophic bacterial populations and activity.

Table 4.10: Summary and literature comparison of AgNMs and $AgNO_3$ effects on CFU and Substrate-Induced Respiration relative to controls

	Particle Type	Particle Size (nm)		Concentration (mg Ag/kg)	Media	Effect on CFU	Effect of Substrate-Induced Respiration
		Supplier	Average Measured				
PVP High	PVP AgNPs	20-30	25	6.2	Soil	Insignificant decrease	Insignificant decrease
Ionic High	$AgNO_3$	N/A	N/A	9.1	Soil	Insignificant decrease	Insignificant decrease
Samarajeewa et al. (2017)	PVP AgNPs	20	31	10	Soil	No effect	No effect
	PVP AgNPs	20	31	49	Soil	Significant decrease of 63%	Significant decrease
Schlich and Hund-Rinke (2015)	AgNP	15		0.56-15	Arable Soils		Minimal dose-response
	$AgNO_3$	N/A	N/A	5-15	Arable Soils		Minimal dose-response
Weathered Low	Released from textile	N/A	110	1.2	Soil	Insignificant decrease	No effect

Metagenomic sequencing of soils from each treatment revealed that exposure to AgNMs or ionic silver had an insignificant effect on the diversity and richness of the microbial community ($p>0.05$). The average Shannon diversity index, species richness and evenness for various treatments are presented in Table 4.11 where different letters have been used to denote significant differences between treatments from a Tukey post-hoc test. Species evenness was increased in the ionic-high and the soap control; however, the increases were not significant relative to the control. Species which accounted for at least 0.5% relative abundance of the average microbial population in any given treatment are shown in Figure 4.11.

Despite insignificant changes to the overall diversity of the bacterial species present in treatments, ionic treatments diverged from other treatments in terms of the quantity of specific species present. The ionic-high treatment resulted in significant increases in the populations of *Frankia alni* and *Arenimonas malthae* with 0.5% and 0.6% abundance, respectively, while the control treatment equated to 0.2% and 0.08% abundance ($p<0.05$). This trend continued further in ionic-maximum where significant increases were found in *Runella limosa*, *F. alni* and *A. malthae* equating to 3.2% abundance compared to 0.2% in the control, 0.7% and 1.0%, respectively. *R. limosa* is a species of aerobic, non-motile bacteria which has been isolated from activated sludge (Ryu, et al. 2006). Given that this species has been isolated from activated sludge where fast-growing heterotrophs out-compete slow growing bacteria (Li and Wu 2014), this suggests that this fast growing bacteria has increased in abundance through niche filling; replacing slower growing bacteria and those which were inhibited by silver. *A. malthae* is also a species of aerobic bacteria first isolated from an oil-contaminated site (Young, et al. 2007). *F. alni* is a species of actinobacteria capable of nitrogen fixation under both symbiotic and free-living aerobic conditions, which differentiates it from most rhizobia (Benson and Silvester 1993).

Table 4.11: Shannon diversity index, bacterial species richness and species evenness after three months exposure. Different letters denote significantly different treatments ($p<0.05$).

Treatment	Shannon Diversity Index (H)	Species Richness (S)	Species Evenness (E)
Control	2.45±0.04	998 ± 103	0.355±0.003[ab]
Soap Control	2.50±0.05	982 ± 158	0.364±0.001[b]
Weathered Low	2.54±0.09	1240 ± 216	0.357±0.005[ab]
Sulphidized Low	2.41±0.04	1090 ± 136	0.345±0.008[a]
Uncoated Low	2.44±0.07	1040 ± 100	0.352±0.007[ab]
PVP Low	2.46±0.03	1090 ± 81.0	0.351±0.007[ab]
Ionic Low	2.44±0.04	1080 ± 23.0	0.350±0.003[ab]
Sulphidized High	2.45±0.03	1170 ± 199	0.348±0.006[ab]
Uncoated High	2.47±0.03	1140 ± 41.0	0.362±0.007[ab]
PVP High	2.49±0.04	1200 ± 158	0.351±0.008[ab]
Ionic High	2.53±0.06	1070 ± 89.0	0.362±0.007[b]
Ionic Maximum	2.45±0.02	980 ± 48.0	0.356±0.002

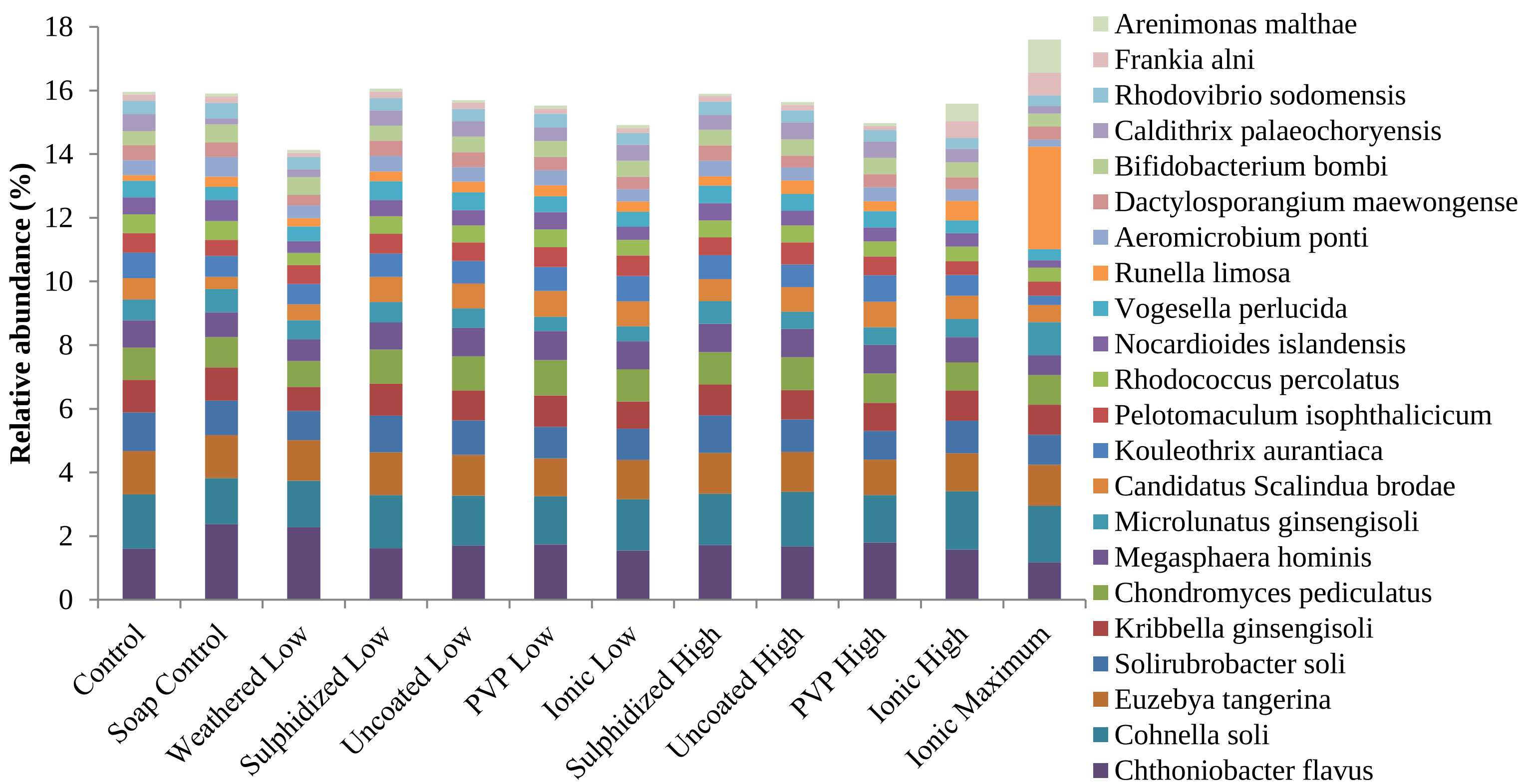

Figure 4.11: Bacterial species relative abundance contributing to ≥0.5% of average treatment microbial populations

A positive dose-response (Figure 4.12) was found for the abundance of *F. alni* treated with ionic silver meaning that this species of bacteria is tolerant of silver and could be increasing in abundance to compensate for inhibition to related classified or unclassified species which perform a similar function. While a linear relationship between ionic silver concentration and relative abundance of *F. alni* only moderately fits the data from the coefficient of determination, a Michaelis-Menten type relationship (Michaelis, et al. 2011) may be able to better approximate this relationship. After three months of exposure, the ionic-maximum also demonstrated a 14-fold increase of a phytopathogen, *Xanthomonas oryzae*, relative to the control ($p<0.05$). This bacterium is known to cause leaf blight most commonly in rice and within members of the *Poaceae* family (Nino-Lui, et al. 2006), meaning that increasing concentrations of silver and nitrates in combination could decrease the microbial community's resistance to phytopathogens.

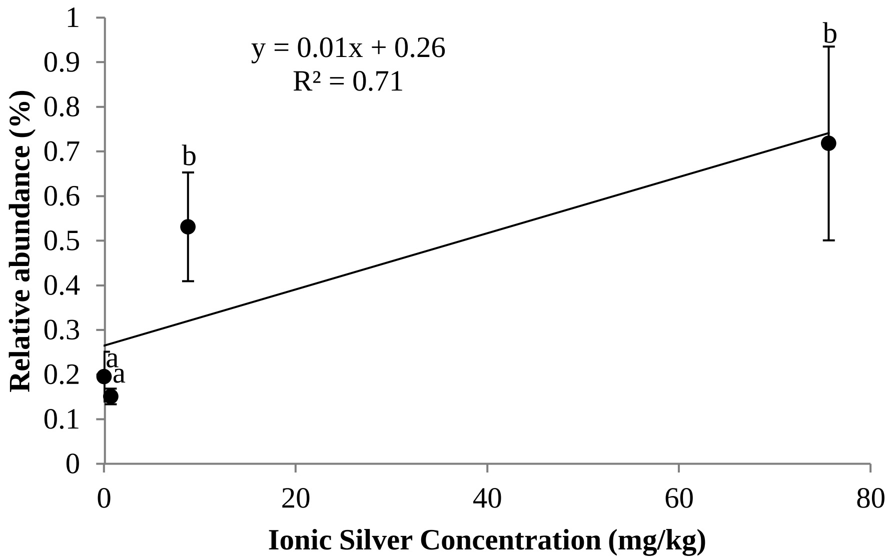

Figure 4.12: Relative abundance of *Frankia alni* after three months' exposure to ionic silver

Table 4.12 summarizes the effect of AgNMs and $AgNO_3$ on nitrogen-fixing bacteria in this study and in literature. Nitrogen-fixing bacteria, including *Rhizobiales*, have been previously shown to be inhibited by the presence of metallic nanoparticles including AgNPs (Ge, et al. 2011; Ge, et al. 2012; Kumar, et al. 2011; Kumar, et al. 2014; Pallavi, et al. 2016). The insignificant effect of AgNMs on nitrogen-fixers observed in this study and significant increases with increasing ionic silver concentrations suggest that inhibition to specific genera could be concentration dependent while moderate concentrations of silver could have a stimulatory effect on silver tolerant species, thus filling the niche of inhibited species. Similar observations with regards to silver tolerant species were made by Samarajeewa et al. (2017) wherein concentrations of 124 and 287 mg AgNPs/kg soil resulted in significantly increased total bacterial counts due to the growth of silver tolerant bacteria after 14 days of incubation. Increased activity of nitrogen-fixers is, in general, beneficial to soil nitrogen cycling since it will allow for greater fixation of atmospheric nitrogen potentially reducing the overall rate of nitrogen losses from soil.

Table 4.12: Summary and literature comparison of AgNMs and $AgNO_3$ effects on nitrogen-fixing bacteria relative to controls

	Particle Type	Particle Size (nm)	Concentration (mg Ag/kg)	Media	Effect on Nitrogen-fixers
Uncoated High	AgNP	36	5.5	Soil	Insignificant decrease
Ionic High	$AgNO_3$	N/A	9.1	Soil	Significant increase
Shah et al. (2014)	AgNP	35	2-174	Soil	No effect
Kumar et al. (2011)	AgNP	20	66	Arctic Soil	Significant decrease in *rhizobiales*
Kumar et al. (2014)	AgNPs	40	66	Arctic Soil	Significant decrease in *rhizobium*
	Micro-Ag	75000	66	Arctic Soil	Significant decrease in *rhizobium*
Pallavi et al. (2016)	Citrate AgNP	35	50	Soil	Significant decrease in diversity, not bacterial count
	Citrate AgNP	35	75	Soil	Significant decrease in diversity and bacterial count which decreased in magnitude over time
Weathered Low	Released from textile	112	1.2	Soil	Insignificant decrease

PCA of normalized species relative abundance, Figure 4.13, indicated that treatments that had the most consistent differences in species abundance compared to other treatments were ionic-high and ionic-maximum as also seen in Figure 4.11. Richness, evenness and diversity of the ionic-high treatment was greater than in the control meaning that this ionic treatment had a greater number of species present without having significantly shifted the community composition towards specific species enough to significantly change the evenness. The ionic-maximum treatment however was less rich than the control and had similar evenness and diversity indicating that a slight decrease in richness resulted in a similar distribution of species throughout the community despite changes to the community composition including increased abundance of *R. limosa*, *F. alni* and *A. malthae*.

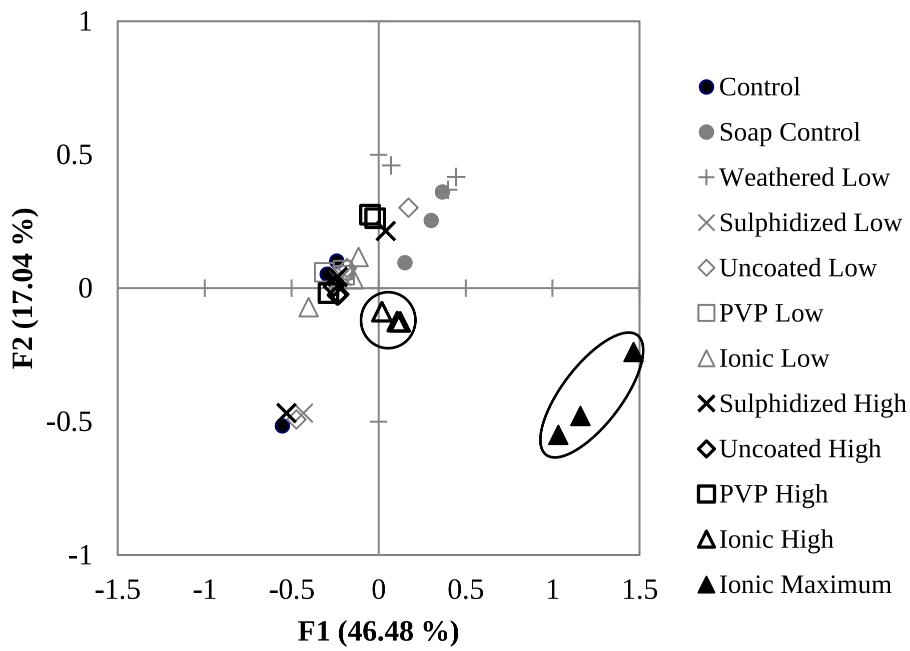

Figure 4.13: Principal component analysis ordinations for normalized species relative abundance determined from sequencing of 16S rRNA after Month 3.

4.3.4 Primary Findings

Significant results of treatments relative to the control for metrics investigated in this study are summarized in Table 4.13 and Table 4.14. After one month, the weathered-low increased utilization of amino acids and root exudates. Ionic-high treatments significantly decreased the microbial activity only after two months through reduced activity of carboxylic and acetic acids, amino acids and root exudates. After three months' time, no silver exposures resulted in significant effects on the microbial activity or community composition, however, the soap control significantly increased the utilization of amines/amides. These differences in utilization indicate that released nanomaterials from a consumer textile in soap had an initially positive effect on the microbial community that was generally opposite in effect to the toxicity of ionic silver. This suggests that the nanomaterials released from washing of socks in tap water and soap solution are not toxic to soil microbial communities and that this form of environmental release did not pose a risk through mechanisms of ionic silver release.

Table 4.13: Significant differences between the treatment and control according to measured physical and biological parameters of soil. Treatments significantly higher than the control (↑) and significantly lower than the control (↓) ($p \leq 0.05$).

Treatment	pH	Conductivity	Moisture Content	Organic Matter	AWCD Month 1	AWCD Month 2	AWCD Month 3	Richness Month 1	Richness Month 2	Richness Month 3
Soap Control	—	↑	—	—	—	—	—	—	—	—
Weathered Low	—	↑	—	—	—	—	—	—	—	—
Sulphidized Low	—	—	—	↓	—	—	—	—	—	—
Uncoated Low	—	—	—	↓	—	—	—	—	—	—
PVP Low	—	—	—	—	—	—	—	—	—	—
Ionic Low	—	—	—	↓	—	—	—	—	—	—
Sulphidized High	—	—	—	↓	—	—	—	—	—	—
Uncoated High	—	—	—	—	—	—	—	—	—	—
PVP High	—	—	—	↓	—	—	—	—	—	—
Ionic High	—	—	—	↓	—	↓	—	—	↓	—

Table 4.14: Significant differences between the treatment and control according to guild carbon utilisation. Treatments significantly higher than the control (↑) and significantly lower than the control (↓) ($p \leq 0.05$).

Month	Treatment	Carbohydrates	Polymers	Carboxylic and acetic acids	Amino acids	Amines/ amides	Root exudates
Month 1	Soap Control	–	–	–	–	–	–
	Weathered Low	–	–	–	↑	–	↑
	Sulphidized Low	–	–	–	–	–	–
	Uncoated Low	–	–	–	–	–	–
	PVP Low	–	–	–	–	–	–
	Ionic Low	–	–	–	–	–	–
	Sulphidized High	–	–	–	–	–	–
	Uncoated High	–	–	–	–	–	–
	PVP High	–	–	–	–	–	–
	Ionic High	–	–	–	–	–	–
Month 2	Soap Control	–	–	–	–	–	–
	Weathered Low	–	–	–	–	–	–
	Sulphidized Low	–	–	–	–	–	–
	Uncoated Low	–	–	–	–	–	–
	PVP Low	–	–	–	–	–	–
	Ionic Low	–	–	–	–	–	–
	Sulphidized High	–	–	–	–	–	–
	Uncoated High	–	–	–	–	–	–
	PVP High	–	–	–	–	–	–
	Ionic High	–	–	↓	↓	–	↓
Month 3	Soap Control	–	–	–	–	↑	–
	Weathered Low	–	–	–	–	–	–
	Sulphidized Low	–	–	–	–	–	–
	Uncoated Low	–	–	–	–	–	–
	PVP Low	–	–	–	–	–	–
	Ionic Low	–	–	–	–	–	–
	Sulphidized High	–	–	–	–	–	–
	Uncoated High	–	–	–	–	–	–
	PVP High	–	–	–	–	–	–
	Ionic High	–	–	–	–	–	–

5 Chapter 5: Fate and effects of pristine and aged silver nanomaterials in agricultural soil planted with *Triticum spp.*

5.1 Introduction

Increasing use of nanomaterials in consumer products has created a growing potential for toxicological effects on environmental receptors such as agricultural soil and plants. Silver nanomaterials which have been proven to remain in biosolids, primarily as silver sulfide and SAgNPs, after wastewater treatment are of particular concern due the toxicity of bulk silver and potential toxicity mechanisms at the nanoscale (Wang, et al. 2016). Silver sulfide is insoluble (K_{sp}= $6.3x10^{-50}$ (Dean 1998)) contributing to the belief that sulphidized silver nanoparticles are not bioavailable, however, previous studies including those of Pradas del Real et al. (2016), Kraas et al. (2017) and Chapter 4 suggest that nanomaterials and their residual form (SAgNPs) in biosolids could have increasing bioavailability over time.

The use of biosolids in agriculture to supply organic matter and macronutrients provides a route of exposure to AgNMs in soil and plants. Due to the symbiotic relationship between plants, bacteria, and mychorrhizal fungi, and their fundamental role in mineral biotransformations vital to plant health, inhibition due to AgNMs could be detrimental. Plant root exudates such as sugars, amino acids and enzymes promote diverse microbial populations in the rhizosphere (Gabreva, et al. 2004) and aid in nutrient cycling (Van Der Heijden, et al. 2008). Rhizosphere bacteria can also inhibit pathogenic microorganisms through both synergistic and antagonistic mechanisms (Raaijmakers, et al. 2009). Since the function of soil microbial communities are imperative to nutrient cycling, changes to the microbial populations or diversity can therefore directly impact plant productivity and health. Bacteria involved in nitrogen cycling have shown particular sensitivity to silver in both bulk and nanomaterial form including nitrifying bacteria (Doolette, et al. 2016; Choi and Hu 2008) and nitrogen-fixing bacteria such as *Rhizobiales* (Kumar, et al. 2011; Kumar, et al. 2014).

Many of the existing studies of nanomaterial toxicity to terrestrial plants have been conducted under hydroponic conditions utilizing pristine nanoparticles. Such hydroponic studies have shown that AgNPs can cause damage to root cell membranes, reduce root elongation, plant biomass and seed germination (Yin, et al. 2011; Barrena, et al. 2009; Stampoulis, et al. 2009; Qian, et al. 2013). A study of agricultural crops exposed to ionic and silver nanoparticles in agar and soil by Lee et al. (2012) indicated that the exposure media can greatly impact the effect of nanoparticles to terrestrial plants. In agar both *Sorghum bicolor* and *Phaseolus radiatus* (mung bean) demonstrated concentration dependent inhibition as a result of AgNP exposure whereas in soil *S. bicolor* was inhibited and *P. radiatus* was unaffected by concentrations up to 2000 mg Ag/kg (Lee, et al. 2012). Wheat plants (*Triticum aestivum*) exposed to various concentrations of AgNPs have shown inconsistent effects on plant growth with decreased shoot and root length and biomass at concentrations of 2.5 mg/kg after 14 days of growth in a sand matrix (Dimkpa, et al. 2013) and no effects on these same parameters at 50 mg/kg after 40 days of growth in soil (Pallavi, et al. 2016). While previous studies have therefore demonstrated that AgNPs have unique implications on phytotoxicity, no studies to date have been conducted using nanomaterials released from consumer products, perhaps the most relevant form, in the assessment of environmental toxicology. This study will therefore examine the fate and effects of pristine, sulphidized and weathered nanomaterials from a consumer textile in agricultural soil planted with *Triticum spp.* (wheat).

Wheat kernel was chosen due to its growing period of approximately three months, as well as for its importance to Canadian agriculture. Spring wheat and winter wheat varieties of wheat kernel are Canada's two largest types of wheat crop while wheat export from Canada constitutes 14% of the globally traded wheat (Government of Canada 2010). Thus, detrimental effects to this plant could have a global impact.

5.2 Materials and Methods

Chapter three describes the materials and methods used in this study. Effects of pristine and aged nanomaterials in agricultural soil planted with wheat kernel were investigated using the experimental design shown in Table 5.1. A three month study was conducted through the use of three randomized sample sets of 11 treatment types (Table 5.1) in triplicate which were destructively sampled over the course of three months. The same concentrations of SAgNPs were added to soil for two different treatments with mean sizes of 120 and 160 nm allowing for investigation of potential size-related toxicity effects. Enzyme analysis, functional diversity, plant biomass and total silver concentrations in soil, roots and shoots were conducted after each month while the full suite of methods described in chapter three was conducted after 12 weeks. Total silver concentrations of each treatment, representing the sum of background silver concentration in the soil and silver added in treatments are summarized in Table 5.2 where significantly different treatments within low or high concentrations are denoted by different letters. The recovery of the standard reference material SRM 2711a was 73±27%.

Table 5.1: Schematic of a sample set of soil including PVP AgNPs, uncoated AgNPs, weathered nanomaterials from sock wash water, sulphidized AgNPs, ionic silver (positive control) and no AgNPs (negative control).

	Concentration Level of Silver Added to Soil					
	Low	**High**	**Low**	**High**	**Low**	
Replicate #1	**PVP**	**PVP**	**Uncoated**	**Uncoated**	**Weathered**	
Replicate #2	**PVP**	**PVP**	**Uncoated**	**Uncoated**	**Weathered**	
Replicate #3	**PVP**	**PVP**	**Uncoated**	**Uncoated**	**Weathered**	
	Low, 120 nm	**Low, 160 nm**	**Low**	**High**	**Maximum**	**0**
Replicate #1	**Sulphidized**	**Sulphidized**	**Ionic**	**Ionic**	**Ionic**	**Control**
Replicate #2	**Sulphidized**	**Sulphidized**	**Ionic**	**Ionic**	**Ionic**	**Control**
Replicate #3	**Sulphidized**	**Sulphidized**	**Ionic**	**Ionic**	**Ionic**	**Control**

Table 5.2: Total concentrations of silver in soil after each treatment (n=18). Significantly different treatment groups within low or high concentration treatments from post-hoc Tukey tests are denoted using different letters.

Concentration Level of Ag Added to Soil	Treatment Type	Soil Concentration (mg Ag/kg)
0	Control	0.23 ± 0.08
Low	Sulphidized, 120 nm	0.87 ± 0.40^{bc}
	Sulphidized, 160 nm	0.86 ± 0.33^{bc}
	Weathered	1.3 ± 0.42^{a}
	Uncoated	0.39 ± 0.14^{d}
	PVP	0.47 ± 0.21^{cd}
	Ionic	0.91 ± 0.36^{ab}
High	Uncoated	4.3 ± 1.8^{a}
	PVP	4.2 ± 2.0^{a}
	Ionic	7.1 ± 3.2^{b}
Maximum	Ionic	67 ± 19

5.3 Results and Discussion

5.3.1 Fate of silver nanomaterials in agricultural soil planted with wheat

The fate and transport of silver nanomaterials in agricultural soil is an important factor in the consideration of plant health and uptake. Since AgNMs have a high sorption capacity, nanomaterials tend to sorb to soil surfaces and root surfaces where they can cause damage to root cell membranes as well as translocate into aboveground biomass (Yin, et al. 2011).

Repeated measure ANOVA of soil silver concentrations of treatments for the three months of the study indicated that there was no effect of time and no interaction effect between treatment and time ($p>0.05$). A student t-test indicated no significant difference between the concentration of silver in the upper and lower regions of soil in treatments ($p>0.05$). Silver concentrations were therefore consistent each month between and within individual treatment replicates as in Chapter 4. This indicates that AgNMs become sorbed to soil and organic matter, preventing dissolution. Due to the lack of mobility of ionic silver, this also means that Ag^{+} sorbs to soil and organic matter. In a silt loam soil approximately 50% of sorbed AgNPs were associated with silt and clay particles while total sorption decreased with increasing AgNP concentrations (Ebeling, et al. 2013). The presence of greater organic matter content such as that found in wetland soils and waste treatment residuals lead to increases in sorption with 100% of AgNPs sorbed to wetland soil medium at initial concentrations of up to 60 mg Ag/L (Ebeling, et al. 2013). Even in hydroponic experiments where AgNMs are highly mobile, many AgNMs become sorbed to plant roots before being taken up into the root directly or in ionic form through oxidative dissolution (Yin, et al. 2011).

5.3.2 Effects of silver nanomaterials on soil properties

Soil characteristics including moisture content, organic matter content, pH and conductivity were assessed to determine if exposure to silver nanomaterials or ionic silver in the presence of wheat plants impacted these characteristics and could therefore influence microbial community structure or activity. The weathered-low treatment had significantly higher moisture content than the control

($p<0.05$) while no other significant differences between treatments were found. Table 5.3 summarizes the physical properties of soil treatments after three months of exposure. The average moisture content of treatments ranged from 27-40%, organic matter content was 7-12%, pH ranged from 6.5-7.2 and conductivity was 30-47 µS/cm. Decreased conductivity of treatments relative to those of the unplanted exposure (Table 4.3) indicates that the presence of plants decreased the ionic content in the soil. This is expected since the amount of soluble salts in the soil should decrease as plants utilize them for nutrients.

The average water holding capacity for the control and ionic-maximum at 24 hours were 0.59 g water/g d.w. soil and 0.55 g water/g d.w. soil, respectively. A student t-test for water holding capacity of negative and positive control treatments at 24 and 48 hours indicated that treatment means did not differ significantly ($p>0.05$). This indicates that even the highest silver concentration examined, 67 mg Ag/kg, did not impact microbial health enough to decrease the volume of biofilm in the soil. Microbial biofilms composed of immobile bacterial communities and extracellular polymeric substances (EPS) help in the formation and stabilization of soil aggregates (Young and Crawford 2004), organic carbon degradation and sequestration (Jass, et al. 2002). Biofilm grown in solutions of citrate-coated AgNPs or ionic silver have previously demonstrated significant decreases in biofilm adhesiveness and EPS carbohydrate and protein components (Schmidt, et al. 2017). These effects on EPS structure and composition would make biofilm more susceptible to detachment and therefore decrease the biofilm volume and water holding capacity (Chenu and Roberson 1996; Rosenzweig, et al. 2012).

Table 5.3: Physical properties of treatments after three months of exposure

Treatment	Moisture Content (%)	Organic Matter (%)	pH	Conductivity (µS/cm)
Control	27.4±8.1	9.2±0.7	6.95±0.27	40.8±8.3
Sulphidized Low, 120 nm	35.6±1.4	6.8±4.0	7.18±0.11	42.1±0.4
Sulphidized Low, 160 nm	32.8±4.0	8.9±0.2	7.06±0.18	45.8±21.3
Weathered Low	40.5±2.5	8.8±0.1	6.53±0.17	33.0±7.3
Uncoated Low	34.7±1.7	10.1±0.1	7.08±0.19	34.4±1.3
PVP Low	37.6±2.1	9.0±0.1	7.11±0.12	30.1±9.2
Ionic Low	35.4±4.1	9.2±0.7	7.01±0.11	39.2±1.5
Uncoated High	37.6±0.3	7.9±1.4	7.07±0.18	35.1±5.9
PVP High	35.1±1.9	10.1±0.8	6.82±0.34	39.2±4.4
Ionic High	32.3±3.6	11.8±4.2	6.78±0.25	47.2±7.9
Ionic Maximum	30.5±1.7	8.5±1.7	6.57±0.27	42.6±7.1

5.3.3 Effects of silver nanomaterials on soil microbial communities

A healthy soil microbial community is of vital importance to terrestrial ecosystems due to its role in nutrient cycling and provision of essential nutrients for plants. The function, activity and diversity of soil microbial communities in soil planted with *Triticum spp.* exposed to silver treatments (AgNMs or ionic) were therefore assessed using several metrics including community level physiological profiling, heterotrophic plate counts, substrate-induced respiration and metagenomic sequencing to determine potential effects on the microbial community.

Functional diversity and metabolic activity were assessed using CLPP. AWCD of treatments are presented in Figure 5.1. Each month was examined individually to determine the differences between treatments at each time point. A post-hoc Dunnett test or t-test identified the ionic-maximum treatment as having significantly decreased AWCD compared to the control in all months. In each month, the ionic-maximum was also significantly lower in activity than the weathered-low and sulphidized-low (120 nm) treatments in addition to PVP-high and uncoated-low at month 1, and ionic-high at month 2 ($p<0.05$). Over the three month duration of the study the ionic-maximum treatment decreased the metabolic activity of the microbial community, indicating a toxic effect. Aged nanomaterials, sulphidized-low (120 nm) and weathered-low, were among treatments with the highest activity each month; indicating a positive effect on the microbial community. The positive effect of weathered treatments was also present in the unplanted exposure (Figure 4.3) indicating that weathered nanomaterials were not toxic even in the absence of soap. The increased activity of the weathered-low treatment could be the result of greater water retention as indicated by the significant increase in moisture content in this treatment. In a previous study, soil with a moisture content of 41% had a greater amount of viable microbial biomass when compared to the same soil at 13, 28, 62, 80 and 100% moisture content (Uhlirova, et al. 2005). Samarajeewa et al. (2017) also found that PVP AgNP concentrations of 10 mg Ag/kg had no toxic effect on soil microbial communities indicating that AgNMs may not be toxic to the microbial community at environmentally relevant concentrations in soil.

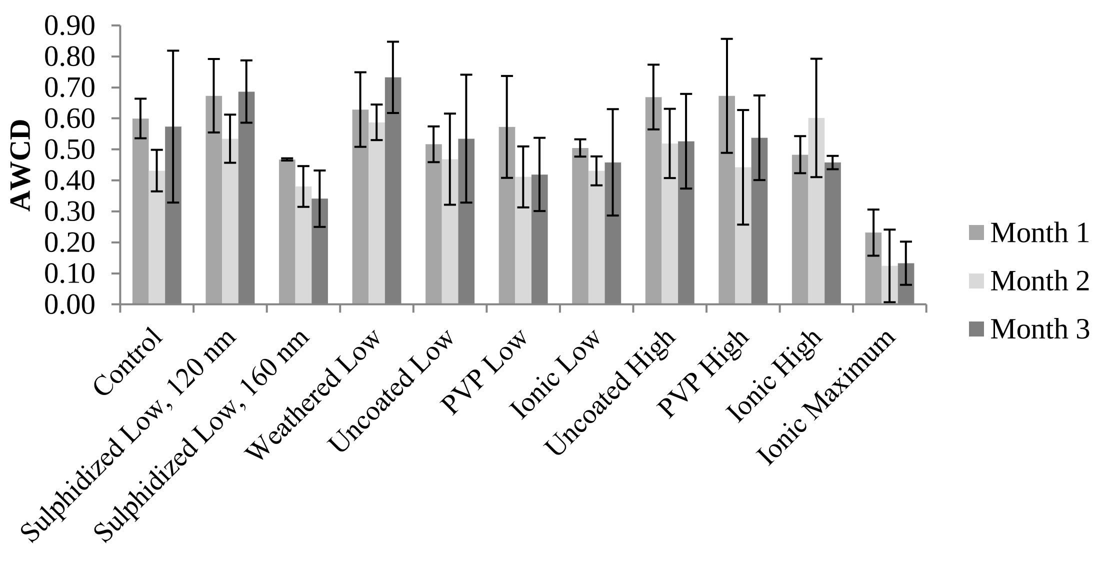

Figure 5.1: Average well colour development of treatments assessed each month

Richness, the number of carbon substrates within Biolog Ecoplates which a treatment was capable of utilizing was assessed to determine potential reductions in microbial functionality. The average richness of each treatment as determined from CLPP is shown in Figure 5.2. In month 1 and month 2, ionic-maximum treatment richness was identified as significantly less than all other treatments ($p<0.05$). In month 3, the ionic-maximum was similar to PVP-low, sulphidized-low (160 nm) and ionic-high treatments and significantly less than all other treatments. Overall, the greatest reduction in richness occurred in the ionic-maximum and was approximately 67% less than the control. The ionic-maximum treatment was therefore able to utilize fewer carbon sources than control treatments, which is indicative of reduced functionality of the microbial community. In an arctic soil treated with 66 mg AgNPs/kg for 176 days, substrate richness was reduced by approximately 79% relative to the control (Kumar, et al. 2011). In the same study by Kumar et al. (2011), soil exposure to AgNPs reduced the ability to utilize all but three substrates, two carboxylic acids and one carbohydrate, indicating completely reduced functionality in utilization of amino acids, polymers and amines.

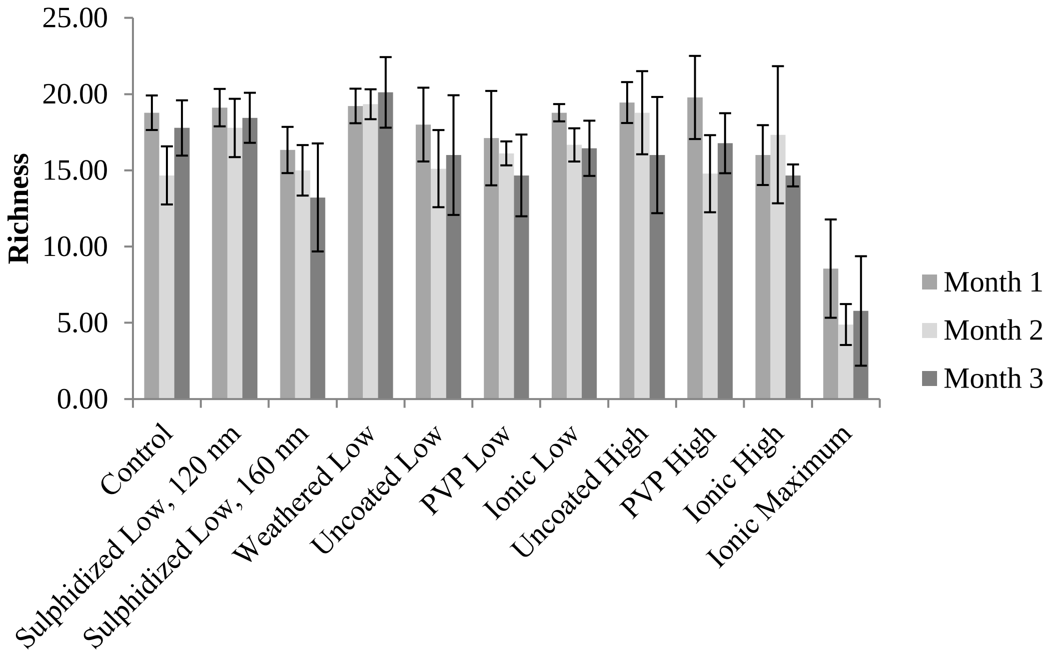

Figure 5.2: Average richness of treatments assessed each month

Guilds/ groupings in the Biolog Ecoplates consisting of carbohydrates, polymers, carboxylic and acetic acids, amino acids, amines/amides and root exudates were examined with one-way ANOVAs of each treatment guild's contribution to the AWCD to further examine how treatments affected CSUPs and microbial function. Statistically significant differences between treatment means were present in month 1 (polymers, amino acids and root exudates), month 2 (polymers, carboxylic and acetic acids and amino acids) and month 3 (polymers, carboxylic and acetic acids, amino acids and root exudates) ($p<0.05$). A post-hoc Tukey test identified similar treatment groups within each guild in cases where ANOVA yielded significant results; these groups are denoted using the same letter in Table 5.4- Table 5.6 which show the AWCD contribution of specific guilds.

In month 1, all treatments were similar to the control with the exception of the ionic-maximum. These findings therefore indicate that the ionic-maximum treatment reduced the ability of the soil microbial community to utilize polymers, amino acids and root exudates.

Table 5.4: Average well colour development contributions of guilds in Month 1. Significantly different treatment groups from a post-hoc Tukey test are identified using different letters ($p<0.05$).

	AWCD Contribution		
Treatment	Polymers	Amino Acids	Root Exudates
Control	$0.080±0.001^{a}$	$0.12±0.01^{a}$	$0.20±0.02^{ab}$
Sulphidized Low, 120 nm	$0.090±0.006^{a}$	$0.11±0.01^{ab}$	$0.24±0.05^{a}$
Sulphidized Low, 160 nm	$0.080±0.005^{a}$	$0.090±0.005^{ab}$	$0.16±0.01^{ab}$
Weathered Low	$0.09±0.01^{a}$	$0.12±0.03^{ab}$	$0.20±0.06^{ab}$
Uncoated Low	$0.090±0.002^{a}$	$0.110±0.001^{ab}$	$0.17±0.02^{ab}$
PVP Low	$0.080±0.003^{a}$	$0.12±0.02^{ab}$	$0.21±0.08^{ab}$
Ionic Low	$0.08±0.01^{a}$	$0.100±0.006^{ab}$	$0.17±0.02^{ab}$
Uncoated High	$0.09±0.02^{a}$	$0.13±0.01^{a}$	$0.24±0.04^{a}$
PVP High	$0.10±0.02^{a}$	$0.12±0.02^{a}$	$0.25±0.06^{a}$
Ionic High	$0.09±0.02^{a}$	$0.10±0.01^{ab}$	$0.16±0.01^{ab}$
Ionic Maximum	$0.030 ± 0.004^{b}$	$0.06 ± 0.03^{b}$	$0.08 ± 0.04^{b}$

In month 2, ionic-maximum treatments again demonstrated decreased contributions to the AWCD of several carbon source guilds; however, this reduction was only significant relative to the control in polymer and amino acid guilds. This finding is in agreement with month 1 guild contributions wherein significant decreases in AWCD contribution of the ionic-maximum relative to control treatments were present in polymer and amino acid guilds. Amino acid AWCD contribution decreased further beyond the last time point indicating a potential increase in inhibition of the microbial community over time.

Table 5.5: Average well colour development contributions of guilds in Month 2. Significantly different treatment groups from a post-hoc Tukey test are identified using different letters (p<0.05).

	AWCD Contribution		
Treatment	Polymers	Carboxylic and Acetic Acids	Amino Acids
Control	0.08±0.01[a]	0.11±0.02[ab]	0.10±0.02[a]
Sulphidized Low, 120 nm	0.09±0.01[a]	0.15±0.05[b]	0.13±0.01[a]
Sulphidized Low, 160 nm	0.06±0.01[ab]	0.11±0.02[ab]	0.08±0.01[ab]
Weathered Low	0.090±0.005[a]	0.15±0.01[b]	0.11±0.02[a]
Uncoated Low	0.09±0.02[a]	0.11±0.03[ab]	0.10±0.02[ab]
PVP Low	0.08±0.02[a]	0.10±0.04[ab]	0.09±0.03[ab]
Ionic Low	0.08±0.01[a]	0.11±0.01[ab]	0.09±0.02[ab]
Uncoated High	0.09±0.02[a]	0.13±0.03[b]	0.13±0.02[a]
PVP High	0.09±0.01[a]	0.10±0.03[ab]	0.08±0.03[ab]
Ionic High	0.09±0.02[a]	0.14±0.06[b]	0.11±0.02[a]
Ionic Maximum	0.030±0.003[b]	0.02±0.01[a]	0.030±0.002[b]

In month 3, the ionic-maximum treatment demonstrated reduced ability to utilize specific carbon sources within the polymer, carboxylic and acetic acids, amino acids and root exudate guilds, however, these reductions were not significantly less than the control guild contributions due to increasing variance of the ionic-maximum treatment. Decreases in the functional diversity of the ionic-maximum were therefore evident at all time points, as also seen from the richness. Guild contributions indicated that significant decreases in richness and microbial activity were most commonly related to decreased utilization of polymers and amino acids. Previously, AgNPs in an arctic soil completely inhibited utilization of amino acids, polymers and amines suggesting silver has an impact on the microbial community's ability to utilize compounds within these guilds (Kumar, et al. 2011). Reduced utilization of these compounds could mean a decreased ability to break down organic matter, reducing bioavailability of essential nutrients for plants and potentially agricultural product yields.

Table 5.6: Average well colour development contribution of guilds in Month 3. Significantly different treatment groups from a post-hoc Tukey test are identified using different letters (p<0.05).

	AWCD Contribution			
Treatment	Polymers	Carboxylic and Acetic Acids	Amino Acids	Root Exudates
Control	0.08±0.01^{ab}	0.16±0.08^{ab}	0.12±0.04^{ab}	0.23±0.13^{ab}
Sulphidized Low, 120 nm	0.09±0.01^{a}	0.16±0.01^{a}	0.15±0.01^{a}	0.26±0.03^{ab}
Sulphidized Low, 160 nm	0.06±0.01^{ab}	0.09±0.02^{ab}	0.06±0.02^{ab}	0.11±0.03^{ab}
Weathered Low	0.10±0.01^{a}	0.21±0.04^{a}	0.14±0.02^{a}	0.27±0.02^{a}
Uncoated Low	0.08±0.03^{a}	0.13±0.04^{ab}	0.11±0.04a^{b}	0.22±0.08^{ab}
PVP Low	0.07±0.01^{ab}	0.13±0.04^{ab}	0.09±0.02^{ab}	0.16±0.04^{ab}
Ionic Low	0.09±0.03^{a}	0.10±0.02^{ab}	0.10±0.03^{ab}	0.17±0.06^{ab}
Uncoated High	0.08±0.01^{ab}	0.15±0.05^{ab}	0.12±0.03^{ab}	0.21±0.07^{ab}
PVP High	0.08±0.01^{ab}	0.13±0.03^{ab}	0.11±0.04^{ab}	0.21±0.06^{ab}
Ionic High	0.08±0.02^{ab}	0.10±0.03^{ab}	0.11±0.01^{ab}	0.17±0.01^{ab}
Ionic Maximum	0.03±0.02^{b}	0.03±0.01^{b}	0.03±0.02^{b}	0.05±0.03^{b}

PCA was performed using Taylor transformed CSUPs extracted from CLPP measurements made at month 1, month 2 and month 3. PCA ordinations are shown in Figure 5.3- Figure 5.5 and constitute 58-72% of the variation in CSUPs. PCA analysis of treatments after one month indicated that most treatments could be grouped together with the exception of sulphidized-low (160 nm), uncoated-low, ionic-low, high and maximum treatments. After two months, PCA ordinations of CSUPs again showed most treatments closely grouped aside from weathered-low, ionic-high and maximum treatments. At three months, all treatments could be loosely grouped with the exception of the ionic-maximum treatment. Overall, PCA analysis indicated that the ionic-maximum treatment's utilization of carbon sources was the least consistent with the CSUPs of all other treatments. The ionic-high also had different CSUPs from other treatments at each time point; however, the CSUPs at three months could generally be explained by the variation between treatment replicates at this time rather than the contribution of the PCA ordinates. The general convergence of CSUPs over time in planted soil treatments was much greater than that of the unplanted soil (Figure 4.8) suggesting that the presence of plants stabilized the microbial community, reducing the effects of silver exposure. The diverse microbial community in rhizosphere soil compared to bulk soil is more resilient to both AgNMs and ionic silver; however, the ionic-maximum treatment's inhibitory effect was greater in magnitude than this stabilizing effect. In a study of maize plants in soil exposed to 100 mg AgNPs/kg, CSUPs of AgNP exposed rhizosphere soil also proved to be more similar to unexposed bulk and rhizosphere soils than AgNP exposed bulk soil indicating this plant-stabilizing effect (Sillen, et al. 2015).

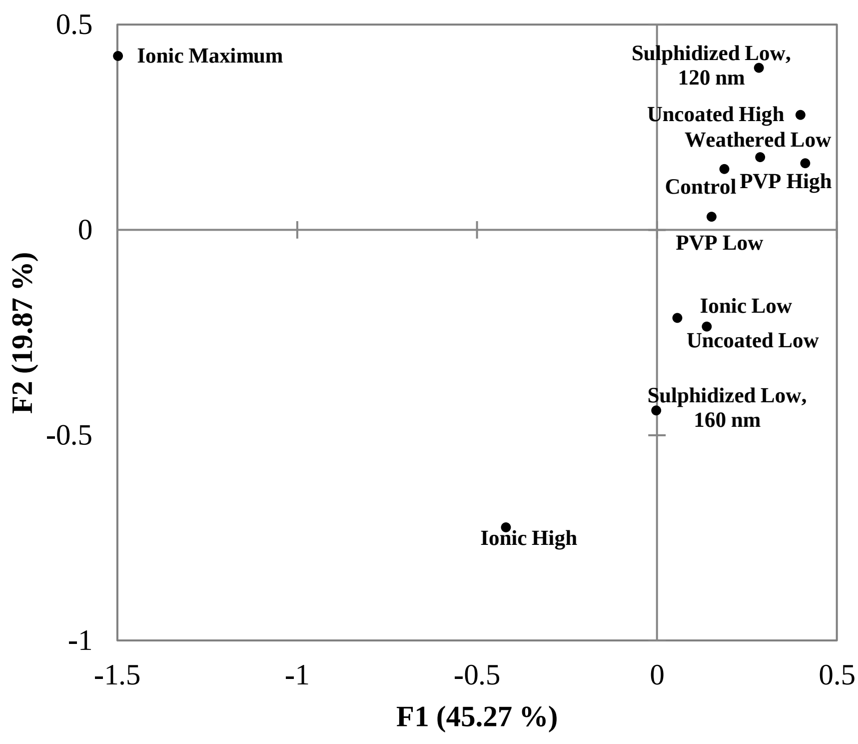

Figure 5.3: Principal component analysis ordinations for carbon source utilization patterns obtained from Biolog Ecoplates for average treatments at Month 1.

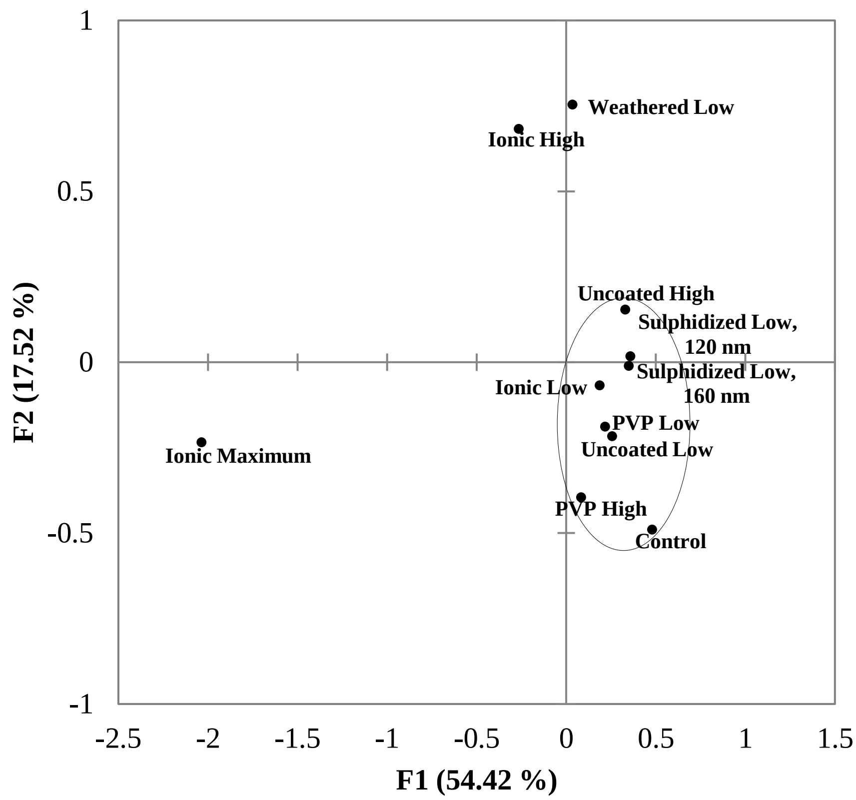

Figure 5.4: Principal component analysis ordinations for carbon source utilization patterns obtained from Biolog Ecoplates for average treatments at Month 2.

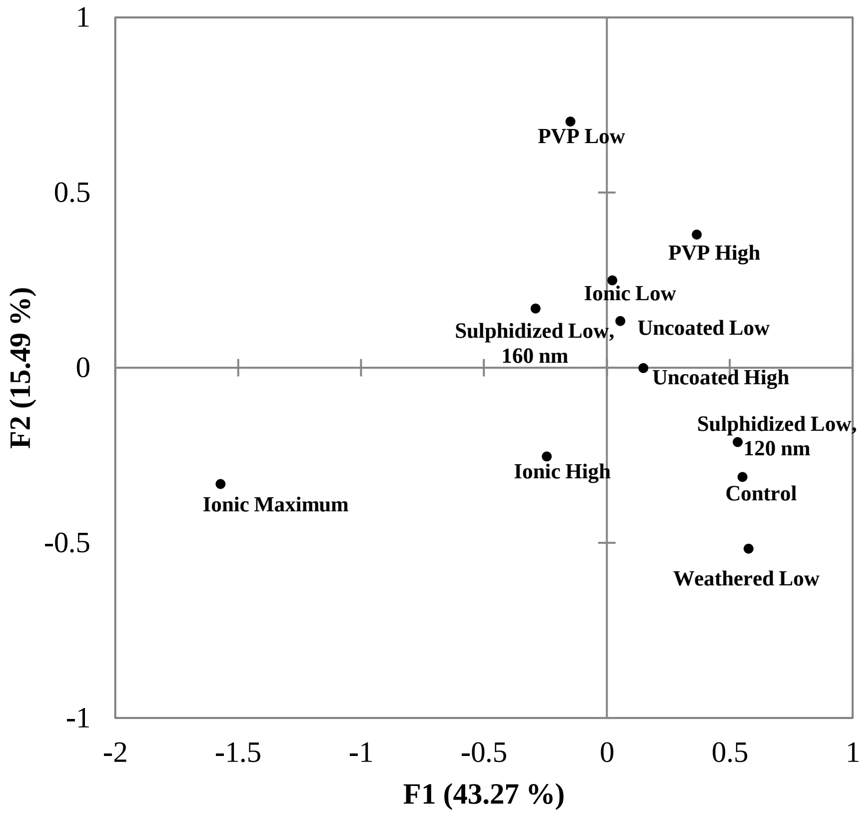

Figure 5.5: Principal component analysis ordinations for carbon source utilization patterns obtained from Biolog Ecoplates for average treatments at Month 3.

Figure 5.6 represents the relative community divergence of CSUPs of treatments from the initial soil microbial community over a three month period. This metric therefore quantitatively represents how much a treatment has diverged from the initial microbial community as well as other treatments in terms of its CSUP. At month 1, uncoated-high and ionic-maximum treatments show the greatest relative community divergence. Two months after treatment, ionic-high and ionic-maximum treatments diverged further from the initial microbial community. After three months, sulphidized-low (160 nm) and ionic-maximum treatments were the most divergent from the initial soil microbial community. At this time, the ionic-low treatment had the lowest relative community divergence indicating that it was most similar to the initial microbial community. Relative community divergence of the control indicates that the control diverges from the initial community with the greatest divergence being at the first timepoint, month 1, as the soil thermally acclimatizes from storage temperatures to greenhouse temperatures. Divergence of the control initially increases over the first month, before gradually decreasing up until the end-point of three months. In general, most treatments showed a similar trend wherein the relative divergence of the treatment was similar to the control at month 3 indicating a stabilization of the microbial community. Increases in the relative community divergence of sulphidized-low (160 nm) and ionic-maximum treatments over time are indicative of inhibition of the microbial community as demonstrated from the decreased AWCD of these treatments at month 3. In both cases this reduction in metabolic activity was caused by decreased utilization of substrates within the polymers, carboxylic and acetic acids and amino acid guilds. Comparison of the relative community divergence of soil planted with wheat to unplanted soil (Figure 4.9) demonstrates the stabilization effect of plants on the microbial community. As seen in Figure 4.9, the relative divergence of treatments at three months' time does not converge to the same extent as in soils with plants present. This stabilization effect means that soils with plants present will be more resistant to changes in the microbial community that could result from exposure to silver.

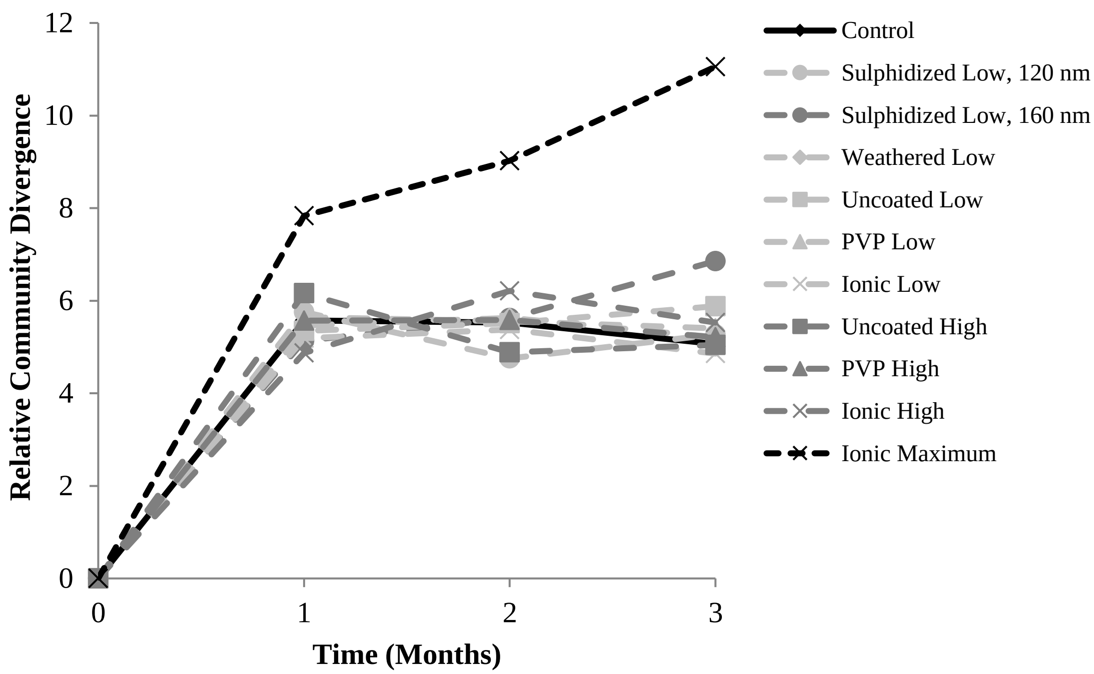

Figure 5.6: Relative community divergence of treatments over three months exposure

Figure 5.7 shows homogenous groups generated from clustering analysis using the squared Euclidean distance between CSUPs measured for samples after 1, 2, and 3 months. Hierarchal cluster analysis of CSUPs at month 1 indicated that all treatments linked together by a linkage distance of 12 with the exception of ionic-maximum treatments. Similarly, at month 2 the linkage distance was the same as month 1 with ionic-maximum replicates grouping last. After 3 months, clusters linked by a linkage distance of 12 with the exception of a sulphidized-low (160 nm) replicate and two ionic-maximum replicates. These results are consistent with those of the AWCD, PCA and relative community divergence where the ionic-maximum treatment showed the greatest inhibition to the microbial community. This inhibition therefore means that increasing concentrations of ionic silver reduce the functionality of the microbial community, altering the microbial community composition in such a way that it could have a reduced ability to degrade organic matter, hindering nutrient cycling and potentially decreasing agricultural yields. As demonstrated in the PCA and relative community divergence, wheat plant growth was able to stabilize the microbial community with the exception of the ionic-maximum treatment's toxicity effect which was greater in magnitude than the rhizosphere effect.

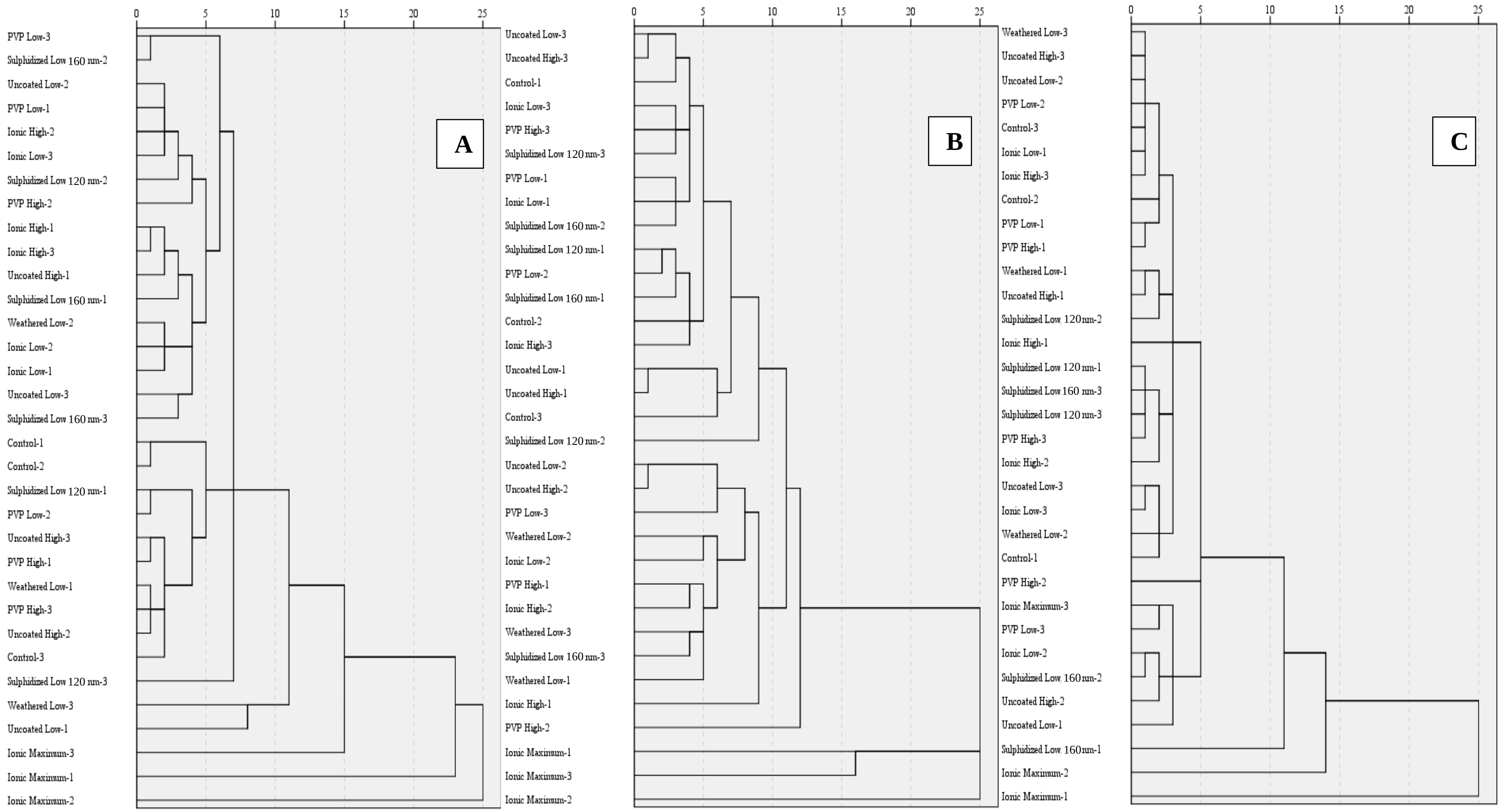

Figure 5.7: Hierarchal cluster analysis of carbon source utilization patterns based on squared Euclidian distances after Month 1 (A), Month 2 (B) and Month 3 (C) of exposure

Enzyme activity of soil enzymes including β-glucosidase, α-glucosidase, xylosidase, cellobiosidase, n-acetylglucosaminadase, phosphatase and leucine amino peptidase were assessed monthly to determine if treatments affected extracellular enzyme activity. Average enzyme activities for each treatment in each month of analysis are shown in Appendix D Table D.58. No significant differences between treatments with respect to enzyme assays or specific nutrient cycles could be determined due to the variation present in this measure. Asadishad et al. (2018) showed that enzyme activities of cellobiohydrolase, xylosidase, acid phosphatase, glucosidase and n-acetylglucosamidase in soils treated with AgNPs or Ag^+ for 30 days were not significantly inhibited by AgNP concentrations of 1-10 mg/kg while Ag^+ inhibited cellobiohydrolase, glucosidase and n-acetylglucosamidase at 100 mg/kg. Extracellular enzyme activity including hydrolytic enzymes have been shown to be significantly greater in the rhizosphere compared to bulk soil due to plant exudates (Koranda, et al. 2011; Kaiser, et al. 2010). Plant growth is therefore likely to reduce the magnitude of effects of ionic silver and AgNPs against extracellular enzymes when compared to bulk soil due to greater activity in the rhizosphere.

Heterotrophic plate counts, substrate-induced respiration values and extracted DNA quantities of treatments after three months are summarized in Table 5.7. Average CFU for the ionic-maximum treatment was significantly less than the control, indicating that the positive control treatment decreased the heterotrophic microbial activity of the soil. Heterotrophic plate counts did not differ significantly between any other treatments due to the variation between replicates. The ionic-maximum treatment also had a significantly decreased substrate-induced respiration rate compared to the control. The ionic-maximum treatment's heterotrophic plate count and respiration rate were both decreased by a factor of approximately two relative to the control. The EC_{50} for substrate-induced respiration of soil in the absence of plants has been reported as 20.7-25.6 mg Ag^+/kg after 28 days of incubation (Schlich and Hund-Rinke 2015). In this case, the EC_{50} occurred at a greater concentration after three months indicating establishment and increased resilience of the microbial community over time, likely as a result of the rhizosphere bacteria. The average quantity of DNA extracted from the ionic-maximum after three months was also significantly less than in the control indicating a reduction in the size of the microbial population.

Table 5.7: Heterotrophic plate counts, substrate-induced respiration measures and DNA quantities extracted from treatments after 3 months of exposure. Significantly different treatment groups from a post-hoc Tukey test are identified using different letters ($p<0.05$).

Treatment	Heterotrophic Plate Count (CFU/ g d.w. soil)	Substrate- Induced Respiration (µl CO_2/ g d.w. soil h)	Extracted DNA Quantity (ng/g d.w. soil)
Control	$2.38x10^7 \pm 4.27x10^6$	15.10 ± 1.52^{abc}	718±80.0
Sulphidized Low, 120 nm	$2.92x10^7 \pm 5.97x10^6$	17.89 ± 0.43^{bc}	768±63.0
Sulphidized Low, 160 nm	$1.58x10^7 \pm 9.10x10^6$	18.86 ± 0.28^{c}	735±153
Weathered Low	$3.05x10^7 \pm 1.80x10^7$	11.25 ± 3.89^{ab}	879±138
Uncoated Low	$2.39x10^7 \pm 1.07x10^7$	18.37 ± 0.95^{c}	768±122
PVP Low	$1.89x10^7 \pm 1.24x10^7$	17.42 ± 2.89^{bc}	871±113
Ionic Low	$1.86x10^7 \pm 1.35x10^7$	15.68 ± 1.38^{bc}	802±155
Uncoated High	$3.33x10^7 \pm 5.72x10^6$	15.47 ± 2.67^{bc}	795±87.0
PVP High	$2.10x10^7 \pm 1.02x10^7$	20.04 ± 2.41^{c}	735±79.0
Ionic High	$2.46x10^7 \pm 6.16x10^6$	15.85 ± 1.12^{bc}	649±222
Ionic Maximum	$8.66x10^6 \pm 5.82x10^6$	8.10 ± 0.28^{a}	454±96.0

Metagenomic sequencing of each soil treatment revealed that silver exposure to AgNMs or ionic silver had a significant effect on the diversity of bacterial species wherein the weathered-low and ionic-maximum treatments had greater diversity than the control ($p<0.05$). Treatment Shannon diversity indices, richness and evenness summarized in Table 5.8 indicate that increases in the diversity of weathered-low and ionic-maximum treatments resulted from increases in the number of species present and the evenness of species distribution in the microbial community. Since the ionic-maximum treatment resulted in increased diversity as well as decreased activity, this suggests that some species have been inhibited by this treatment and that other species have filled this niche or shifted within the microbial community, given that the evenness also increased. The weathered-low treatment which previously demonstrated the highest activity and richness from CLPP (Figure 5.1 and Figure 5.2) also had significantly increased diversity indicating that the microbial community shifted in favour of a more even community composition and that this had a positive effect on the metabolic activity.

Table 5.8: Shannon diversity index, bacterial species richness and species evenness after three months exposure. Different letters denote significantly different treatments ($p<0.05$).

Treatment	Shannon Diversity Index (H)	Species Richness (S)	Species Evenness (E)
Control	2.40±0.06^{a}	1020 ± 30.0	0.346±0.007
Sulphidized Low, 120 nm	2.41±0.00^{ab}	1110 ± 118	0.344±0.005
Sulphidized Low, 160 nm	2.43±0.01^{abc}	1080 ± 29.0	0.348±0.003
Weathered Low	2.50±0.05^{bc}	1070 ± 79.0	0.359±0.009
Uncoated Low	2.45±0.01^{abc}	1070 ± 72.0	0.352±0.003
PVP Low	2.44±0.02^{abc}	1050 ± 331	0.353±0.020
Ionic Low	2.45±0.04^{abc}	1040 ± 45.0	0.352±0.006
Uncoated High	2.45±0.04^{abc}	980 ± 216	0.357±0.007
PVP High	2.44±0.02^{abc}	1050 ± 49.0	0.351±0.003
Ionic High	2.46±0.02^{abc}	1080 ± 6.00	0.352±0.003
Ionic Maximum	2.50±0.03^{c}	1110 ± 110	0.357±0.002

Species which accounted for at least 0.5% relative abundance of the microbial population for any given treatment at month 3 are shown in Figure 5.8. Increasing ionic silver concentrations were determined to have resulted in divergence from other treatments in terms of the abundance of specific species present. Relative abundance of these prominent species in comparison to those present in the unplanted exposure (Figure 4.11) again indicates the stabilizing effect of the plant rhizosphere particularly in the ionic-high treatment where the shift demonstrated in the unplanted community composition has been mediated.

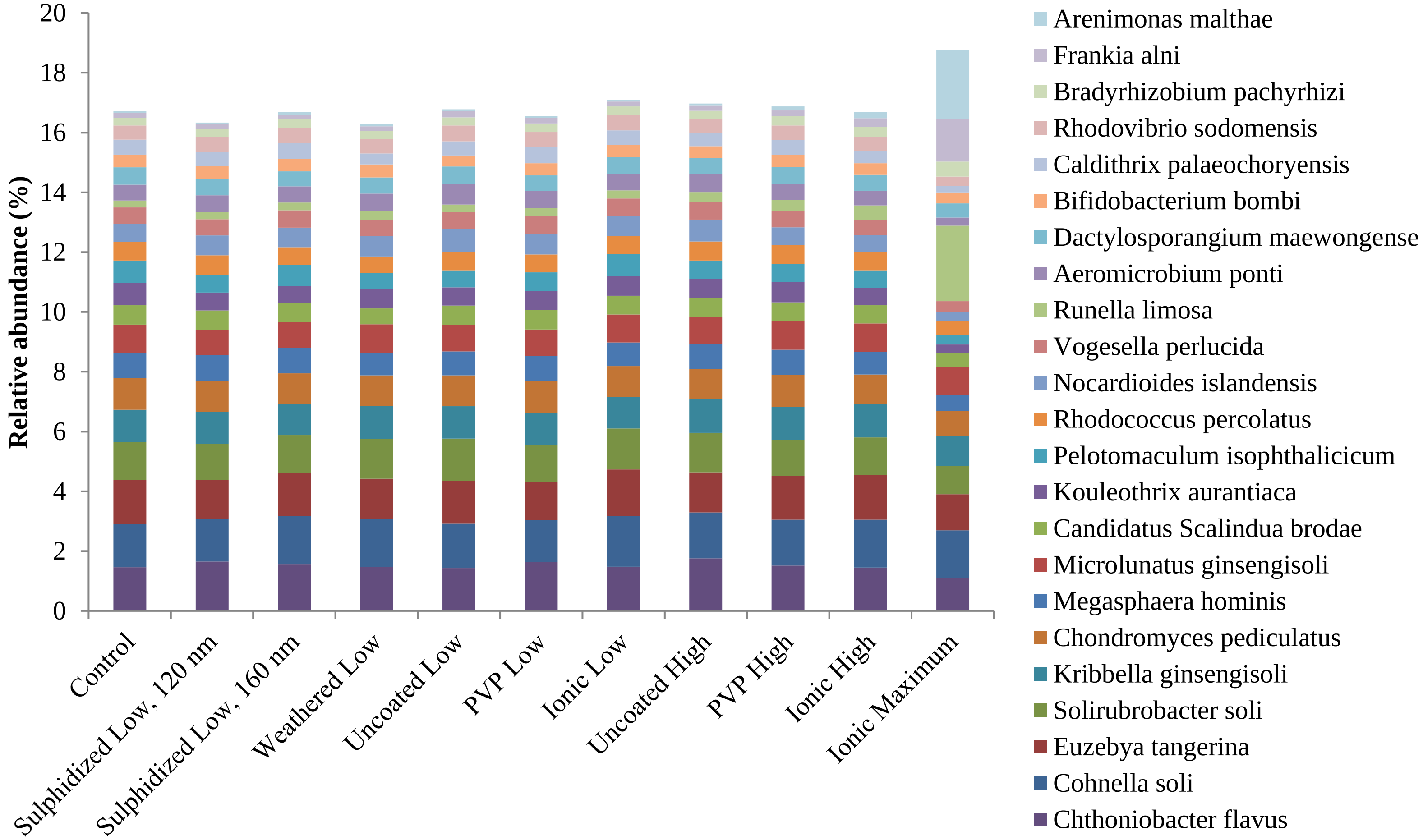

Figure 5.8: Bacterial species relative abundance contributing ≥ 0.5% of average treatment microbial populations

The ionic-maximum treatment had significantly increased populations of *R. limosa*, *F. alni*, *A. malthae* and *Bradyrhizobium pachyrhizi* relative to the control. After three months *R. limosa* accounted for 2.5% relative abundance compared to 0.2% in the control. *R. limosa* is a species of aerobic bacteria which has been isolated from activated sludge (Ryu, et al. 2006). *F. alni*, a species of nitrogen-fixing actinobacteria (Benson and Silvester 1993), had a relative abundance of 1.4% in the ionic-maximum compared to 0.2% in the control. *A. malthae,* a species of aerobic bacteria first isolated from an oil-contaminated site (Young, et al. 2007) also demonstrated a significant increase in abundance in ionic-maximum treatments, 2.3% relative to the control's 0.06%. *B. pachyrhizi*, a nitrogen-fixing member of *Rhizobiales*, had a relative abundance of 0.5% in ionic-maximum compared to 0.3% in the control. These findings indicate that AgNMs did not inhibit several species of *Rhizobiales* and increasing concentrations of $AgNO_3$ demonstrated a positive dose-response in growth of nitrogen-fixing bacteria *B. pachyrhizi* and *F. alni* as seen in Figure 5.9.

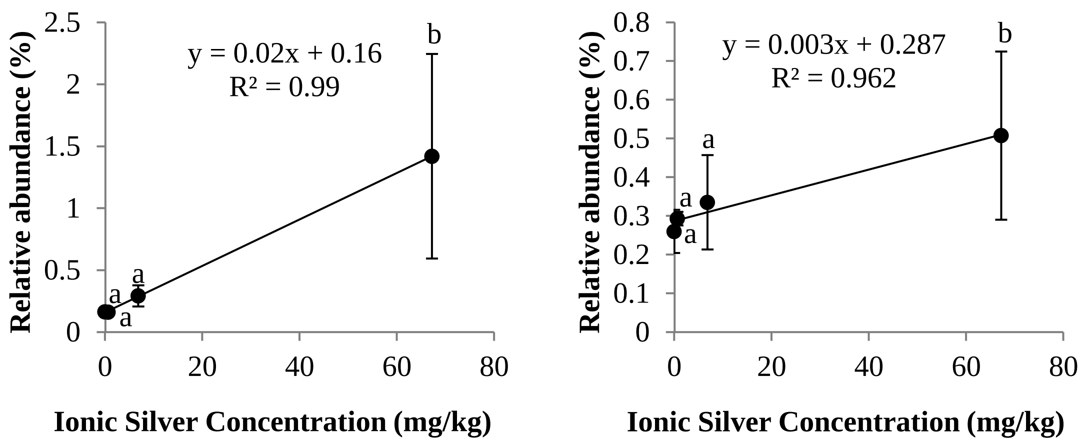

Figure 5.9: Relative abundance of nitrogen-fixers *Frankia alni* (left) and *Bradyzhizobhium pachyrhizi* (right) at month 3 of exposure to ionic silver.

Nitrogen-fixing bacteria, including *Rhizobiales*, have been previously shown to be inhibited by the presence of metallic nanoparticles such as AgNPs (Ge, et al. 2011; Ge, et al. 2012; Kumar, et al. 2011; Kumar, et al. 2014; Pallavi, et al. 2016). Pallavi et al. (2016) found that AgNP exposures in soil with wheat plants significantly reduced the population of nitrogen-fixers after 40 days at 75 mg/kg while treatments with 50 mg/kg were unaffected. PVP AgNPs, SAgNPs and Ag^+ at concentrations of 1, 10 and 100 mg/kg soil were also found to have not significantly affected symbiosis between nitrogen-fixing bacteria and *Medicago truncala* (barrelclover) (Judy, et al. 2016). The effect of AgNPs on nitrogen-fixing bacteria is therefore seemingly dependent upon exposure conditions in terms of media, plant species and concentrations. Aside from effects on nitrogen-fixing bacteria, PVP AgNPs, SAgNPs and Ag^+ (each at 100 mg/kg) were previously found to decrease populations of gram-negative bacteria, gram positive bacteria and actinomycetes in soil planted with tomatoes (Judy, et al. 2015), however, these effects were not demonstrated by the positive control in this current study.

In this study, three months of exposure to ionic-maximum treatments resulted in a 38-fold increase of phytopathogen, *X. oryzae,* relative to the control ($p<0.05$). *X. oryzae* causes bacterial leaf blight which most commonly affects rice but can also affect other hosts including *Triticeae* (grasses such as wheat) (NAPPFAST NCSU APHIS Plant Pest Forecasting System 2008). This pathogen begins to affect growth during the tillering stage, when plants begin to grow tiller shoots after the initial shoot has formed, water-soaked spots at the tips and margins of leaves become visible before expanding along the veins and causing chlorosis followed by necrosis (NAPPFAST NCSU APHIS Plant Pest Forecasting System 2008). In rice plants, increased nitrogen fertilization rates as well as increasing moisture and humidity increased the severity of bacterial leaf blight and lead to decreased crop yields (Reddy, et al. 1979). The increasing abundance of this phytopathogen with increased $AgNO_3$ concentrations could therefore result from the increased nitrate concentrations provided by this treatment, however, this species of bacteria has still proven tolerant of ionic silver.

PCA ordinations of bacterial species abundance, Figure 5.10, indicate that the ionic-maximum was the only treatment that had significantly different species relative abundance from other treatments. This can also be seen from Figure 5.8 wherein a shift in the microbial community composition of the ionic-maximum treatment has occurred with increases in abundance of species including *R. limosa*, *F. alni*, *A. malthae*, and *B. pachyrhizi*. Overall, comparison to the unplanted exposure PCA (Figure 4.13) indicates that the microbial community composition of treatments with plants were more convergent with the control, indicating less susceptibility to the effects of AgNMs and moderate ionic silver concentrations.

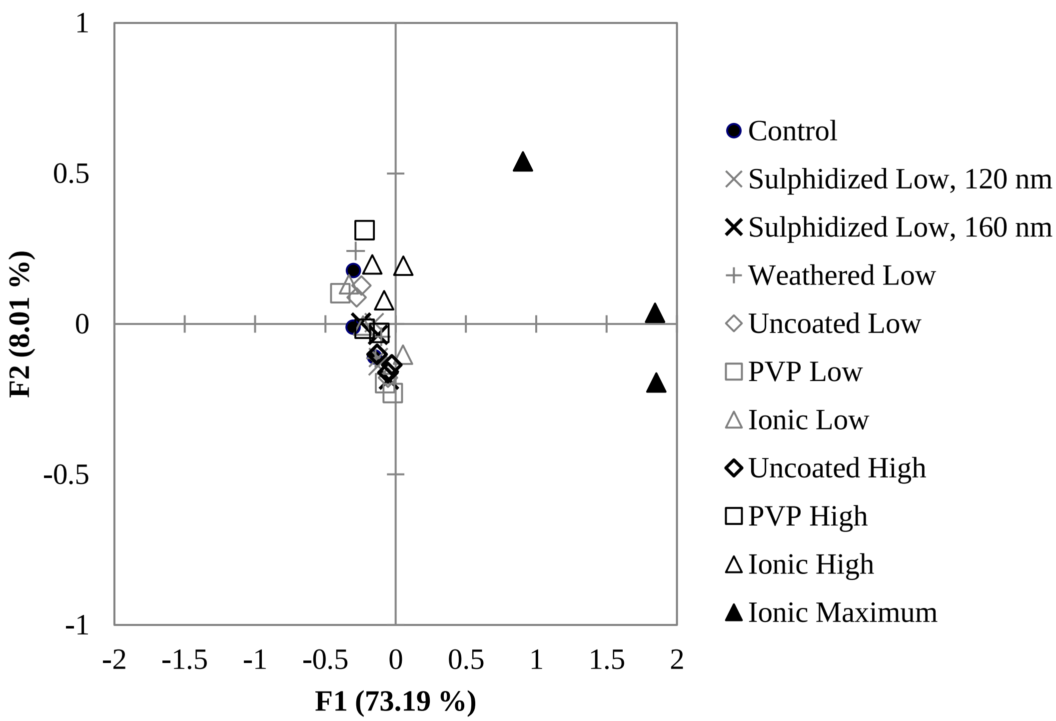

Figure 5.10: Principal component analysis ordinations for normalized species relative abundance determined from sequencing of 16S rRNA after Month 3.

5.3.4 Effect of silver nanomaterials on wheat plants

Due to the potential for AgNMs to negatively impact plants, plant health was assessed using biomass measures, concentrations of silver in roots, shoots and seeds (when available) at 4 week intervals. In terms of crop plants such as wheat where only the grain is harvested, translocation of silver into aboveground biomass including seeds could result in consumption throughout the food chain and potential bioaccumulation. Additionally, decreases in yield could negatively impact production of this globally relevant crop. Physical soil properties were well-suited for wheat production since pH was generally within range of the optimal pH for wheat growth 6.0-7.0 (Vitosh 1998) and organic matter content was moderately high.

The average proportion of seeds germinated within the first week of planting, root biomass at month 3 and shoot biomass of wheat plants over three months are shown in Table 5.9 with statistically different treatment groups being denoted by different letters. Proportions of seed germination as well as shoot biomass at each measurement were consistent ($p>0.05$). Due to discrepancies between removal of soil from plant roots, belowground biomass from months 1 and 2 have been excluded from analysis. No treatment root biomasses were significantly different from the control treatment; however, the uncoated-high treatment had the lowest root biomass and was significantly less than the PVP-high treatment. PVP coating therefore negated the inhibitory effect on root growth seen in uncoated AgNPs, which indicated an effect on root growth potentially due to oxidative dissolution of nanoparticles on the root surface. Observations of plant roots when sampling indicated that the ionic-maximum treatment had less rootlets than other treatments as seen in Figure 5.11. Although decreases in rootlets/root hairs did not impact overall root biomass, due to their negligible mass, these roots provide significant amounts of root surface area which aid in acquisition of soil water and nutrients (Wasson, et al. 2012). In a previous study of wheat plants grown in soil for 40 days with AgNPs (50 and 75 mg/kg) root and shoot biomass were also unaffected (Pallavi, et al. 2016). In wetland plants, *Carex spp.* and *Eupatorium fistulosum* in soil exposed to PVP AgNPs and $AgNO_3$ for seven weeks were unaffected when treated with a 40 mg Ag/L while *P. americana* aboveground biomass was significantly reduced by PVP AgNP (Yin, et al. 2012). Low doses of AgNPs and Ag^+, 0.14 mg/kg soil and 0.56 mg/kg soil respectively, did not affect biomass of several species *Carex Lurida*, *Juncus effuses*, *Lobelia cardinalis*, *Pancium virgalum* while both treatments significantly reduced aboveground biomass in *M. vimineum* and increased belowground biomass (Colman, et al. 2013). These findings therefore indicate that effects of AgNPs on plant biomass are more dependent on plant species rather than concentration dependent effects alone. Despite the reduction in the uncoated-high treatment's root biomass demonstrated here, neither root biomass nor shoot biomass measured over the course of three months significantly differed from the control. Therefore, plant growth of wheat for three months was not inhibited by AgNMs or ionic silver at concentrations up to 4 mg/kg and 67 mg/kg, respectively. Reduction in rootlets such as in the ionic-maximum treatment could however negatively affect the distribution of organic matter in the soil resulting in detrimental effects to future generations of plant growth. Similarly to the study conducted by Yin et al. (2012), germination was also unaffected by AgNMs or $AgNO_3$.

Table 5.9: Proportion of seeds germinated, root biomass after 3 months and shoot biomass of treatments over three months. Significantly different treatment groups from a post-hoc Tukey test are identified using different letters (p<0.05).

Treatment	Proportion of seeds germinated within 1 week	Root biomass at Month 3 (g d.w.)	Shoot Biomass (g d.w.)		
			Month 1	Month 2	Month 3
Control	0.62±0.14	$0.87±0.15^{ab}$	0.51±0.02	0.96±0.15	1.24±0.15
Sulphidized Low, 120 nm	0.65±0.10	$0.88±0.17^{ab}$	0.53±0.04	1.07±0.06	1.08±0.19
Sulphidized Low, 160 nm	0.63±0.12	$1.10±0.21^{ab}$	0.54±0.07	1.26±0.12	1.29±0.12
Weathered Low	0.67±0.12	$0.80±0.09^{ab}$	0.58±0.03	1.19±0.12	1.14±0.15
Uncoated Low	0.63±0.11	$0.85±0.07^{ab}$	0.53±0.07	1.16±0.04	1.14±0.15
PVP Low	0.60±0.12	$1.14±0.35^{ab}$	0.58±0.08	1.11±0.04	1.23±0.05
Ionic Low	0.67±0.12	$1.02±0.27^{ab}$	0.53±0.03	1.17±0.06	1.06±0.07
Uncoated High	0.63±0.19	$0.53±0.04^{a}$	0.49±0.04	1.01±0.10	1.10±0.20
PVP High	0.54±0.17	$1.23±0.24^{b}$	0.56±0.06	0.97±0.10	1.07±0.22
Ionic High	0.60±0.13	$1.16±0.14^{ab}$	0.52±0.06	1.12±0.09	1.15±0.11
Ionic Maximum	0.54±0.14	$0.82±0.15^{ab}$	0.57±0.04	0.96±0.13	1.09±0.03

Figure 5.11: Root structures of ionic low (left) and ionic maximum (right) treatments after one month of growth.

All treatments contained concentrations of silver in wheat shoots at each interval measured (Figure 5.12). After one month, shoot Ag concentrations were significantly higher than the control in the sulphidized-low (160 nm), ionic-low and ionic-maximum treatments, indicating the highest quantities of translocation. At month two, the ionic-maximum also had significantly higher shoot silver concentrations than in the control. At month 3, no treatments had significantly different silver concentrations from the control ($p>0.05$). Increased translocation of ionic silver treatments in comparison to AgNMs could be expected due to some silver quantities remaining mobile in pore water. Ag treatments did not result in significant bioaccumulation of Ag in shoots relative to the control in most treatments and was insignificant after three months of exposure. The greater uptake of ionic silver as well as the increased shoot concentrations measured in month 2 suggest that the uptake of silver was related to plant growth and water uptake, the greatest rate of plant growth occurring from month 1 to month 2. Shoot silver concentrations generally increased with increasing soil silver concentrations, however, variation in shoot Ag concentrations, including in the control due to the initial silver concentration in the control soil, meant that treatment shoot concentrations were often insignificant relative to the control. This initial control soil concentration was however consistent with uncontaminated soil concentrations found to range between 0.06-0.4 mg Ag/kg (United States Environmental Protection Agency 1981; Rasmussen, et al. 2001). Colman et al. (2013) also found that Ag bioaccumulation in aboveground biomass was greater in ionic treatments than AgNP treatments and that a high variability in Ag concentrations often obscured statistical differences in AgNP treatments. Dimkpa, et al. (2013) found significant increases in wheat shoot Ag concentrations occurred after 14 days growth in treatments of 2.5 mg Ag/kg AgNPs or $AgNO_3$, however, this was conducted in a sand matrix which has a lower sorption capacity than soils with higher organic matter and clay content (Cornelis, et al. 2010).

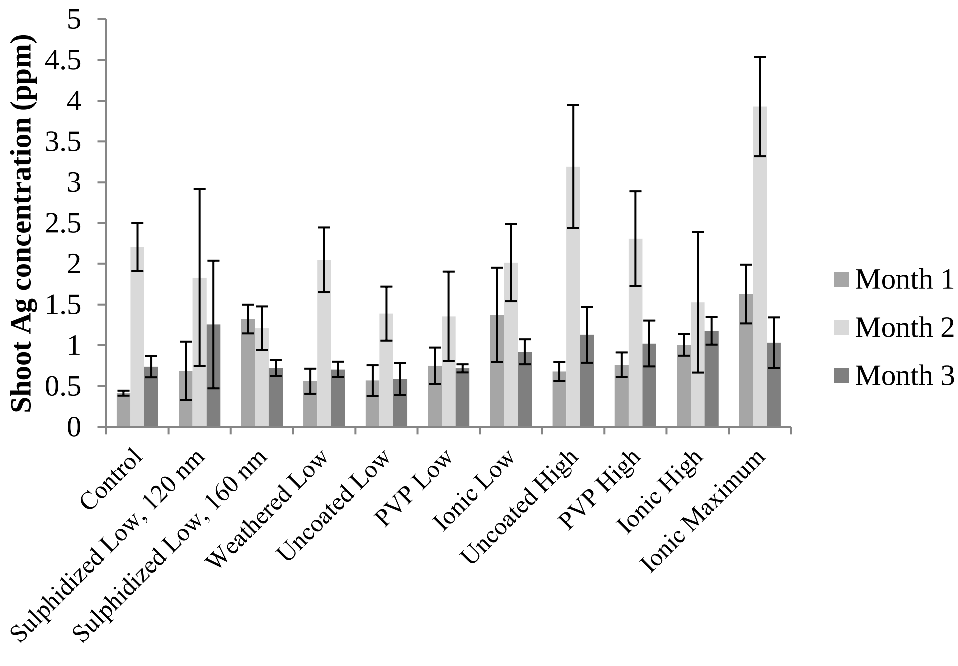

Figure 5.12: Silver concentrations in wheat shoots over three months of growth

Root silver concentrations (Figure 5.13) increased with soil silver concentrations, potentially due to large amounts of sorption to root surfaces as observed by Yin et al. (2011). After one month, root Ag concentrations were significantly higher than the control in uncoated-high, PVP-high, ionic-high and ionic-maximum treatments. After two months, ionic-high, PVP-high and ionic-maximum treatments had significantly higher root Ag concentrations while only the ionic-high and ionic-maximum roots had significantly higher concentrations than the control after three months. Since soil was only fully removed from roots at three months, month 1 and month 2 concentrations could be skewed due to sorption. After three months of exposure, root concentrations were a factor of 1-23 times greater than in aboveground biomass at low and high concentration treatments. The ionic-maximum treatment's root concentration was a factor of 260 times greater than in the shoots. Despite the significantly increased root concentrations in ionic-maximum treatments, shoot concentrations were not significantly increased after three months of growth, indicating that bioavailable forms of silver were generally immobilized in the plant roots. Agricultural crops and grasses have been shown to preferentially accumulate silver in the roots with less translocation to aboveground biomass (Klein 1978; Hirsch 1998; Kramer, et al. 1994; Kramer, et al. 1996). In agricultural plants, silver concentrations in roots have been found to be up to 50 times greater than aboveground biomass concentrations when exposed to silver sulfide (106 mg Ag/kg soil) (Hirsch 1998). Limited translocation of silver species to aboveground biomass has been suggested to result from deposition of silver phosphates, chlorides and sulphides in plant roots (Ward, et al. 1979; Kabata-Pendias and Pendias 2001). This limited translocation means a reduced risk for bioaccumulation in crops harvested from aboveground biomass.

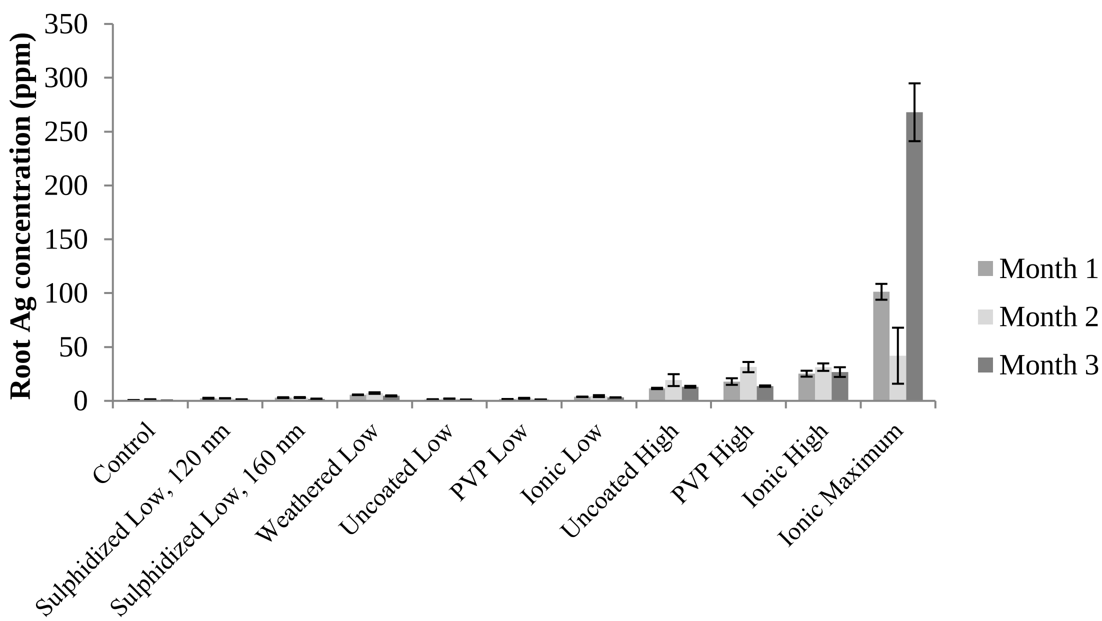

Figure 5.13: Silver concentrations in wheat roots over three months of growth

Seeds produced from the wheat plants were bulked on a per treatment basis at the conclusion of the experiment to assess translocation to seed kernels and to ensure that the mass of analyte would be above the ICP-MS detection limit. Due to differences in plant growth including heading, booting and fertilization rates, it cannot be concluded as to what extent differences in yield could be directly related to treatments. Average number of flowering plants and average number of seeds produced at month 3 are shown in Table 5.10. No statistically significant differences between treatment flowering was found ($p>0.05$). Sulphidized and weathered nanomaterials were among treatments with the highest yields, potentially indicating increased production rates. It is also notable that the ionic-maximum treatment yielded half the number of seeds produced by the control treatment while all other treatments produced more seeds than the control. This could indicate that the ionic-maximum treatment had an impact on the wheat plants ability to reproduce which could substantially impact grain production. This decreased yield could however also be the result of the phytopathogen, *X. oryzae*, which has been previously shown to decrease rice yields with increasing nitrate concentrations (Reddy, et al. 1979). A previous study of *Borago officinalis L.* (starflower) also found that increasing concentration of silver nitrate reduced seed yield and AgNMs increased seed yield such that the lowest yield was produced by control plants (Seif Sahandi, et al. 2011).

Table 5.10: Average number of flowering plants and seeds produced for wheat plants exposed to treatments for three months.

Treatment	Average number of flowering plants	Average number of seeds produced
Control	5.33 ± 1.25	2.00
Sulphidized Low, 120 nm	3.66 ± 1.25	4.33
Sulphidized Low, 160 nm	4.33 ± 1.89	5.67
Weathered Low	6.00 ± 2.16	7.00
Uncoated Low	6.00 ± 0.82	6.67
PVP Low	3.67 ± 0.94	3.67
Ionic Low	5.00 ± 0.82	4.00
Uncoated High	4.67 ± 1.25	2.67
PVP High	4.00 ± 0.82	4.33
Ionic High	4.00 ± 0.00	3.00
Ionic Maximum	2.67 ± 1.25	1.00

Seed silver concentrations (Figure 5.14) indicated that control treatments had approximately 100 µg Ag/kg while the sulphidized-low (120 nm) treatment had the lowest concentration at 50 µg Ag/kg. Seed concentrations generally increased with increasing soil silver concentrations again indicating that plant uptake and translocation was concentration dependent. Since differences in shoot concentrations were often insignificant over time due the variance between replicates, it is reasonable to assume that differences in seed concentrations could also be quite variable; however, this could not be determined due to the lack of replicates. Cereal and grain products from various uncontaminated sites in the United States between 1979-1980 were found to contain 8-140 µg Ag/kg (Cunningham and Stoube 1987). This therefore places the concentrations found in uncoated-high and ionic-high and maximum treatments above the range of silver concentrations found in cereal products from uncontaminated sites and could be hazardous for consumption and bioaccumulation or prove detrimental to production of the next generation of plants.

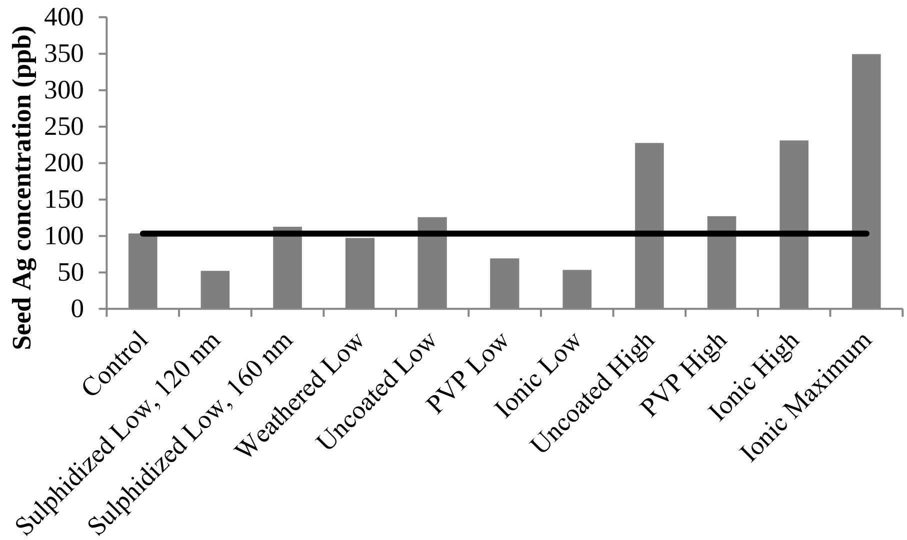

Figure 5.14: Concentration of silver in harvested seeds pooled from treatments after three months of exposure. The black line represents the concentration of silver in the control seeds.

5.3.5 Primary Findings

Significant results of treatments relative to the control for metrics investigated in this exposure are summarized in Table 5.11- Table 5.14. The ionic-maximum treatment consistently demonstrated significant decreases in AWCD and richness as a result of decreases in utilization of polymers, carboxylic and acetic acids and amino acids across all months. These decreases in metabolic activity were coupled with decreased heterotrophic bacteria activity measured through heterotrophic plate counts and substrate-induced respiration. Decreased quantities of DNA extracted from the ionic-maximum and its increased diversity suggest that some species of bacteria were inhibited by this treatment and that the microbial community composition shifted (towards a slightly greater species richness) to fill this niche. Increased activity of the weathered-low treatment (Figure 5.1) and its significant increase in microbial community diversity likely resulted from this treatment's moisture content providing a suitable environment for a more diverse community of bacteria since this treatment also had the highest quantity of extracted DNA.

Uptake of silver into plant biomass was concentration-dependent with the effect decreasing as root age and total biomass increased. Ionic treatments demonstrated the greatest uptake and translocation at the maximum treatment concentration. Translocation of silver from roots to shoots was seemingly dependent on the mobility of the silver species (the greatest translocation being in ionic silver treatments) and was only significant during the first two months of growth indicating that uptake was likely related to plant water uptake. Concentrations of silver translocated to seeds were also soil concentration-dependent.

Table 5.11: Significant differences between the treatment and control according to measured physical and biological parameters of soil. Treatments significantly higher than the control (↑) and significantly lower than the control (↓) ($p \leq 0.05$).

Treatment	pH	Conductivity	Moisture Content	Organic Matter	AWCD Month 1	AWCD Month 2	AWCD Month 3	Richness Month 1	Richness Month 2	Richness Month 3
Weathered Low	–	–	↑	–	–	–	–	–	–	–
Sulphidized Low, 120 nm	–	–	–	–	–	–	–	–	–	–
Sulphidized Low, 160 nm	–	–	–	–	–	–	–	–	–	–
Uncoated Low	–	–	–	–	–	–	–	–	–	–
PVP Low	–	–	–	–	–	–	–	–	–	–
Ionic Low	–	–	–	–	–	–	–	–	–	–
Uncoated High	–	–	–	–	–	–	–	–	–	–
PVP High	–	–	–	–	–	–	–	–	–	–
Ionic High	–	–	–	–	–	–	–	–	–	–
Ionic Maximum	–	–	–	–	↓	↓	↓	↓	↓	↓

Table 5.12: Significant differences between the treatment and control according to guild AWCD contribution. Treatments significantly higher than the control (↑) and significantly lower than the control (↓) ($p \leq 0.05$).

Month	Treatment	Carbohydrates	Polymers	Carboxylic and acetic acids	Amino acids	Amines/ amides	Root exudates
Month 1	Weathered Low	—	—	—	—	—	—
	Sulphidized Low, 120 nm	—	—	—	—	—	—
	Sulphidized Low, 160 nm	—	—	—	—	—	—
	Uncoated Low	—	—	—	—	—	—
	PVP Low	—	—	—	—	—	—
	Ionic Low	—	—	—	—	—	—
	Uncoated High	—	—	—	—	—	—
	PVP High	—	—	—	—	—	—
	Ionic High	—	—	—	—	—	—
	Ionic Maximum	—	↓	↓	↓	—	—
Month 2	Weathered Low	—	—	—	—	—	—
	Sulphidized Low, 120 nm	—	—	—	—	—	—
	Sulphidized Low, 160 nm	—	—	—	—	—	—
	Uncoated Low	—	—	—	—	—	—
	PVP Low	—	—	—	—	—	—
	Ionic Low	—	—	—	—	—	—
	Uncoated High	—	—	—	—	—	—
	PVP High	—	—	—	—	—	—
	Ionic High	—	—	—	—	—	—
	Ionic Maximum	—	↓	↓	↓	—	—
Month 3	Weathered Low	—	—	—	—	—	—
	Sulphidized Low, 120 nm	—	—	—	—	—	—
	Sulphidized Low, 160 nm	—	—	—	—	—	—
	Uncoated Low	—	—	—	—	—	—
	PVP Low	—	—	—	—	—	—
	Ionic Low	—	—	—	—	—	—
	Uncoated High	—	—	—	—	—	—
	PVP High	—	—	—	—	—	—
	Ionic High	—	—	—	—	—	—
	Ionic Maximum	—	↓	↓	↓	↓	—

Table 5.13: Significant differences between the treatment and control according to microbial population size, activity and diversity in soil. Treatments significantly higher than the control (↑) and significantly lower than the control (↓) ($p \leq 0.05$).

Treatment	CFU	Substrate-Induced Respiration	DNA Extracted	Shannon Diversity Index	Species Richness	Evenness
Weathered Low	—	—	—	↑	—	—
Sulphidized Low, 120 nm	—	—	—	—	—	—
Sulphidized Low, 160 nm	—	—	—	—	—	—
Uncoated Low	—	—	—	—	—	—
PVP Low	—	—	—	—	—	—
Ionic Low	—	—	—	—	—	—
Uncoated High	—	—	—	—	—	—
PVP High	—	—	—	—	—	—
Ionic High	—	—	—	—	—	—
Ionic Maximum	↓	↓	↓	↑	—	—

Table 5.14: Significant differences between the treatment and control according to root and shoot silver concentrations. Treatments significantly higher than the control (↑) and significantly lower than the control (↓) ($p \leq 0.05$).

Month	Treatment	Root Silver Concentration	Shoot Silver Concentration
Month 1	Weathered Low	—	—
	Sulphidized Low, 120 nm	—	—
	Sulphidized Low, 160 nm	—	↑
	Uncoated Low	—	—
	PVP Low	—	—
	Ionic Low	—	↑
	Uncoated High	↑	—
	PVP High	↑	—
	Ionic High	↑	—
	Ionic Maximum	↑	↑
Month 2	Weathered Low	—	—
	Sulphidized Low, 120 nm	—	—
	Sulphidized Low, 160 nm	—	—
	Uncoated Low	—	—
	PVP Low	—	—
	Ionic Low	—	—
	Uncoated High	—	—
	PVP High	↑	—
	Ionic High	↑	—
	Ionic Maximum	↑	↑
Month 3	Weathered Low	—	—
	Sulphidized Low, 120 nm	—	—
	Sulphidized Low, 160 nm	—	—
	Uncoated Low	—	—
	PVP Low	—	—
	Ionic Low	—	—
	Uncoated High	—	—
	PVP High	—	—
	Ionic High	↑	—
	Ionic Maximum	↑	—

6 Chapter 6: Outcomes and Recommendations

6.1 Research Objectives

The main objective of this thesis was to examine the effects of silver nanomaterials in agricultural soil-plant systems, namely:

1. Determine the toxicity of AgNMs originating from commercial textiles on soil microbial communities.
2. Determine the fate of AgNMs including those originating from commercial textiles in agricultural soils.
3. Quantify the effect and uptake of pristine and aged AgNMs in an agricultural crop (*Triticum spp.*).

6.1.1 Objective 1: Determine the toxicity of AgNMs originating from commercial textiles on soil microbial communities.

Exposure to silver nanomaterials resulted in no significant changes to physical characteristics of the soil including soil particle size, moisture content, water holding capacity and pH relative to the control. Thus, any effects on the microbial community were not linked to changes in the soil physical properties.

Negative effects of treatments on soil microbial activity were most often caused by a reduced ability to utilize root exudates and their primary constituents, amino acids. Low concentrations of SAgNPs (0.5 mg/kg soil) demonstrated decreased activity of amino acids after three months, indicating that they could be becoming biologically available over time. Visible changes to the morphology of sulphidized nanoparticles during STEM analysis prior to and after soil exposure are potentially indicative of amorphous sulphur bonds which may have differing behaviour over time compared to crystalline silver sulfide. Relative community divergence and hierarchal cluster analysis of CSUPs from CLPP both indicated that treatments had fairly low divergence from control treatments at all time points. Enzymatic assays, heterotrophic plate counts and substrate-induced respiration assessed at multiple endpoints indicated no statistically significant differences between treatments, meaning that silver nanomaterial treatments did not impact the heterotrophic microbial activity or soil enzymatic activity at the concentrations examined. Released nanomaterials from a consumer textile in soap had an initially positive effect on the microbial community that was generally opposite in effect to the toxicity of ionic silver. This suggests that the nanomaterials released from washing of socks in tap water and soap solution are not toxic to soil microbial communities and that this form of environmental release did not pose a risk through mechanisms of ionic silver release. Metagenomic sequencing also indicated no significant impact of treatment on the overall bacterial diversity, however, species *R. limosa*, nitrogen-fixer *F. alni* and *A. malthae* demonstrated concentration dependent increases in abundance when exposed to ionic silver. The maximum concentration of ionic silver (76 mg/kg) was also determined to significantly increase the abundance of a phytopathogen, *X. oryzae*. These findings therefore indicate that under the experimental conditions examined, environmentally relevant concentrations (70-860 years of biosolid loadings) and nanomaterial forms did not cause alarming toxicity to the soil microbial community or alter the microbial community composition. Despite the lack of toxicity found under these conditions, changes to the ecosystem such as interactions with vegetation as well as increasing exposure times could influence the stability and therefore toxicity of nanomaterials.

6.1.2 Objective 2: Determine the fate of AgNMs including those originating from commercial textiles in agricultural soils.

Potted soil with submerged nanomaterial samples adhered to carbon conductive tabs were examined to observe potential transformations or changes in morphology using STEM and EDS. Sulphidized, uncoated and PVP AgNPs were examined without soil, with soil and after three months of soil exposure. This was conducted in addition to monthly total silver analysis of upper and lower regions of potted soils exposed to silver treatments for three months to assess nanomaterial mobility.

Analysis of silver concentrations in the upper and lower regions of soil over the course of three months indicated that the AgNMs and ionic silver sorbed to the surfaces of soil and organic matter, remaining relatively immobile under the conditions examined as no statistically significant differences existed between regions at any time ($p>0.05$). EDS analysis of sulphidized, uncoated and PVP AgNPs indicated that no further sulphidation of particles or disassociation of sulphur from the AgNPs occurred over this same duration. Visible changes to the morphology of sulphidized nanoparticles during STEM analysis prior to and after soil exposure are potentially indicative of amorphous sulphur bonds which have been suggested to have different behaviour over time.

6.1.3 Objective 3: Quantify the effect and uptake of pristine and aged AgNMs in an agricultural crop (*Triticum spp.*).

Pristine and aged silver nanomaterials were shown to have insignificant effects on wheat health and soil microorganisms when exposed to environmentally relevant concentrations. This finding shows that agricultural soil exposed to relevant transformations of silver nanomaterials at concentrations representing 100 years equivalent of biosolid applications had little measurable effect on the agricultural ecosystem, wheat productivity and bioaccumulation in wheat crop under the examined conditions. While increasing soil concentrations of pristine nanomaterials greater than 4 mg/kg resulted in increasing concentrations of silver in harvested wheat grains, these loading concentrations are representative of multiple biosolid applications which would likely be immobilized by soil interactions over time, thus reducing their risk potential.

Only the ionic control at concentrations of 67 mg Ag/kg demonstrated measurable toxic effects. The ionic-maximum demonstrated reduced metabolic activity over three months' exposure from CLPP. This reduction in activity was correlated to a reduced ability to utilize carbon sources including polymers, carboxylic and acetic acids, and amino acids across the entire duration of the study, indicating decreased functional diversity. The ionic-maximum resulted in significant decreases to both heterotrophic plate counts and substrate-induced respiration when compared to the control. Despite this decrease in activity, ionic-maximum treatments had the highest Shannon diversity index of bacterial species from metagenomic sequencing. This shift in community composition of the ionic-maximum treatment resulted from increased richness and evenness suggesting niche filling. Species abundance of *R. limosa, A. malthae*, nitrogen-fixers *B. pachyrhizi* and *F. alni* as well as phytopathogen *X. oryzae* were also significantly more abundant than in the control treatment. In addition to this effect on microbial community composition, the ionic-maximum decreased yield, producing half as many seed kernels as the control and also decreased rootlet quantities, potentially as a result of this phytopathogen's effects.

Although AgNMs demonstrated no toxic effects in any of the measurements made, some differences did exist between treatments indicating differences in the effects of these nanomaterials on the soil microbial community and plant growth. Aged nanomaterials, weathered-low and sulphidized-low (120 nm) commonly demonstrated greater metabolic activity from CLPP than their pristine counterparts. Sulphidized-low (160 nm) treatments also demonstrated an apparent decrease in activity relative to the sulphidized-low (120 nm) treatment from CLPP and heterotrophic plate counts. Aged nanomaterials, weathered-low and sulphidized-low (160 nm) resulted in greater numbers of flowering plants than the control and the highest yields among treatments including the control. Bioaccumulation of silver in seeds within aged nanomaterial treatments were at most 9 µg Ag/kg greater than the control. These findings therefore all indicate that both pristine and aged nanomaterials entering agricultural soils at environmentally relevant concentrations did not negatively affect the microbial population or plant growth and do not pose a risk for significant bioaccumulation.

6.2 Scientific Contribution

This thesis contributes to the first toxicological assessment of weathered nanomaterials from commercial products in soil. This analysis was conducted alongside pristine and sulphidized nanomaterials which have been previously studied to provide a baseline for their behaviour in soil and plants. Significant knowledge was gained with respect to the effects of nanomaterials on soil microbial community composition, the role of plants in the mediating of these effects and the risk potential for translocation of AgNMs into biomass and cereal products. This research has shown that despite a lack of inhibitory effects on plant growth, yield, heterotrophic microbial activity and community composition, increasing silver nanomaterial concentrations can still result in translocation within plants including seed kernels. Additionally, silver nitrate treatment which is consistent with combined silver exposure and nitrogen fertilization was found to increase phytopathogen abundance and decrease yield and rootlets at increasing concentrations. These effects while most prominent at the maximum concentration examined could prove detrimental to long-term agricultural productivity.

6.3 Future Research

Based on the findings discussed in this thesis, recommendations for future research include in-depth taxonomic analysis of bacterial species sequenced from the soil at multiple end-points, long-term soil studies and multi-generational plant studies with nanomaterials. In the studies previously discussed, sulphidized nanomaterials demonstrated differing effects depending on concentration, size and the presence of plants which cannot be accounted for without identification of the changes to operational taxa units. Comparing the microbial composition of silver exposed treatments to control soil through operational taxa units will allow for determination of whether these effects are due primarily to decreases in activity based on reduced functionality or subtle changes in diversity and therefore what toxicity mechanisms may be involved. Long-term studies of soil exposed to silver nanomaterials including sequential loading would allow for examination of the potential effects of nanomaterials to soil microbial communities over cycles of time relevant to agricultural practices. Similarly, multi-generational plant studies, germinating and growing the kernels of plants grown in exposed soil will help to determine whether growth of plants in nanomaterial exposed ecosystems is sustainable or has implications on plant growth, health and reproduction, as seen with high concentration ionic silver treatments.

7 Bibliography

Adriano, D. 1986. *Trace Elements in Terrestrial Environments.*

Ahamed, M., M. AlSalhi, and M. Siddiqui. 2010. "Silver nanoparticle applications and human health." *Clinica Chmica Acta* 411 (23): 1841-1848.

Ahmadi, T., Z. Wang, A. Henglein, and M. El-Sayed. 1996. "Cubic Colloidal Platinum Nanoparticles." *Chemistry of Materials* 8 (6): 1161-1163.

Ahrland, S., J. Chatt, and N. Davies. 1958. "The relative affinities of ligand atoms for acceptor molecules and ions." *Quarterly Review, Chemical Society* 12: 265-276.

Aillon, K., Y. Xie, N. El-Gendy, C. Berkland, and M. Forrest. 2009. "Effects of nanomaterial physicochemical properties on in vivo toxicity." *Advanced Drug Delivery Reviews* 61 (6): 457-466.

Aitken, R., M. Chaudhry, A. Boxall, and M. Hull. 2006. "Manufacture and use of nanomaterials: current status in the UK and global trends." *Occupational Medicine* 56 (5): 300-306.

Alcamo, E. 1997. *Fundamentals of Microbiology.* Reading, MA: Addison Wesley Longman Inc.

Alloway, B., and B. Davies. 1971. "Trace element content of soils affected by base metal mining in Wales." *Geoderma* 5 (3): 197-208.

American Public Health Association. 1999. "Standard Methods for the Examination of Water and Wastewater: Part 9000 Microbiological Examination."

Anjum, N., S. Gill, A. Duarte, E. Pereira, and I. Ahmad. 2013. "Silver nanoparticles in soil-plant systems." *Journal of Nanoparticle Research* 15:1896.

Arora, S., J. Jain, J. Rajwade, and K. Paknikar. 2009. "Interactions of silver nanoparticles with primary mouse fibroblasts and liver cells." *Toxicology and Applied Pharmacology* 236 (3): 310-318.

Asadishad, B., S. Chalal, A. Akbari, V. Cianciarelli, M. Azodi, S. Ghoshal, and N. Tufenkji. 2018. "Amendment of Agricultural Soil with Metal Nanoparticles: Effects on Soil Enzyme Activity and Microbial Community Composition." *Environmental Science & Technology.*

Asghari, S., S. Johari, Y. Kim, Y. Jeon, H. Choi, M. Moon, and I. Yu. 2012. "Toxicity of various silver nanoparticles compared to silver ions in Daphnia magna." *Journal of Nanobiotechnology* 10 (14).

Ashrani, P., W. Lian, and S. Valiyaveettil. 2008. "Toxicity of silver nanoparticles in zebrafish models." *Nanotechnology* 19 (25).

Bar-Ilan, O., R. Albrecht, V. Fako, and D. Furgeson. 2009. "Toxicity Assessments of Multisized Gold and Silver Nanoparticles in Zebrafish Embryos." *Small* 5 (16): 897-910.

Barrena, R., E. Casals, J. Colon, X. Font, A. Sanchez, and V. Puntes. 2009. "Evaluation of the ecotoxicity of model nanoparticles." *Chemosphere* 75 (7): 850-857.

Barton, L., L. Schipper, C. McLay, and C. Smith. 1999. "Annual denitrification rates in agricultural and forest soils: A review." *Australian Journal of Soil Research* 37 (6): 1073-1093.

Bell, C., B. Fricks, J. Rocca, J. Steinweg, S. McMahon, and M. Wallenstein. 2013. "High-throughput Fluormetric Measurement of Potential Soil Extracellular Enzyme Activities." *Journal of Visualized Experiments* 81.

Benn, T., and P. Westerhoff. 2008. "Nanoparticle silver released into water from comercially available sock fabrics." *Environmental Science & Technology* 42: 4133-4139.

Benoit, R., K. Wilkinson, and S. Sauve. 2013. "Partitioning of silver and chemical speciation of free Ag in soils amended with nanoparticles." *Chemistry Central Journal* 7 (1): 75.

Benson, D., and W. Silvester. 1993. "Biology of Frankia strains, actinomycete symbionts of actinorhizal plants." *Microbiology and Molecular Biology Reviews* 57: 293-319.

2002. "Section 24.1 Nitrogen Fixation: Microorganisms Use ATP and a Powerful Reductant to Reduce Atmospheric Nitrogen to Ammonia." In *Biochemistry, 5th Edition,* by J. Berg, J. Tymoczko and L. Stryer. New York: W H Freeman.

Bevivino, A., P. Paganin, G. Bacci, A. Florio, M. Pellicer, M. Papaleo, A. Mengoni, et al. 2014. "Soil bacterial community response to differences in agricultural management along with seasonal changes in a mediterranean region." *PLoS One* 9 (8).

Blagodatskaya, E., S. Blagodatsky, N. Khomyakov, O. Myachina, and Y. Kuzyakov. 2016. "Temperature sensitivity and enzymatic mechanisms of soil organic matter decomposition along an altitudinal gradient on Mount Kilimanjaro." *Scientific Reports* 22240.

Blaser, S., M. Scheringer, M. MacLeod, and K. Hungerbuhler. 2008. "Estimation of cumulative aquatic exposure and risk due to silver: contribution of nanofunctionalized plastics and textiles." *Science of the Total Environment* 390:396-409.

Bot, A., and J. Benites. 2005. *The importance of soil organic matter- Key to drought-resistnat soil and sustained food producation: Annex 1.* Rome: Food and Agriculture Organization of the United Nations.

Boxall, A., K. Tiede, and Q. Chaudry. 2007. "Engineered nanomaterials in soils and water: how do they behave and could they pose a risk to human health?" *Nanomedicine* 2:919-927.

Brunetti, G., E. Donner, G. Laera, R. Sekine, K. Scheckel, M. Khaksar, K. Vasilev, G. De Mastro, and E. Lombi. 2015. "Fate of zinc and silver engineered nanoparticles in sewerage networks." *Water Research* 77: 72-84.

Button, M, H Auvinen, F Van Koetsem, B Hosseinkhani, D Rousseau, K Weber, and G Du Laing. 2016. "Susceptability of constructed wetland microbial communities to silver nanoparticles: A microcosm study." *Ecological Engineering* 80: 162-171.

Button, M, K Weber, J Nivala, T Aubron, and R Muller. 2015. "Community-Level Physiological Profiling of Microbial Communities in Constructed Wetlands: Effects of Sample Preparation." *Applied Biochemistry and Biotechnology.*

Button, M., I. Koch, and K. Reimer. 2012. "Arsenic resistance and cycling earthworms residing at a former gold mine in Canada." *Environmental Pollution* 169: 74-80.

Buzea, C., I. Blandino, and K. Robbie. 2007. "Nanomaterials and nanoparticles: Sources and toxicity." *Biointerphases* 2 (4): 17-172.

Canada Department of Agriculture and Ontario Agricultural College. 1966. *Soil Survey of Frontenac County.* Report No. 39 of the Ontario Soil Survey, Ottawa: Canada Department of Agriculture.

Canadian Council of Ministers of the Environment. 2010. "A Review of the Current Canadian Legislative Framework of Wastewater Biosolids."

Cao, C., J. Huang, W. Cai, C. Yan, Y. Jiang, and C. Peng. 2016. "Effect of Silver Nanoparticles on the Rhizosphere Exoenzyme of T. orientalis Presl." *Advances in Civil, Environmental, and Materials Research.* Jeju Island, Korea: ACEM16.

Carlson, C., S. Hussain, A. Schrand, L. Braydich-Stolle, K. Hess, R. Jones, and J. Schlager. 2008. "Unique cellular interaction of silver nanoparticles: size-dependent generation of reactive oxygen species." *The Journal of Physical Chemistry B* 112 (43): 13608-13619.

Chapman, S. 1989. "Oxidation of micronized elemental sulphur in soil." *Plant and Soil* 116: 69-76.

Chen, Z., H. Meng, X. Gengmei, C. Chen, Y. Zhao, G. Jia, T. Wang, et al. 2006. "Acute toxicological effects of copper nanoparticles in vivo." *Toxicology Letters* 163 (2): 109-120.

Cheng, H., R. Qi, H. Paudel, and H. Zhu. 2011. "Regulation and Function of Protein Kinases and Phophatases." *Enzyme Research.*

Chenu, C., and E. Roberson. 1996. "Diffusion of glucose in microbial extracellular polysaccharide as affected by water potential." *Soil Biology & Biochemistry* 28:877-884.

Choi, O, and Z Hu. 2008. "Size dependent and reactive oxygen species related to nanosilver toxicity to nitifying bacteria." *Environmental Science and Technology* 42: 4583-4588.

Choi, O, K Deng, N Kim, L Ross, R Surampalli, and Z Hu. 2008. "The inhibitory effects of silver nanoparticles, silver ions, and silver chloride colloids on microbial growth." *Water Research* 42:(12) 3066-3074.

Choi, O., B. Deng, R. Surampalli, L. Ross, Z. Hu, and T. Clevenger. 2009. "Role of sulfide and ligand strength in controlling nanosilver toxicity." *Water Research* 43:1879-1886.

Collins, D., T. Luxton, N. Kumar, S. Shah, V. Walker, and V. Shah. 2012. "Assessing the Impact of Copper and Zinc Oxide Nanoparticles on Soil: A Field Study." *Public Library of Science ONE* 7 (8): e42663.

Colman, B., C. Arnaout, S. Anciaux, C. Gunsch, M. Hochella, B. Kim, G. Lowry, et al. 2013. "Low concentrations of silver nanoparticles in biosolids cause adverse ecosystem responses under realistic field scenario." *Public Library of Science* 8 (2).

Contera, S. 2012. "Azonano." 05 28. Accessed 04 20, 2017. http://www.azonano.com/article.aspx?ArticleID=3012.

Cornelis, G, L Pang, C Doolette, J Kirby, and M McLaughlin. 2013. "Transport of silver nanoparticles in saturated columns of natural soils." *Science of The Total Environment* 463: 120-130.

Cornelis, G., J. Kirby, D. Beak, D. Chittleborough, and M. McLaughlin. 2010. "A method for determination of retention of silver and cerium oxide manufactured nanoparticles in soils." *Environmental Chemistry* 7 (3): 298-308.

Coutris, C., E. Joner, and D. Oughton. 2012. "Aging and soil organic matter content affect the fate of silver nanoparticles in soil." *Science of the Total Environment* 420: 327-333.

Crohn, D. 2004. "Nitrogen Mineralization and its Importance in Organic Waste Recyling." *National Alfalfa Symposium.* San Diego: University of California Davis.

Csuros, M., and C. Csuros. 1999. *Microbiological examination water and wastewater.* Washington, D.C.: CRC/Lewis Publishers.

Cunningham, W., and W. Stoube. 1987. "Application of an instrumental neutron activation analysis procedure to analysis of food." *Science of the Total Environment* 63:29-43.

Dean, J. 1998. *Lange's Handbook of Chemistry, 15th Edition.* New York City, NY, USA: McGraw-Hill Professional.

Debaditya, P., and K. Rajinder. 2005. "Nanotechnology and potential of microorganisms." *Critical Reviews in Biotechnology* 25: 199-204.

Dibroc, P., J. Dzioba, K. Gosink, and C. Hase. 2002. "Chemiosmotic mechanism of antimicrobial activity of Ag+ in Vibrio chlorae." *Antimicrobial Agents and Chemotherapy* 46: 4228-2670.

Dimkpa, C, J McLean, N Martineau, D Britt, R Haverkamp, and A Anderson. 2013. "Silver Nanoparticles Disrupt Wheat (Triticum aestivum L.) Growth in a Sand Matrix." *Environmental Science and Technology* 47: 1082-1090.

Dimkpa, C. 2014. "Can nanotechnology deliver the promised benefits without negatively impacting soil microbial life?" *Journal of Basic Microbiology* 54:889-904.

Doolette, C., V. Gupta, Y. Lu, J. Payne, D. Batstone, and J. Kirby. 2016. "Quantifying the sensitivity of soil microbial communities to silver sulfide nanoparticles using metagenome sequencing." *PLos One* 11 (8).

Ebeling, A., V. Hartmann, A. Rockman, A. Armstrong, R. Balza, J. Erbe, and D. Ebeling. 2013. "Silver Nanoparticle Adsorption to Soil and Water Treatment Residuals and Impact on Zebrafish in a Lab-scale Constructed Wetland." *Computational Water, Energy, and Environmental Engineering* 2:16-25.

Echavarri-Bravo, V., L. Paterson, T. Aspray, J. Porter, M. Winson, B. Thornton, and M. Hartl. 2015. "Shifts in the metabolic function of benthic estuarine microbial community following a single pulse exposure to silver nanoparticles." *Environmental Pollution* 201:91-99.

Edwards, P. 1998. *Sulphur Cycling, Retention, and Mobility in Soils: A Review.* General Technical Report NE-250, Radnor, PA: USDA Forest Service.

Elechiguerra, J., J. Burt, J. Morones, A. Camacho-Bragado, X. Gao, and H. Lara. 2005. "Interaction of silver nanoparticles with HIV-1." *Journal of Nanobiotechnology* 3: 6.

Environment and Climate Change Canada. 2016. "Proposed prioiritization approach for nanoscale forms of substances on the Domestic substances list." July. Accessed 4 1, 2017. http://www.ec.gc.ca/lcpe-cepa/default.asp?lang=En&n=FA3C8DBF-1.

Eriksen, J., M. Murphy, and E. Schnug. 1998. "The Soil Sulphur Cycle." In *Sulphur in Agroecosystems,* by E Schnug, 39-73. Dordrecht: Kluwer Academic Publishers.

EUGRIS: portal for soil and water management in Europe. 2016. "Further description:- Receptor: Ecological." Accessed 09 30, 2016. http://www.eugris.info/FurtherDescription.asp?Ca=2&Cy=0&T=Receptor:%20Ecological&e=34.

Fajardo, C., L. Ortiz, M. Rodriguez-Membibre, M. Nande, M. Lobo, and M. Martin. 2012. "Assessing the impact of zero-valent iron (ZVI) nanotechnology on soil microbial structure and functionality: A molecular approach." *Chemosphere* 86: 802-808.

Fauss, E. 2008. "The Silver Nanotechnology Commercial Inventory." September. Accessed October 3, 2016. http://www.nanotechproject.org/process/assets/files/6718/fauss_final.pdf.

Feng, Q., J. Wu, G. Chen, F. Cui, T. Kim, and J. Kim. 2000. "A mechanistic study of the antibacterial effect of silver ions on Escherichia coli and Staphylococcus aureus." *Journal of Biomedical Materials Research* 52:662-668.

Forster, B., M. Garcia, and H. SchallnaB. 2006. "Respiration rates of soil invertebrates from temperate and tropical zones as measured by infrared gas analysis." *Ecotropica* 12: 27-33.

Frens, G. 1973. "Controlled nucleation for the regulation of the particle sinze in monodisperse gold suspensions." *Nature Physical Science* 241: 20-22.

Gabreva, P., J. van Veen, and J. van Elsas. 2004. "Microbial Diversity in Soil: Selection of Microbial Populations by Plant and Soil Type and Implications for Disease Suppressiveness." *Annual Review of Phytopathology* 42: 243-270.

Gaderer, R., V. Seidl-Seiboth, and L. Kappel. 2017. "Chitin and N-acetylglucosamine Metabolism in Fungi- A Compex Machinery Harnessed for the Design of Chitin-Based High Value Products." *Current Biotechnology* 6: 178-193.

Gagnon, V., M. Button, D. O'Carroll, and K. Weber. 2017. "Influence of wear and tear on the release of silver nanoparticles from sports socks." *253rd American Chemical Society National Meeting & Exposition.* San Francisco, USA: American Chemical Society.

Galiano, K., C. Pleifer, and K. Engelhardt. 2008. "Silver segregation and bacterial growth of intraventricular cathetars impregnated with silver nanoparticles in cerebrospinal fluid drainages." *Neurological Research* 30 (3): 285-287.

Garland, J. 1997. "Analysis and interpretation of community-level physiological profiles in microbial ecology." *FEMS Microbiology Ecology* 24: 289-300.

Gatoo, M., S. Naseem, M. Arfat, A. Dar, K. Qasim, and S. Zubair. 2014. "Physicochemical Properties of Nanomaterials: Implication in Associated Toxic Manifestations." *BioMed Research International* (BioMed Research International) 14.

Ge, L., Q. Li, M. Wang, J. Ouyang, X. Li, and M. Xing. 2014. "Nanosilver particles in medical applications: synthesis, performance, and toxicity." *International Journal of Nanomedicine* 9: 2399-2407.

Ge, Y., J. Schimel, and P. Holden. 2011. "Evidence for negative effects of TiO2 and ZnO nanoparticles on soil bacterial communities." *Environmental Science & Technology* 45: 1659-1664.

Ge, Y., J. Schimel, and P. Holden. 2012. "The identification of soil bacteria susceptible to TiO2 and ZnO nanoparticles." *Applied Environmental Microbiology* 78: 6749-6758.

Geissmann, Q. 2013. "OpenCFU, a new Free and Open-Source Software to Count Cell Colonies and Other Circular Objects." *PLOS One* 8(2): e54072.

Gomez, E., J. Garland, and M. Conti. 2004. "Reproducibility in the response of soil bacterial community-level physiological profiles from a land use intensification gradient." *Applied Soil Ecology* 26: 21-30.

Gottschalk, F., T. Sonerer, R. Scholz, and B. Nowak. 2009. "Modeled environmental concentratios of engineered nanomaterials (TiO2, ZnO, CNT, fullerenes) for different regions." *Enviornmental Science and Technology* 43: 9216-9222.

Government of Canada. 2010. *Wheat Sector Profile Part One: Overview.* Agriculture and Agri-Food Canada.

Government of Ontario. 2017. "Nutrient Management Act, 2002 Ontario Regulation 267/03 General."

Gray, E., and D. Smith. 2005. "Intracellular and extracellular PGPR: commonalities and distinctions in the plant-bacterium signaling processes." *Soil Biology and Biochemistry* 30: 369-378.

Hansch, M., and C. Emmerling. 2010. "Effects of silver nanoparticles on the microbiota and enzyme activity in soil." *Journal of Plant Nutrition and Soil Science* 173: 554-558.

Hardy, R., and R. Burns. 1968. "Biological Nitrogen Fixation." *Annual Reviews of Biochemistry* (E.I. du Pont deNemours & Co., Inc.) 331-352.

Harley, J., and S. Smith. 1983. *Mycorrhizal symbiosis.* New York: Academic Press.

Hatchett, D., and S. Henry. 1996. "Electrochemistry of sulfur adlayers on the low-index faces of silver." *Journal of Physical Chemistry* 100:9854-9859.

Haun, J. 1973. "Visual Quantification of Wheat Development." *Agronomy Journal* 65(1).

Hawkesford, M., W. Horst, T. Kichey, P. White, J. Schjoerring, I. Skrumsager Moller, and H. Lambers. 2012. "Chapter 6: Functions of Macronutrients." In *Marschner's Mineral Nutrition of Higher Plants*, by H Marschner, 135-189. Amsterdam: Elsevier.

He, S., Y. Feng, H. Ren, Y. Zhang, N. Gu, and X. Lin. 2011. "The impact of iron oxide magnetic nanoparticles on the soil bacterial community." *Journal of Soils and Sediments* 11 (8): 1408-1417.

Hedberg, J., S. Skoglund, M. Karlsson, S. Wold, I. Wallinder, and Y. Hedberg. 2014. "Sequential Studies of Silver Released from Silver Nanoparticles in Aqueous Media Simulating Sweat, Laundry Detergent Solutions and Surface Water." *Environmental Science and Technology* 48: 7314-7322.

Hirsch, M. 1998. "Availability of sludge-borne silver to agricultural crops." *Environmental Toxicology and Chemistry* 17:610-624.

Hofstra, N., and A. Bouwman. 2005. "Denitrification in agricultural soils: summarizing published data and estimating global annual rates." *Nutrient Cycling in Agroecosytems* 72: 267-278.

Holt, K., and A. Bard. 2005. "Interaction of silver(I) ions with the respiratory chain of Escherichia coli: an electrochemical and scanning electrochemical microscopy study of the antimicrobial mechanism of micromolar Ag." *Biochemistry* 44: 13214-13223.

Hoogsteen, M., E. Lantinga, E. Bakker, J. Groot, and P. Tittonell. 2015. "Estimating soil organic carbon through loss on ignition: effects if ignition conditions and structural water loss." *European Journal of Soil Science.*

Hu, Z. 2010. *Impact of Silver Nanoparticles on Wastewater Treatment.* London, UK: IWA Publishing.

Hwang, E., J. Lee, Y. Chae, Y. Kim, B. Kim, B. Sang, and M. Gu. 2008. "Analysis of the toxic mode of action of silver nanoparticles using stress-specific bioluminescent bacteria." *Small* 4: 746-750.

Iravani, S., H. Korbekandi, S. Mirmohammadi, and B. Zolfaghari. 2014. "Synthesis of silver nanoparticles: chemical, physical and biological methods." *Research in Pharmaceutical Sciences* 9 (6): 385-406.

2015. "ISO/TS 80004-2:2015 Nanotechnologies- Vocabulary- Part 2: Nano-objects." *ISO Online Browsing Platform*. June. Accessed January 8, 2018. https://www.iso.org/obp/ui/#iso:std:iso:ts:80004:-2:ed-1:v1:en.

Jacoby, R., M. Peukert, A. Succorro, A. Koprivova, and S. Kopriva. 2017. "The Role of Soil Microorganisms in Plant Mineral Nutrition- Current Knowledge and Future Directions." *Frontiers in Plant Science* 8: 1617.

Jana, N., L. Gearheart, and C. Murphy. 2001. "Seed- mediated growth approach for shape-controlled synthesis of spheroidal and rod-like gold nanopaticles using a surfactant template." *Advanced Materials* 1389-1393.

Jass, J., S. Roberts, and H. Lappin-Scott. 2002. "Enzymes in the Environment." In *Activity, Ecology and Applications*, by R Burns and R Dick. Florida, US: CRC Press.

Jeffries, P., and J. Barea. 1994. "Biogeochemical cycling and arbuscular mycorrhizas in the sustainability of plant-soil systesms." In *Impact of arbuscular mycorrhizas on sustainable agriculture and natural ecosystems*, by S Gianinazzi and H Schuepp, 101-115. Basel: Birkhauser.

Jiang, H., M. Li, F. Chang, and L. Yin. 2012. "Physiological analysis of silver nanoparticles and AgNO3 toxicity to Spirodela polyrhiza." *Environmental Toxicology and Chemistry* 31 (8): 1180-1886.

Jin, X., M. Li, J. Wang, C. Marambio-Jones, F. Peng, X. Huang, R. Damoiseaux, and E. Hoek. 2010. "High-Throughput Screening of Silver Nanoparticle Stability and Bacterial Inactivation in Aquatic Media: Influence of Specific Ions." *Environmental Science & Technology* 44 (19): 7321-7328.

Judy, J., J. Kirby, C. Creamer, M. McLaughlin, C. Fiebiger, C. Wright, T. Cavagnaro, and P. Bertsch. 2015. "Effects of silver sulfide nanomaterials on mycorrhizal colonization of tomato plants and soil microbial communities in biosolid-amended soil." *Environmental Pollution* 206: 256-263.

Judy, J., J. Kirby, M. McLaughlin, and P. Bertsch. 2016. "Symbiosis between nitrogen-fixing bacteria and Medicago truncatula is not significantly affected by silver and silver sulfide nanomaterials." *Environmental Pollution* 214:731-736.

Jung, M. 2008. "Heavy metal concentrations in soil and factors affecting metal uptake in plants in the vicinity of a Korean Cu-W mine." *Sensors* 8 (4): 2413-2423.

Jung, W., H. Jeon, J. Kim, and J. Youk. 2008. "Antibacterial activity and mechanism of action of the silver ion in Staphylococcus aureus and Escherichia coli." *Applied Environmental Microbiology* 74: 2171-2178.

Kabata-Pendias, A., and H. Pendias. 2001. *Trace elements in soils and plants.* Boca Raton, FL, USA: CRC Press.

Kaegi, R., A. Voeglin, B. Sinnet, S. Zuleeg, H. Hagendorfer, M. Burkhardt, and H. Siegrist. 2011. "Behaviour of metallic silver nanoparticles in a pilot wastewatertreatment plant." *Environmental Science and Technology* 45(9): 3902-3908.

Kaiser, C., M. Koranda, B. Kitzler, L. Fuchsleuger, J. Schnecker, P. Schweiger, F. Rasche, S. Zechmeister-Boltenstern, A. Sessitsch, and A. Richter. 2010. "Belowground carbon allocation by trees drive seasonal pattern of extracellular enzyme activities by altering the microbial community composition in a beech forest soil." *New Phytologist* 187:843-858.

Kaveh, R., Y. Li, S. Ranjbar, R. Therani, C. Brueck, and B. Van Aken. 2013. "Changes in Arabidopsis thaliana Gene Expression in Response to Silver Nanoparticles and Silver Ions." *Environmental Science and Technology* 47 (18): 10637-10644.

Kelly, L., E. Coronado, L. Zhao, and G. Schatz. 2003. "The optical properties of metal nanoparticles: the influence of size, shpae and dielectric environment." *Journal OF Physical Chemistry B* 107: 668-677.

Khodashenas, B., and H. Ghorbani. 2015. "Synthesis of silver nanoparticles with different shapes." *Arabian Jounral of Chemistry.*

Kim, B., C. Park, M. Murayama, and M. Hoachella. 2010. "Discover and characterization of silver sulfide nanoparticles in final sewage sludge products." *Environmental Science and Technology* 44(19): 7509-7514.

Kim, J., E. Kuk, K. Yu, J. Kim, S. Park, H. Lee, S. Kim, Y. Park, Y. Park, and C. Hwang. 2007. "Antimicrobial effects of silver nanoparticles." *Nanomedecine: Nanotechnology, Biology and Medicine* 3:95-101.

Kim, K., W. Sung, S. Moon, J. Choi, J. Kim, and D. Lee. 2008. "Antifungal effect of silver nanoparticles on dermatophytes." *Journal of Microbiol Biotechnology* 18 (8): 1482-1484.

Kim, T., C. Lee, S. Joo, and K. Lee. 2008. "Kinetics of gold nanoparticle aggregation: Experiments and modeling." *Journal of Colloid and Interface Science* 318 (2): 238-243.

Kiss, S., M. Dragan-Bularda, and D. Radulescu. 1978. "Soil polysaccharidases: activity and agricultural importancce." In *Soil Enzymes*, by R. Burns, 117-147. London: Academic.

Klaine, S., P. Alvarez, G. Batley, T. Fernandes, R. Handy, D. Lyon, S. Mahendra, M. McLaughlin, and J. Lead. 2008. "Nanomaterials in the environment: behaviour, fate, bioavailability and effects." *Environmental Toxicology and Chemistry* 27:1825-1851.

Klein, D. 1978. "Plant effects." In *Environmental Impacts of Artificial Ice Nucleating Agents*, by D Klein, 109-126. Stoutsburg, PA, USA: Hutchinson and Ross.

Koranda, M., J. Schnecker, C. Kaiser, L. Fuchslueger, B. Kitzler, C. Stange, A. Sessitsch, S. Zechmesiter-Boltenstern, and A. Richter. 2011. "Microbail processes and community composition in the rhizosphere of European beech- The influence of plant C exudater." *Soil Biology and Biochemistry* 43(3):551-558.

Kraas, M., K. Schlich, B. Knopf, F. Wege, R. Kagi, K. Terytze, and K. Hund-Rinke. 2017. "Long-Term Effects of Sulfidized Silver Nanoparticles in Sewage Sludge on Soil Microflora." *Environmental Toxicology and Chemistry.*

Kramer, J, R Bell, P Collins, S Malcolmson, and C Rogers. 1994. "Nature and Fate of silver-Part I." *2nd Argentum International Conference on the Transport, Fate, and Effects of Silver in the Environment.* Madison, WI, USA. 51-56.

Kramer, J., N. Adams, H. Manolopoulos, and P. Collins. 1996. "Fate of silver in sediments and plant material in and old mining camp, Cobalt, Ontario." *4th Argentum International Conference on the Transport, Fate and Effects of Silver in the Environment.* Madison, WI, USA. 79-88.

Kreibig, U., and M. Vollmer. 1995. *Optical properties of metal clusters.* Berlin: Springer.

Krutyakov, Y., A. Kudrinskiy, A. Olenin, and G. Lisichkin. 2008. "Synthesis and properties of silver nanoparticles: advances and prospects." *Russian Chemical Reviews* 77 (3).

Kumar, N, G Palmer, V Shah, and V Walker. 2014. "The Effect of Silver Nanoparticles on Seasonal Change in Arctic Tundra Bacterial and Fungal Assemblages." *PLoS ONE* 9(5): e99953.

Kumar, N, V Shah, and V Walker. 2011. "Perturbation of an artic soil microbial community by metal nanoparticles." *Journal of Hazardous Materials* 190 (1-3): 816-822.

Kumar, N., V. Shah, and V. Walker. 2012. "Influence of nanoparticle mixture on an artic soil community." *Environmental Toxicology and Chemistry* 31: 131-135.

Lapen, D., E. Topp, C. Metcalfe, H. Li, M. Edwards, N. Gottschall, P. Bolton, W. Curnoe, and A. Beck. 2008. "Pharmaceutical and person care products in tile drainage following land applications of municipal biosolids." *Science of the Total Environment* 399 (1): 50-65.

Lawrence, J., and J. Germida. 1991. "Microbial and chemical characteristics of elemental sulfur beads in agricultural soils." *Soil Science Society of America Journal* 23: 617-622.

Lee, W., J. Kwak, and Y. An. 2012. "Effect of silver nanoparticles in crop plants Phaseolus radiatus and Sorghum bicolor: media effect on phytotoxicity." *Chemosphere* 86 (5): 491-499.

Legendre, P., and L. Legendre. 1998. *Numerical Ecology, second ed.* Amsterdam, NL: Elsevier B.V.

Legge, R., and K. Weber. 2010. "Method for the detachment of culturable bacteria from wetland gravel." *Journal of Microbiological Methods* 80 (3): 242-250.

Lettl, A., O. Langkramer, and V. Lochman. 1981. "Dynamics of oxidation of inorganic suphur compounds in upper soil horizons of spruce forests." *Folia Microbiology* 26: 24-28.

Levard, C., B. Reinsch, F. Michel, C. Oumahi, G. Lowry, and G. Brown. 2011. "Sulfidation Processes of PVP-Coated Silver Nanoparticles in Aqueous Solution: Impact on Dissolution Rate." *Environmental Science & Technology* 45: 5260-5266.

Li, B., and G. Wu. 2014. "Effects of Sludge Retention Times on Nutrient Removal and Nitrous Oxide Emission in Biological Nutrient Removal Processes." *International Journal of Environmental Research and Public Health* 11(4): 3553-3569.

Liau, S., D. Read, W. Pugh, J. Furr, and A. Russell. 1997. "Interaction of silver nitrate with readily identifiable groups: relationship to the antibacterial action of silver ions." *Letters in Applied Microbiology* 25:279-283.

Lok, C., C. Ho, R. Chen, Q. He, W. Yu, H. Sun, P. Tam, J. Chiu, and C. Che. 2006. "Proteomic analysis of the mode of anibacterial action of silver nanoparticles." *Journal of Proteome Research* 5 (4): 916-924.

Lui, J., and R. Hurt. 2010. "Ion release kinetics and particle persistence in aqueous nano-silver colloids." *Environmental Science and Technology* 44 (6): 2169-2175.

Lupwayi, N., M. Monreal, G. Clayton, C. Grant, A. Johnston, and W. Rice. 2001. "Soil microbial biomass and diversity respond to tillage and sulphur fertilizers." *Canadian Journal of Soil Science* 577-589.

Ma, X., J. Geisser-Lee, Y. Deng, and A. Kolmakov. 2010. "Interactions between engineered nanoparticles (ENPs) and plants: phytotoxicity, uptake and accumulation." *Science of the Total Environment* 408:3053-3061.

Marambio-Jones, C., and E. Hoek. 2010. "A review of the anti-bacterial effects of silver nanomaterials and potential implications for human health and the environment." *Journal of Nanoparticle Research* 12 (5): 1531-1551.

Matsui, M., L. Walling, and J. Fowler. 2006. "Leucine aminopeptidases: diversity in structure and function." *Biological Chemistry* 387 (12): 1535-1544.

McGill, W. 2007. "The Physiology and Biochemistry of Soil Organisms." In *Soil Microbiology, Ecology and Biochemistry*, by E Paul, 231-256. Amsterdam: Elsevier.

Mendis, E., N. Rajapakse, H. Byun, and S. Kim. 2005. "Investigation of jumbo squid (Dosidicus gigas) skin gelatin peptides for their in vitro antioxidant effects." *Life Sciences* 77 (17): 2166-2178.

Metcalf & Eddy, Inc. 2003. "Wastewater Engineering: Treaatment and Reuse (4th Ed.)." 1449. USA: McGraw Hill.

Michaelis, L., M. Menten, R. Goody, and K. Johnson. 2011. "The original Michaelis constant: translation of the 1913 Michaelis-Menten paper." *Biochemistry* 50(39):8264-8269.

Ministry of Environment. 1996. *Guidelines for the utilization of biosolids and other wastes on agricultural land.* Ministry of Agriculture, Food and Rural Affairs.

Mock, J., M. Barbic, D. Smith, D. Schultz, and S. Schultz. 2002. "Shape effects in plasmon resonance of individual colloidal silver nanoparticles." *Journal of Chemical Physics* 116 (5): 6755.

Monteith, H., L. Sterne, S. Dong, W. Parker, and C. Metcalfe. 2009. *Emerging Substances of Concern in Biosolids: Concentration and Effects of Treatment Processes.* Final Report-Literature Review, Winnipeg: Canadian Council of Ministers of the Environment.

Morones, J., J. Elechiguerra, A. Camacho, K. Holt, J. Kouri, J. Ramirez, and M. Yacaman. 2005. "The bactericidal effect of silver nanoparticles." *Nanotechnology* 16:2346-2353.

Mu, Q., G. Jiang, L. Chen, H. Zhou, D. Fourches, A. Tropsha, and B. Yan. 2014. "Chemical Basis of Interactions Between Engineered Nanoparticles and Biological Systems." *Chemical Reviews* 114 (15): 7740-7781.

Mueller, N., and B. Nowak. 2008. "Exposure modeling of engineered nanoparticles in the environment." *Environmental Science and Technology* 42:4447-53.

Murphy, K., S. Long, M. Rearick, and O. Ertas. 2002. "The accurate determination of potassium and calcium using isotope dilution inductively coupled "cold" plasma mass spectrometrey." *Journal of Analytic Atomic Spectroscopy* 17: 469-477.

Musante, C., and J. White. 2012. "Toxicity of Silver and Copper to Cucurbita pepo: Differential Effects of Nano and Bulk-Size Particles." *Environmental Toxicology* 27: 510-517.

Nakamoto, T., and S. Wakahara. 2004. "Development of substrate induced respiration (SIR) method combined with selective inhibition for estimating fungal and bacterial biomass in humic andosols." *Plant Production Science* 7:1 70-76.

NanoHorizons, Inc. 2016. "SmartSilver Products." Accessed October 3, 2016. http://www.smartsilver.com/products.

NAPPFAST NCSU APHIS Plant Pest Forecasting System. 2008. "Weather-Based Pest Risk Mapping Project Risk Assessment: Xanthomonas oryzae pv. oryzae, bacterial leaf blight or kresek disease." *nappfast.org.* September 03. Accessed February 16, 2018. https://web.archive.org/web/20120425231216/http://www.nappfast.org/pest%20reports/Xanthomonas_oryzae_rice_OR_MAP_20080903_ver01.pdf.

Navarro, E., F. Piccapietra, B. Wagner, F. Macroni, R. Kaegi, N. Odzak, L. Sigg, and R. Behra. 2008. "Toxicity of silver nanoparticles in chlamydomonas reinhardtii." *Environmental Science and Technology* 42: 8959-8964.

Nicholson, F., S. Smith, B. Alloway, C. Carlton-Smith, and B. Chambers. 2003. "An inventory of heavy metals inputs to agricultural soils in England and Wales." *Science of the Total Environment* 311:205-219.

Nino-Lui, D., P. Ronald, and A. Bogdanove. 2006. "Xanthomonas oryzae pathovars: model pathogens of a model crop." *Molecular Plant Pathology* 7(5):303-324.

Noji, M., and K. Saito. 2003. "Sulphur Amino Acids: Biosynthesis of Cysteine and Methionine." In *Sulphur in Plants*, by Y. Abrol and A. Ahmad, 135-144. Dordrecht: Springer.

Nowack, B., and D. Mitrano. 2018. "Procedures for the production and use of synthetically aged and product released nanomaterials for further environmental and ecotoxicity testing." *NanoImpact* 10: 70-80.

Nowack, B., H. Krug, and M. Height. 2011. "120 Years of Nanosilver History: Implications for Policy Makers." *Environmental Science and Technology* 45(4): 1177-1183.

Nyberg, L., R. Turco, and L. Nies. 2008. "Assessing the Impact of Nanomaterials on Anaerobic Microbial Communities." *Environmental Science and Technology* 42 (6): 1938-1943.

Ogilvie, L. 2017. *Influence of Establishment Periods, Aeration, and Silver Nanoparticles on the Function of Vertical Flow Constructed Wetlands.* Thesis, Kingston, ON: Royal Military College of Canada.

Ontario Ministry of Agriculture, Food and Rural Affairs. 2009. "OMAFRA Publication 811: Agronomy Guide for Field Crops." April 30. Accessed October 3, 2016. http://www.omafra.gov.on.ca/english/crops/pub811/1tillage.htm.

Pallavi, M., C. Mehta, R. Srivastrava, S. Arora, and A. Sharma. 2016. "Impact assessment of silver nanoparticles on plant growth and soil bacterial diversity." *3 Biotech* 6(2): 254.

Panacek, A., M. Kolar, M. Vecerova, R. Prucek, J. Soukupova, V. Krystof, P. Hamal, R. Zboril, and L. Kvitek. 2009. "Antifungal activity of silver nanoparticles against Canduda spp." *Biomaterials* 30:6333-6340.

Paungfoo-Lonhienne, C., T. Lonhienne, D. Rentsch, N. Robinson, M. Christie, R. Webb, H. Gamage, B. Carroll, P. Schenk, and S. Schmidt. 2008. "Plants can use protein as a nitrogen source without assistance from other organisms." *National Academy of Sciences.* USA. 4524-4529.

Peyrot, C., K. Wilkinson, M. Desrosiers, and S. Sauve. 2014. "Effects of silver nanoparticles on soil enzyme activities with and without added organic matter." *Environmental Toxicology and Chemistry* 33 (1): 115-125.

Pokhrel, L., and B. Dubey. 2013. "Evaluation of developmental responses of two crop plants exposed to silver and zinc oxide nanoparticles." *Science of the Total Environment* 452: 321-332.

1979. In *The sulphate-reducing bacteria*, by J. Postgate, 1-8. Cambridge: Cambridge University Press.

Pradas del Real, A., H. Castilloi-Michel, R. Kaegi, B. Sinnet, V. Magnin, N. Findling, J. Villanova, et al. 2016. "Fate of Ag-NPs in Sewage Sludge after Application on Agricultural Soils." *Environmental Science & Technology* 50: 1759-1768.

Priester, J., Y. Ge, R. Mielke, A. Horst, S. Moritz, K. Espinosa, J. Gelb, et al. 2012. "Soybean susceptibility to manufactured nanomaterials with evidence for food quality and soil fertility interruption." *Ptoceeding of the Nation Academy of Sciences of the United States of America* 109 (37): 2451-2456.

Qian, H., X. Peng, X. Han, J. Ren, L. Sun, and Z. Fu. 2013. "Comparison of the toxicity of silver nanoparticles and silver ions on the growth of terrestrial plant model Aripidopsis thaliana." *Journal of Environmental Sciences* 25(9): 1947-1955.

Raab, G., M. Bartling, M. Stapanian, W. Cole, R. Tidwell, and K. Cappo. 1990. "The Homogenization of Environmental Soil Samples in Bulk." In *Hazardous Waste Measurements*. Lewis Pub.

Raaijmakers, J., T. Paulitz, C. Steinberg, C. Alabouvette, and Y. Moenne-Loccoz. 2009. "The rhizosphere: a playground and battlefield for soilborne pathogens and beneficial microorganisms." *Plant and Soil* 321: 341-361.

Raffi, M., F. Hussain, T. Bhatti, J. Akhter, A. Hameed, and M. Hasan. 2008. "Antibacterial characterization of silver nanoparticles against E. Coli ATCC-15224." *Journal of Material Science and Technology* 24: 192-196.

Rashid, M., L. Mujawar, T. Shahzad, T. Almeelbi, I. Ismail, and M. Oves. 2016. "Bacteria and fungi can contribute to nutrients bioavailability and aggregate formation in degraded soils." *Microbiological Research* 183: 26-41.

Rasmussen, P., K. Subramanian, and B. Jessiman. 2001. "A multi-element profile of housedust in relation to exterior dust and soils in the city of Ottawa, Canada." *The Science of the Total Environment* 267: 125-140.

Ray, P., H. Yu, and P. Fu. 2009. "Toxicity and Environmental Risks of Nanomaterials: Challenges and Future Needs." *Journal of Environmental Science and Health, Part C: Environmental Carcinogenesis and Exotoxicology Reviews* 27 (1): 1-35.

Reddy, A., J. Katyal, D. Rouse, and D. Mackenzie. 1979. "Relationship Between Nitrogen Fertilization, Bacterial Leaf Blight Severity, and Yield of Rice." *Phytopathology* 970-973.

Robertson, G., and P. Groffman. 2007. "Chapter 13: Nitrogen Transformations." In *Soil Microbiology, Ecology and Biochemistry*, by E Paul, 341-364. Amsterdam: Elsevier.

Roesch, L., R. Fulthrope, A. Riva, G. Casella, A. Hadwin, A. Kent, S. Daroub, F. Camargo, W. Farmerie, and E. Triplett. 2007. "Pyrosequencing enumerates and contrasts soil microbial diversity." *ISME Journal* 1(4): 283-290.

Rosenzweig, R., U. Shavit, and A. Furman. 2012. "Water Retention Curves of Biofilm-Affected Soils using Xanthan as an Analogue." *Soil Science Society of America Journal* 76:61-69.

Ryu, S., T. Nguyen, W. Park, C. Kim, and C. Jeon. 2006. "Runella limosa sp. nov., isolated from activated sludge." *International Journal of Systematic and Evolutionary Microbiology* 56: 2757-2760.

Sahverdi, A., S. Minaeian, H. Sahverdi, and A. Nohi. 2007. "Rapid synthesis of silver nanoparticles using culture supernatants of Enterobacteria: A novel biological approach." *Process Biochemistry* 42 (5): 919-923.

Samarajeewa, A., J. Velicogna, J. Princz, R. Subasinghe, R. Scroggins, and L. Beaudette. 2017. "Effect of silver nano-particles on soil microbial growth, activity and community diversity in a sandy loam soil." *Environmental Pollution* 220: 504-513.

Schimel, J., and J. Bennett. 2004. "Nitrogen mineraliztion: challenges of a changing paradigm." *Ecology* 85: 591-602.

Schlich, K., and K. Hund-Rinke. 2015. "Influence of soil properties on the effect of silver nanomaterials on microbial activity in five soils." *Environmental Pollution* 321-330.

Schmidt, H., M. Thom, M. Madzgalla, S. Gernersdorf, G. Metreveli, and W. Manz. 2017. "Exposure to Silver Nanoparticles Affects Biofilm Structure and Adhesiveness." *Journal of Aquatic Pollution and Toxiciology* 1(2):9.

Schreurs, W., and H. Rosenberg. 1982. "Effects of silver ions on transport and retention of phosphate by Escherichia coli." *Journal of Bacteriology* 152: 7-13.

Schulz, S., R. Brankatschk, A. Dumig, I. Kogel-Knabner, M. Schloter, and J. Zeyer. 2013. "The role of microorganisms at different stages of ecosystem development for soil formation." *Biogeosciences* 10: 3983-3996.

Seif Sahandi, M., A. Sorooshzadeh, H. Rezazadeh, and H. Naghdibadi. 2011. "Effect of nano silver and silver nitrate on seed yield of borage." *Journal of Medicinal Plants Research* 5: 706-710.

Sekine, R., G. Brunetti, E. Donner, M. Khaksar, K. Vasilev, A. Jamting, K. Scheckel, P. Kappen, H. Zhang, and E. Lombi. 2015. "Speciation and lability of Ag-, AgCl- and Ag2S-nanoparticles in soil determined by X-ray absorption spectroscopy and diffusive gradients in thin films." *Environmental Science & Technology* 49(2): 897-905.

Senapati, S. 2005. "Ph.D. Thesis: Biosynthesis and immobilization of nanoparticles and their applications." India: University of Pune.

Settimio, L., M. McLaughlin, J. Kirby, K. Langdon, E. Lombi, E. Donner, and K. Scheckel. 2014. "Fate and lability of silver in soils: Effect of ageing." *Environmental Pollution* 191: 151-157.

Shah, V., D. Collins, V. Walker, and S. Shah. 2014. "The impact of engineered cobalt, iron, nickel and silver nanoparticles on soil bacterial diversity under field conditions." *Environmental Research Letters* 9 024001.

Shahrokh, S., and G. Emitiazi. 2009. "Toxicity and Unusual Behaviour of Nanosilver on Gram Positive and Negative Bacteria Assayed by Microtiter-Plate." *European Journal of Biological Sciences* 3:28-31.

Shoults-Wilson, W., B. Reinsch, O. Tsyusko, P. Bertsch, G. Lowry, and J. Unrine. 2010. "Role of Particle Size and Soil Type in Toxicity of Silver Nanoparticles to Earthworms." *Soil Science Society of Americal* 75: 2011.

Sibbald, R., J. Contreras-Ruiz, P. Coutts, M. Fierheller, A. Rothman, and K. Woo. 2007. "Baceriology, inflammation, and healing: a study of nanocrystalline silver dressing in chronic venous leg ulcers." *Advances in Skin & Wound Care* 20 (10): 549-558.

Sillen, W., S. Thijs, G. Abbamondi, J. Janassen, N. Weyens, J. White, and J. Vangronsveld. 2015. "Effects of silver nanoparticles on soil microorganisms and maize biomass are linked in the rhizosphere." *Soil Biology & Biochemistry* 91: 14-22.

Sinsabaugh, R. 1994. "Enzymatic analysis of microbial pattern and process." *Biology and Fertility of Soils* 17: 69-74.

Six, J., H. Bossuyt, S. Degryze, and K. Denef. 2004. "A history of research on the link between (micro) aggregates, soil biota, and soil organic matter dynamics." *Soil Tillage Research* 79: 7-31.

Skirtach, A., J. Munoz, and O. Kreft. 2006. "Laser-Induced release of encapsulated materials inside living cells." *Angewandte Chemie International Edition* 45 (28): 4612-4617.

Smetana, A., K. Klabunde, G. Marchin, and C. Sorenson. 2008. "Biocidal activity of nanocrystalline silver powders and particles." *Langmuir* 24: 7457-7464.

Sondi, I., and B. Salopek-Sondi. 2004. "Silver nanopartices as antimicrobial agent: a case study on E. Coli as a model for Gram-negative bacteria." *Journal of Colloid and Interface Science* 275:177-182.

Sprent, J. 2001. *Nodulation in Legumes.* Kew, UK: Royal Botanical Gardens.

Stampoulis, D., S. Sinha, and J. White. 2009. "Assay-Dependent Phytotoxicity of Nanoparticles to Plants." *Environmental Science & Technology* 43(24): 9473-9479.

Stegemeier, J., F. Schwab, B. Colman, S. Webb, M. Newville, A. Lanzirotti, C. Winkler, M. Wiesner, and G. Lowry. 2015. "Speciation Matters: Biovailability of Silver and Silver Sulfide." *Environmental Science & Technology* 49: 8451-8460.

Strickland, T., and J. Fitzgerald. 1984. "Formation and mineralization of organic sulfur in forest soils." *Biogeochemistry* 1: 79-95.

Stursova, M., and P. Baldrian. 2011. "Effects of soil properties and management on the activity of soil organic matter transforming enzymes and the quantification of soil-bound and free activity." *Plant and Soil* 338: 99-110.

Sun, X., Z. Sheng, and Y. Lui. 2013. "Effects of silver nanoparticles on microbial community structure in activated sludge." *Science of the Total Environment* 443: 828-835.

Suresh, A., D. Pelletier, and M. Doktycz. 2013. "Relating nanomaterial properties and microbial toxicity." *Nanoscale* 5: 463-474.

Tarboton, D. 2003. "Chapter 4: Soil Properties." In *Rainfall Runoff Processes*. Civil and Environmental Engineering Faculty Publications, Utah State University.

Terrasan, C., J. Guisan, and E. Caarmona. 2016. "Xylanase and B-xylosidase from Penicillium janczewskii: Purification, characterization and hydrolysis of substrates." *Electronic Journal of Biotechnology* 23: 54-62.

The Weather Network. 2018. "Statistics: Kingston,Ontario." https://www.theweathernetwork.com/api/sitewrapper/index?url=%2Fstatistics%2Fcaon0349%2F%2F%2F%2F%3F.

Thien, S. 1979. "A flow diagram for teaching texture by feel analysis." *Journal of Agronomic Education* 8: 54-55.

Throback, I., M. Johansson, M. Rosenquist, M. Pell, M. Hansson, and S. Hallin. 2007. "Silver (Ag+) reduces denitrification and induces enrickment of novel nirK genotypes in soil." *FEMS Microbiology Letters* 270 (2): 189-194.

Tietjen, T., and R. Wetzel. 2003. "Extracellular enzyme-clay mineral complexes: Enzyme adsorption, alteration of enzyme activitym and protection from photodegradation." *Aquatic Ecology* 331-339.

Torsvik, V., R. Sorheim, and J. Goksoyr. 1996. "Total bacterial diversity in soil and sediment communities- a review." *Journal of Industrial Microbiology* 17: 170-178.

Tran, Q., V. Nguyen, and A. Le. 2013. "Silver nanoparticles: synthesis, properties, toxicology, applications and perspectives." *Advances in Natural Sciences: Nanoscience and Nanotechnology* 4 (3).

Uchida, R. 2000. "Essential Nutrients for Plant Growth: Nutrient Functions and Deficiency Symptoms." In *Plant and Nutrient Management in Hawaii's Soils, Approaches for Tropical and Subtropical Agriculture*, by J Silva and R Uchida, 31-55. University of Hawaii at Manoa: College of Tropical Agriculture and Human Resources.

Uchihara, T. 2007. "Silver Diagnosis in neuropathy: principles, practice and revised interpretation." *Acta Nuropathologica* 113 (5): 483-499.

Uhlirova, E., D. Elhottova, J. Triska, and H Santruckova. 2005. "Physiology and Microbial Community Structure in Soil at Extreme Water Content." *Folia Microbiologica* 50 (2): 161-166.

United States Department of Agriculture. 2016. "Estimating Moist Bulk Density by Texture." Accessed October 3, 2016.

http://www.nrcs.usda.gov/wps/portal/nrcs/detail/soils/survey/office/ssr10/tr/?cid=nrcs144p2_074844.

United States Environmental Protection Agency. 1981. *An Exposure and Risk Assessment for Silver: EPA-440/4-81-017*. Risk Assessment, Washington, DC: Office of Water Regulations and Standards .

—. 2010. "Emerging Contaminants- Nanomaterials." 10. Accessed 03 26, 2017. https://archive.epa.gov/region9/mediacenter/web/pdf/emerging_contaminant_nanomaterials.pdf.

United States Environmental Protection Agency. 2002. *Nitrification.* Distribution System Issue Paper, Washington, D.C: Office of Ground Water and Drinking Water.

Van Der Heijden, M., R. Bardgett, and N. Van Straalen. 2008. "The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems." *Ecology Letters* 11 (3): 298-310.

Vance, M., T. Kuiken, E. Vejerano, S. McGinnis, M. Hochella, D. Rejeski, and M. Hull. 2015. "Nanotechnology in the real world: Redeveloping the nanomaterial consumer products inventory." *Journal of Nanotechnology* 6: 1769-1780.

VandeVoort, A., and Y. Arai. 2012. "Effect of silver nanoparticles on soil denitrification kinetics." *Industrial Biotechnology* 358-365.

Vannini, C., G. Domingo, E. Onelli, F. De Mattia, I. Bruni, M. Marsoni, and M. Bracale. 2014. "Phytotoxic and genotoxic effects of silver nanoparticles exposure on germinating wheat seedlings." *Journal of Plant Physiology* 171: 1142-1148.

Vitanov, T., and A. Popov. 1983. "Adsorption of SO4 2- on growth of (111) and (100) faces of silver single crystals." *Journal of Electroanalytical Chemistry and Interfacial Electrochemistry* 159:437-441.

Vitosh, M. 1998. "Wheat Facts: Wheat Fertility and Fertilization." *Michigan State University Field Crops Team.* May. Accessed April 18, 2018. http://fieldcrop.msu.edu/uploads/documents/E2526.pdf.

Wainwright, M., W. Nevell, and S. Grayston. 1986. "Effects of organic matter on sulphur oxidation in soil and influence of sulphur oxidation on soil nitrification." *Plant and Soil* 96: 369-376.

Wallenstein, M., and M. Weintraub. 2008. "Emerging tools for measuring and modeling the in situ activity of soil extracellular enzymes." *Soil Biology & Biochemistry* 40: 2098-2106.

Wang, G., and D. Or. 2010. "Aqueous films limit bacterial cell motility and colony explansion on partially saturated rough surfaces." *Environmental Microbiology* 12: 1363-1373.

Wang, J., and H. Gu. 2015. "Novel Metal Nanomaterials and Their Catalytic Applications." *Molecules* 20: 17070-17092.

Wang, P, N Menzies, E Lombi, R Sekine, F Blamey, M Hernandez-Soriano, M Cheng, et al. 2015. "Silver sulfide nanoparticles (Ag2S-NPs) are taken up by plants and are phytotoxic." *Nanotoxicology.*

Wang, P., N. Menzies, P. Dennis, J. Guo, C. Forstner, R. Sekine, E. Lombi, P. Bertsch, and P. Kopittke. 2016. "Silver nanoparticles entering soils via the wastewater-sludge-soil pathway pose low risk to plants but elevated Cl concentrations increase Ag bioavailability." *Environmental Science & Technology* 50: 8274-8281.

Ward, N., E. Roberts, and R. Brooks. 1979. "Silver uptake by seedlings of Lolium perenne L. and Trifolium repens L." *New Zealand Journal of Science* 22:129-132.

Wasson, A., R. Richards, R. Chatrath, S. Misra, S. Prasad, G. Rebetzke, J. Kirkegaard, J. Christopher, and M. Watt. 2012. "Traits and selection strategies to improve root systems and water uptake in water-limited wheat crops." *Journal of Experimental Botany* 68:3485-3498.

Water Environment Association of Ontario. 2009. "Frequently Asked Questions on Biosolids Management." *Canadian Water and Wastewater Association.* July. Accessed December 11, 2017. http://www.cwwa.ca/cbp-pcb/pdf%20files/FAQ_on_Biosolids_Management.pdf.

Weber, K, and R Legge. 2010. "Community-Level Physiological Profiling." In *Bioremediation. Methods in Molecular Biology (Methods and Protocols), vol 599,* by S P Cummings, 263-281. NY, NY: Humana Press.

Weber, K, and R Legge. 2009. "One-dimensional metric for tracking bacterial community divergence using sole carbon source utilization patterns." *Journal of Microbial Methods* 79 (1): 55-61.

Weber, K, J Grove, M Gehder, W Anderson, and R Legge. 2007. "Data transformation in the analysis of community-level substrate utilization data from microplates." *Journal of Microbial Methods* 69 (3): 461-469.

Weber, K., M. Gehder, and R. Legge. 2008. "Assessment of changes in the microbial community of constructed wetland mesocosms in response to acid main drainage exposure." *Water Research* 180-188.

Wei, H., B. Guenet, S. Vicca, N. Nunan, H. AbdElgawad, V. Pouteau, W. Shen, and I. Janssens. 2014. "Thermal acclimation of organic matter decomposition in artifical forest soil is related to shifts in microbial community composition." *Soil Biology and Biochemistry* 71:1-12.

Weintraub, M., L. Scott-Denton, S. Schmidt, and R. Monson. 2007. "The effects of tree rhizodeposition on soil exoenzyme activity, dissolved organic carbon, and nutrient availability in a subalpine forest ecosystem." *Oecologia* 327-338.

Xu, X., W. Bronlow, S. Kyriacou, Q. Wan, and J. Viola. 2004. "Real-time probing of membrane transport in living microbial cells using single nanoparticle optics and living cell imaging." *Biochemistry* 43: 10400-10413.

Yang, W., C. Shen, Q. Ji, H. An, J. Wang, Q. Lui, and Z. Zhang. 2009. "Food storage material silver nanoparticles interfere with DNA replication fidelity and bind with DNA." *Nanotechnology* 20 (8).

Yin, L., B. Colman, B. McGill, J. Wright, and E. Bernhardt. 2012. "Effects of silver nanoparticle exposure on germination and early growth of eleven wetland plants." *Public Library of Science* 7 (10).

Yin, L., Y. Cheng, B. Epinasse, B. Colman, M. Auffan, M. Wiesner, J. Rose, and E. Bernhardt. 2011. "More than the ions: the effects of silver nanoparticles on lolium multiflorum." *Environmental Science and Technology* 45 (6): 2360-2367.

Yoo-iam, M., R. Chaichana, and T. Satapanajaru. 2014. "Toxicity, bioaccumulation and biomagnification of silver nanoparticles in green algae (Chlorella sp.), water flea (Moina macrocopa), bloodworm (Chironomus spp.) and silver barb (Barbonymus gonionotus)." *Chemical Speciation & Bioavailabilty* 26 (4): 257-265.

Young, C., P. Kampfer, M. Ho, H. Busse, B. Huber, A. Arun, F. Shen, W. Lai, and P. Rekha. 2007. "Arenimonas malthae sp. nov., a gammaproteobacterium isolated from an oil-contaminated site." *International Journal of Systematic and Evolutionary Microbiology* 57: 2790-2793.

Young, I., and J. Crawford. 2004. "Interactions and self-organization in the soil microbe complex." *Science* 304:1634-1637.

Yu, S., Y. Yin, and J. Liu. 2013. "Silver nanoparticles in the environment." *Environ Sci Process Impacts* 15:78-92.

Yurkov, G., A. Fionov, Y. Koksharov, and S. Gubin. 2007. "Electrical and magnetic properties of nanomaterials containing iron or cobalt nanoparticles." *Inorganic Materials* 43 (8): 834-844.

Zak, J., M. Willig, D. Moorhead, and H. Wildman. 1994. "Functional diversity of microbial communities: a quantitative approach." *Soil Biology and Biochemistry* 26: 1101-1108.

Zhai, Y., E. Hunting, W. Peijnenburg, M. Vijver, and M. Wouters. 2016. "Silver Nanoparticles, Ions, and Shape Governing Soil Microbial Functional Diversity: Nano Shapes Micro." *Frontiers in Microbiology* 7: 1123.

Zhao, C., F. Degryse, V. Gupta, and M. McLaughlin. 2014. "Elemental Sulfur Oxidation in Australian Cropping Soils." *Soil Science Society of America Journal* 79: 89-96.

8 Appendices

8.1 Appendix A

The maximum concentration of nanomaterials added to soil in biosolid amendments was calculated as follows.

Based on Ontario's biosolid application rates, 120,000 tonnes of dry biosolids to approximately 150 km^2 annually (Lapen et al. 2008).

$$\frac{Mass\ of\ biosolids}{m^2} = \frac{120000\ t}{150\ km^2} * \frac{1\ km^2}{1000000\ \ m^2} * \frac{1000\ kg}{1\ t} = \frac{0.8\ kg}{m^2}$$

Assuming a 15 cm furrow depth, the volumetric mass loading of the applied biosolids can be calculated.

$$Volumetric\ mass\ loading = \frac{0.8\ kg}{m^2} * \frac{1}{0.15\ m} = \frac{5.3\ kg\ biosolids}{m^3}$$

Based on U.S. predicted maximum concentration of AgNP in STP sludge (Gottschalk, et al. 2009) and assuming a soil bulk density of clay loam, 1450 kg/m^3 (United States Department of Agriculture 2016.), the concentration of nanomaterials added to soil per biosolid amendment can be determined.

$$\begin{aligned} &Concentration\ of\ AgNM\ added\ to\ soil\ per\ biosolids\ application \\ &= \frac{5.86\ mg\ AgNM}{kg\ biosolids} * \frac{5.3\ kg\ biosolids}{m^3} * \frac{m^3}{1450\ kg\ soil} * \frac{1000\ \mu g}{mg} \\ &= \frac{21.4\ \mu g\ AgNM}{kg\ soil} \end{aligned}$$

Table A.1: Summary of percent inhibition, exposure concentration and nanoparticle size in various species

Species	Inhibition (%)	Exposure Concentration (mg/L)	Nanoparticle Size (nm)	Reference
Escherichia coli	100	60	16	Raffi et al. 2008
E. coli	100	15	1000	Smetana et al. 2008
Staphylococcus aureus	99.97	15	1000	Smetana et al. 2008
E. coli	100	1.5	1000	Smetana et al. 2008
S. aureus	99.01	1.5	1000	Smetana et al. 2008
Chlamydomonas reinhardtii	50	0.35	25	Navarro et al. 2008
Candida albicans	80	3	3	Kim et al. 2008
Candida tropicalis	80	7	3	Kim et al. 2008
Candida globrata	80	4	3	Kim et al. 2008
Candida Parapsilosis	80	18	3	Kim et al. 2008
Candida krusei	80	13	3	Kim et al. 2008
Trichophyton mentagrophytes	80	2.5	3	Kim et al. 2008
Chlorella sp.	50	0.89	85	Yoo-iam et al. 2014
Moina macrocopa	50	1.11	85	Yoo-iam et al. 2014
Chrionomus spp.	50	1.08	85	Yoo-iam et al. 2014
Barbonysmus gonionotus	50	1.76	85	Yoo-iam et al. 2014
Spirodela polyrhiza	50	13.67	7.8	Jiang et al. 2012
Polyboroides radiatus	50	13	10	Lee et al. 2012
Sorghum bicolor	50	26	10	Lee et al. 2012
Cucumis sativus	24	100	30	Barrena et al. 2009
Lactuca sativa	5	100	30	Barrena et al. 2009
Mice fibroblast	43.4	50	16.6	Arora et al. 2009
Human colon cancer	40.2	100	16.6	Arora et al. 2009
Mice fibroblast	50	61	16.6	Arora et al. 2009
Daphnia magna	50	0.004	15	Asghari et al. 2012
D. magna	50	0.002	16.6	Asghari et al. 2012
D. magna	50	0.187	20	Asghari et al. 2012

8.2 Appendix B

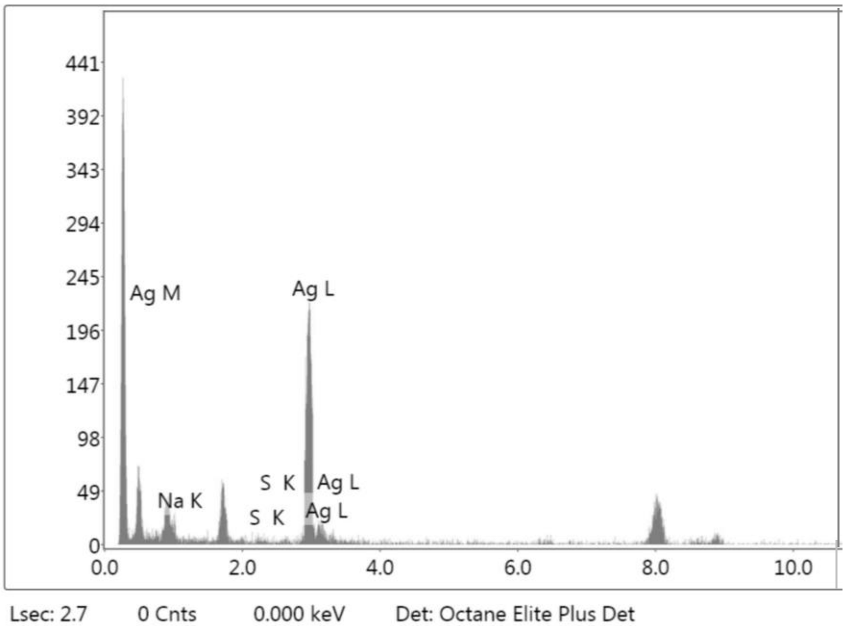

Figure B.1: Spectra of PVP coated silver nanoparticle from EDS Analysis

Table B.1: Smart Quant results of PVP coated silver nanoparticle EDS Analysis

Element	Weight (%)	Atomic (%)
Na	46.84	79.46
S	1.55	1.88
Ag	51.61	18.66

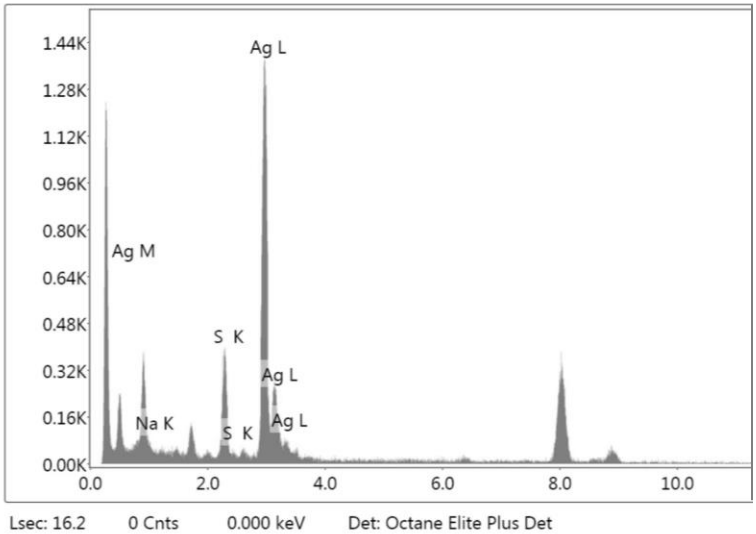

Figure B.2: Spectra of sulphidized silver nanoparticle from EDS Analysis

Table B.2: Smart Quant results of sulphidized silver nanoparticle EDS Analysis

Element	Weight (%)	Atomic (%)
Na	41.50	70.39
S	9.90	12.04
Ag	48.59	17.56

Table B.3: Levene's test for homogeneity of variance between treatments

Treatment	Type	Levene's Test		
		Statistic	Degrees of Freedom (DOF)	Significance
Ionic silver 1 mg/kg	Talc	0.031	1, 8	0.864
	Solution			
Ionic silver 10 mg/kg	Talc	0.86	1, 8	0.381
	Solution			
Uncoated AgNPs 1 mg/kg	Talc	4.866	1, 8	0.058
	Solution			
Uncoated AgNPs 10 mg/kg	Talc	2.584	1, 8	0.147
	Solution			

Table B.4: Shapiro-Wilk test of normality of treatments and Kruskal-Wallis one-way variance testing for non-normally distributed samples

Treatment	Type	Shapiro-Wilk			Kruskal-Wallis		
		Statistic	DOF	Significance	X^2	DOF	Significance
Ionic silver 1 mg/kg	Talc	0.927	5	0.575			
	Solution	0.901	5	0.413			
Ionic silver 10 mg/kg	Talc	0.889	5	0.350			
	Solution	0.859	5	0.225			
Uncoated AgNPs 1 mg/kg	Talc	0.675	5	0.005	4.811	1	0.028
	Solution	0.821	5	0.119			
Uncoated AgNPs 10 mg/kg	Talc	0.833	5	0.146	1.844	1	0.175
	Solution	0.76	5	0.037			

Table B.5: ANOVA results for comparison between talc and solution treatment concentrations of AgNPs in soil (n=5)

Treatment	DOF	F	Significance
Ionic silver 1 mg/kg	1	1.619	0.239
Ionic silver 10 mg/kg	1	1.135	0.318

Table B.6: Kruskal-Wallis one-way variance test between concentrations of ionic silver and uncoated AgNPs in talc or solution (n=5)

Type	Desired Concentration (mg/kg)	X^2	DOF	Significance
Talc	1	2.455	1	0.117
	10	0.884	1	0.347
Solution	1	4.811	1	0.028
	10	0.273	1	0.602

Table B.7: ANOVA results for comparison between uncoated AgNPs and ionic silver in soil treated with talc or solution (n=5)

Type	Desired Concentration (mg/kg)	DOF	F	Significance
Talc	1	1	3.179	0.112
	10	1	1.437	0.265
Solution	1	1	15.388	0.004
	10	1	0.030	0.868

8.3 Appendix C

Table C.1: Repeated measure ANOVA comparing total silver concentrations in each month's treatments

Effect	Wilk's Lambda Statistic Value	F	Hypothesis DOF	Error DOF	Significance
Time	0.981	0.530	2	54	0.592
Time*Treatment	0.815	0.580	20	108	0.919

Table C.2: T-test of treatment upper and lower region silver concentrations

Treatment	t	DOF	Significance (2-tailed)	Mean Difference	95% Confidence Interval	
					Lower	Upper
Control	0.971	16	0.346	0.076	-0.090	0.241
Soap Control	0.074	16	0.346	0.003	-0.078	0.084
Weathered Low	-0.827	16	0.942	-0.083	-0.295	0.130
Sulphidized Low	-0.414	16	0.942	-0.048	-0.292	0.197
Sulphidized High	-1.087	16	0.420	-0.415	-1.225	0.394
Ionic Low	0.002	16	0.422	0.000	-0.146	0.147
Ionic High	-0.632	16	0.684	-0.730	-3.178	1.719
PVP Low	0.132	16	0.684	0.013	-0.196	0.222
PVP High	0.212	15	0.293	0.300	-2.721	3.322
Uncoated Low	0.497	15	0.299	0.031	-0.103	0.165
Uncoated High	1.021	16	0.999	0.987	-1.063	3.037

Table C.3: One-way ANOVA for silver concentrations of low and high concentration treatments

		Sum of Squares	DOF	Mean Square	F	Significance
Low	Between Groups	4.568	4	1.142	10.329	0.000
	Within Groups	9.398	85	0.111		
	Total	13.966	89			
High	Between Groups	300.647	3	100.216	13.268	0.000
	Within Groups	513.607	68	7.553		
	Total	814.253	71			

Table C.4: Multiple comparisons for low concentration treatments using Tukey's test

Treatment (I)	Treatment (J)	Mean Difference (I-J)	Significance	95% Confidence Interval	
				Lower Bound	Upper Bound
Weathered Low	Sulphidized Low	0.42939*	0.002	0.1205	0.7383
	Ionic Low	0.20564	0.349	-0.1033	0.5146
	PVP Low	0.60744*	0.000	0.2985	0.9164
	Uncoated Low	0.54529*	0.000	0.2364	0.8542
Sulphidized Low	Weathered Low	-0.42939*	0.002	-0.7383	-0.1205
	Ionic Low	-0.22375	0.266	-0.5327	0.0852
	PVP Low	0.17805	0.498	-0.1309	0.4870
	Uncoated Low	0.11590	0.833	-0.1930	0.4248
Ionic Low	Weathered Low	-0.20564	0.349	-0.5146	0.1033
	Sulphidized Low	0.22375	0.266	-0.0852	0.5327
	PVP Low	0.40180*	0.004	0.0929	0.7107
	Uncoated Low	0.33965*	0.024	0.0307	0.6486
PVP Low	Weathered Low	-0.60744*	0.000	-0.9164	-0.2985
	Sulphidized Low	-0.17805	0.498	-0.4870	0.1309
	Ionic Low	-0.40180*	0.004	-0.7107	-0.0929
	Uncoated Low	-0.06215	0.980	-0.3711	0.2468
Uncoated Low	Weathered Low	-0.54529*	0.000	-0.8542	-0.2364
	Sulphidized Low	-0.11590	0.833	-0.4248	0.1930
	Ionic Low	-0.33965*	0.024	-0.6486	-0.0307
	PVP Low	0.06215	0.980	-0.2468	0.3711

Table C.5: Tukey test subset treatment groups for low concentration treatments

Treatment	N	Subset		
		1	2	3
PVP Low	18	0.3058		
Uncoated Low	18	0.3679		
Sulphidized Low	18	0.4838	0.4838	
Ionic Low	18		0.7076	0.7076
Weathered Low	18			0.9132
Significance		0.498	0.266	0.349

Table C.6: Multiple comparisons for high concentration treatments using Tukey's test

Treatment (I)	Treatment (J)	Mean Difference (I-J)	Significance	95% Confidence Interval	
				Lower Bound	Upper Bound
Sulphidized High	Ionic High	-5.70410*	0.000	-8.1168	-3.2914
	PVP High	-2.77926*	0.018	-5.1920	-0.3665
	Uncoated High	-2.06997	0.118	-4.4827	0.3428
Ionic High	Sulphidized High	5.70410*	0.000	3.2914	8.1168

	PVP High	2.92483*	0.011	0.5121	5.3376
	Uncoated High	3.63413*	0.001	1.2214	6.0469
PVP High	Sulphidized High	2.77926*	0.018	0.3665	5.1920
	Ionic High	-2.92483*	0.011	-5.3376	-0.5121
	Uncoated High	0.70929	0.866	-1.7034	3.1220
Uncoated High	Sulphidized High	2.06997	0.118	-0.3428	4.4827
	Ionic High	-3.63413*	0.001	-6.0469	-1.2214
	PVP High	-0.70929	0.866	-3.1220	1.7034

Table C.7: Tukey test subset treatment groups for high concentration treatments

Treatment	N	Subsets		
		1	2	3
Sulphidized High	18	3.0726		
Uncoated High	18	5.1426	5.1426	
PVP High	18		5.8519	
Ionic High	18			8.7767
Significance		0.118	0.866	1.000

Table C.8: Levene's test for homogeneity of variance of nanoparticle compositions from EDS

Nanoparticle Type	Levene Statistic	DOF 1	DOF 2	Significance
Uncoated	3.858	2	21	0.037
PVP	0.648	2	21	0.533
Sulphidized	3.295	2	21	0.057

Table C.9: Kruskal-Wallis non-parametric test of variance for uncoated nanoparticle composition

	Uncoated Composition
X^2	1.995
DOF	2
Significance	0.369

Table C.10: One-way ANOVA for PVP nanoparticle composition

	Sum of Squares	DOF	Mean Square	F	Significance
Between Groups	166.153	2	83.076	4.348	0.026
Within Groups	401.272	21	19.108		
Total	567.425	23			

Table C.11: Multiple comparisons of treatments from Tukey test of PVP AgNP composition

Condition (I)		Mean Difference (I-J)	Significance	95% Confidence Interval	
				Lower Bound	Upper Bound
No Soil	Initial Soil	6.44125*	0.020	0.9322	11.9503
	Three Months	3.03000	0.366	-2.4791	8.5391
Initial Soil	No Soil	-6.44125*	0.020	-11.9503	-0.9322
	Three Months	-3.41125	0.284	-8.9203	2.0978
Three Months	No Soil	-3.03000	0.366	-8.5391	2.4791
	Initial Soil	3.41125	0.284	-2.0978	8.9203

Table C.12: One-way ANOVA for sulphidized nanoparticle composition

	Sum of Squares	DOF	Mean Square	F	Significance
Between Groups	180.190	2	90.095	4.246	0.028
Within Groups	445.557	21	21.217		
Total	625.747	23			

Table C.13: Multiple comparisons of treatments from Tukey test of sulphidized AgNP composition

Condition (I)		Mean Difference (I-J)	Significance	95% Confidence Interval	
				Lower Bound	Upper Bound
No Soil	Initial Soil	5.09000	0.093	0.093	-0.7151
	Three Months	6.33375*	0.031	0.031	0.5286
Initial Soil	No Soil	-5.09000	0.093	0.093	-10.8951
	Three Months	1.24375	0.853	0.853	-4.5614
Three Months	No Soil	-6.33375*	0.031	0.031	-12.1389
	Initial Soil	-1.24375	0.853	0.853	-7.0489

Table C.14: One-way ANOVA for moisture content

	Sum of Squares	DOF	Mean Square	F	Significance
Between Groups	82.511	10	8.251	2.889	0.018
Within Groups	62.829	22	2.856		
Total	145.339	32			

Table C.15: Multiple comparisons of treatments from Tukey test of moisture content

Treatment (I)	Treatment (J)	Mean Difference (I-J)	Significance	95% Confidence Interval	
				Lower Bound	Upper Bound
Control	Ionic Low	-0.3341	1.000	-5.2667	4.5985
	Ionic High	-1.0634	0.999	-5.9960	3.8692
	PVP Low	1.2690	0.997	-3.6636	6.2016
	PVP High	0.5611	1.000	-4.3715	5.4937
	Soap	-1.2377	0.997	-6.1703	3.6949
	Sulphidized Low	0.7235	1.000	-4.2091	5.6561
	Sulphidized High	2.2575	0.851	-2.6751	7.1901
	Uncoated Low	0.7505	1.000	-4.1821	5.6831
	Uncoated High	-0.4846	1.000	-5.4172	4.4480
	Weathered Low	-4.0779	0.168	-9.0105	.8547
Ionic Low	Control	0.3341	1.000	-4.5985	5.2667
	Ionic High	-0.7293	1.000	-5.6619	4.2033
	PVP Low	1.6031	0.981	-3.3295	6.5357
	PVP High	0.8952	1.000	-4.0374	5.8278
	Soap	-0.9036	1.000	-5.8362	4.0289
	Sulphidized Low	1.0575	0.999	-3.8751	5.9901
	Sulphidized High	2.5916	0.724	-2.3410	7.5241
	Uncoated Low	1.0846	0.999	-3.8480	6.0172
	Uncoated High	-0.1506	1.000	-5.0832	4.7820
	Weathered Low	-3.7439	0.255	-8.6765	1.1887
Ionic High	Control	1.0634	0.999	-3.8692	5.9960
	Ionic Low	0.7293	1.000	-4.2033	5.6619
	PVP Low	2.3324	0.825	-2.6002	7.2650
	PVP High	1.6245	0.979	-3.3081	6.5571
	Soap	-0.1743	1.000	-5.1069	4.7583
	Sulphidized Low	1.7869	0.960	-3.1457	6.7195
	Sulphidized High	3.3209	0.405	-1.6117	8.2535
	Uncoated Low	1.8139	0.956	-3.1187	6.7465
	Uncoated High	.5788	1.000	-4.3538	5.5114
	Weathered Low	-3.0145	0.536	-7.9471	1.9181
PVP Low	Control	-1.2690	0.997	-6.2016	3.6636
	Ionic Low	-1.6031	0.981	-6.5357	3.3295
	Ionic High	-2.3324	0.825	-7.2650	2.6002
	PVP High	-0.7079	1.000	-5.6405	4.2247
	Soap	-2.5067	0.759	-7.4393	2.4259
	Sulphidized Low	-0.5455	1.000	-5.4781	4.3871
	Sulphidized High	.09885	1.000	-3.9441	5.9211
	Uncoated Low	-0.5185	1.000	-5.4511	4.4141
	Uncoated High	-1.7536	0.965	-6.6862	3.1790
	Weathered Low	-5.3469*	0.026	-10.2795	-.4143
PVP High	Control	-0.5611	1.000	-5.4937	4.3715
	Ionic Low	-0.8952	1.000	-5.8278	4.0374

	Ionic High	-1.6245	0.979	-6.5571	3.3081
	PVP Low	0.7079	1.000	-4.2247	5.6405
	Soap	-1.7988	0.958	-6.7314	3.1338
	Sulphidized Low	0.1624	1.000	-4.7702	5.0950
	Sulphidized High	1.6964	0.972	-3.2362	6.6290
	Uncoated Low	0.1894	1.000	-4.7432	5.1220
	Uncoated High	-1.0457	0.999	-5.9783	3.8869
	Weathered Low	-4.6390	0.077	-9.5716	.2935
Soap Control	Control	1.2377	0.997	-3.6949	6.1703
	Ionic Low	0.9036	1.000	-4.0289	5.8362
	Ionic High	0.1743	1.000	-4.7583	5.1069
	PVP Low	2.5067	0.759	-2.4259	7.4393
	PVP High	1.7988	0.958	-3.1338	6.7314
	Sulphidized Low	1.9612	0.930	-2.9714	6.8938
	Sulphidized High	3.4952	0.338	-1.4374	8.4278
	Uncoated Low	1.9882	0.924	-2.9444	6.9208
	Uncoated High	0.7531	1.000	-4.1795	5.6857
	Weathered Low	-2.8402	0.615	-7.7728	2.0924
Sulphidized Low	Control	-0.7235	1.000	-5.6561	4.2091
	Ionic Low	-1.0575	0.999	-5.9901	3.8751
	Ionic High	-1.7869	0.960	-6.7195	3.1457
	PVP Low	0.5455	1.000	-4.3871	5.4781
	PVP High	-0.1624	1.000	-5.0950	4.7702
	Soap	-1.9612	0.930	-6.8938	2.9714
	Sulphidized High	1.5340	0.986	-3.3986	6.4666
	Uncoated Low	0.0270	1.000	-4.9056	4.9596
	Uncoated High	-1.2081	0.998	-6.1407	3.7245
	Weathered Low	-4.8014	0.061	-9.7340	0.1312
Sulphidized High	Control	-2.2575	0.851	-7.1901	2.6751
	Ionic Low	-2.5916	0.724	-7.5241	2.3410
	Ionic High	-3.3209	0.405	-8.2535	1.6117
	PVP Low	-0.9885	1.000	-5.9211	3.9441
	PVP High	-1.6964	0.972	-6.6290	3.2362
	Soap	-3.4952	0.338	-8.4278	1.4374
	Sulphidized Low	-1.5340	0.986	-6.4666	3.3986
	Uncoated Low	-1.5070	0.987	-6.4396	3.4256
	Uncoated High	-2.7421	0.659	-7.6747	2.1905
	Weathered Low	-6.3354*	0.005	-11.2680	-1.4028
Uncoated Low	Control	-0.7505	1.000	-5.6831	4.1821
	Ionic Low	-1.0846	0.999	-6.0172	3.8480
	Ionic High	-1.8139	0.956	-6.7465	3.1187
	PVP Low	0.5185	1.000	-4.4141	5.4511
	PVP High	-0.1894	1.000	-5.1220	4.7432
	Soap	-1.9882	0.924	-6.9208	2.9444
	Sulphidized Low	-0.0270	1.000	-4.9596	4.9056
	Sulphidized High	1.5070	0.987	-3.4256	6.4396

	Uncoated High	-1.2351	0.997	-6.1677	3.6975
	Weathered Low	-4.8284	0.059	-9.7610	.1042
Uncoated High	Control	0.4846	1.000	-4.4480	5.4172
	Ionic Low	0.1506	1.000	-4.7820	5.0832
	Ionic High	-0.5788	1.000	-5.5114	4.3538
	PVP Low	1.7536	0.965	-3.1790	6.6862
	PVP High	1.0457	0.999	-3.8869	5.9783
	Soap	-0.7531	1.000	-5.6857	4.1795
	Sulphidized Low	1.2081	0.998	-3.7245	6.1407
	Sulphidized High	2.7421	0.659	-2.1905	7.6747
	Uncoated Low	1.2351	0.997	-3.6975	6.1677
	Weathered Low	-3.5933	0.304	-8.5259	1.3393
Weathered Low	Control	4.0779	0.168	-.8547	9.0105
	Ionic Low	3.7439	0.255	-1.1887	8.6765
	Ionic High	3.0145	0.536	-1.9181	7.9471
	PVP Low	5.3469*	0.026	.4143	10.2795
	PVP High	4.6390	0.077	-.2935	9.5716
	Soap	2.8402	0.615	-2.0924	7.7728
	Sulphidized Low	4.8014	0.061	-.1312	9.7340
	Sulphidized High	6.3354*	0.005	1.4028	11.2680
	Uncoated Low	4.8284	0.059	-.1042	9.7610
	Uncoated High	3.5933	0.304	-1.3393	8.5259

Table C.16: Subsets of treatment groups from post-hoc Tukey test

Treatment	N	Subset	
		1	2
Sulphidized High	3	20.4306	
PVP Low	3	21.4191	
Uncoated Low	3	21.9376	21.9376
Sulphidized Low	3	21.9646	21.9646
PVP High	3	22.1270	22.1270
Control	3	22.6881	22.6881
Ionic Low	3	23.0221	23.0221
Uncoated High	3	23.1727	23.1727
Ionic High	3	23.7515	23.7515
Soap Control	3	23.9258	23.9258
Weathered Low	3		26.7660
Significance		0.338	0.059

Table C.17: One-way ANOVA for organic matter

	Sum of Squares	DOF	Mean Square	F	Significance
Between Groups	23.174	10	2.317	2.100	0.073
Within Groups	23.178	21	1.104		
Total	46.352	31			

Table C.18: One-way ANOVA for water holding capacity at 24 hours

	Sum of Squares	DOF	Mean Square	F	Significance
Between Groups	0.009	10	0.001	0.349	0.956
Within Groups	0.058	22	0.003		
Total	0.067	32			

Table C.19: One-way ANOVA for water holding capacity at 48 hours

	Sum of Squares	DOF	Mean Square	F	Significance
Between Groups	0.048	10	0.005	0.481	0.885
Within Groups	0.219	22	0.010		
Total	0.266	32			

Table C.20: One-way ANOVA for pH

	Sum of Squares	DOF	Mean Square	F	Significance
Between Groups	0.193	10	0.019	0.581	0.812
Within Groups	0.730	22	0.033		
Total	0.923	32			

Table C.21: One-way ANOVA for conductivity

	Sum of Squares	DOF	Mean Square	F	Significance
Between Groups	17537.488	10	1753.749	11.804	0.000
Within Groups	3268.462	22	148.566		
Total	20805.950	32			

Table C.22: Multiple comparisons of treatments from Tukey test of conductivity

Treatment (I)	Treatment (J)	Mean Difference (I-J)	Significance	95% Confidence Interval	
				Lower Bound	Upper Bound
Control	Ionic Low	3.4800	1.000	-32.0969	39.0569
	Ionic High	-22.0633	0.517	-57.6402	13.5136
	PVP Low	6.8033	1.000	-28.7736	42.3802
	PVP High	6.4900	1.000	-29.0869	42.0669
	Soap	-63.0300*	0.000	-98.6069	-27.4531
	Sulphidized Low	-4.1300	1.000	-39.7069	31.4469
	Sulphidized High	0.3367	1.000	-35.2402	35.9136
	Uncoated Low	-1.0967	1.000	-36.6736	34.4802
	Uncoated High	-4.3300	1.000	-39.9069	31.2469
	Weathered Low	-52.8633*	0.001	-88.4402	-17.2864
Ionic Low	Control	-3.4800	1.000	-39.0569	32.0969
	Ionic High	-25.5433	0.322	-61.1202	10.0336
	PVP Low	3.3233	1.000	-32.2536	38.9002
	PVP High	3.0100	1.000	-32.5669	38.5869
	Soap	-66.5100*	0.000	-102.0869	-30.9331
	Sulphidized Low	-7.6100	0.999	-43.1869	27.9669
	Sulphidized High	-3.1433	1.000	-38.7202	32.4336
	Uncoated Low	-4.5767	1.000	-40.1536	31.0002
	Uncoated High	-7.8100	0.999	-43.3869	27.7669
	Weathered Low	-56.3433*	0.000	-91.9202	-20.7664
Ionic High	Control	22.0633	0.517	-13.5136	57.6402
	Ionic Low	25.5433	0.322	-10.0336	61.1202
	PVP Low	28.8667	0.186	-6.7102	64.4436
	PVP High	28.5533	0.196	-7.0236	64.1302
	Soap	-40.9667*	0.015	-76.5436	-5.3898
	Sulphidized Low	17.9333	0.767	-17.6436	53.5102
	Sulphidized High	22.4000	0.496	-13.1769	57.9769
	Uncoated Low	20.9667	0.585	-14.6102	56.5436
	Uncoated High	17.7333	0.778	-17.8436	53.3102
	Weathered Low	-30.8000	0.130	-66.3769	4.7769
PVP Low	Control	-6.8033	1.000	-42.3802	28.7736
	Ionic Low	-3.3233	1.000	-38.9002	32.2536
	Ionic High	-28.8667	0.186	-64.4436	6.7102
	PVP High	-0.3133	1.000	-35.8902	35.2636
	Soap	-69.8333*	0.000	-105.4102	-34.2564
	Sulphidized Low	-10.9333	0.987	-46.5102	24.6436
	Sulphidized High	-6.4667	1.000	-42.0436	29.1102
	Uncoated Low	-7.9000	0.999	-43.4769	27.6769
	Uncoated High	-11.1333	0.985	-46.7102	24.4436
	Weathered Low	-59.6667*	0.000	-95.2436	-24.0898
PVP High	Control	-6.4900	1.000	-42.0669	29.0869
	Ionic Low	-3.0100	1.000	-38.5869	32.5669

	Ionic High	-28.5533	0.196	-64.1302	7.0236
	PVP Low	0.3133	1.000	-35.2636	35.8902
	Soap	-69.5200*	0.000	-105.0969	-33.9431
	Sulphidized Low	-10.6200	0.989	-46.1969	24.9569
	Sulphidized High	-6.1533	1.000	-41.7302	29.4236
	Uncoated Low	-7.5867	0.999	-43.1636	27.9902
	Uncoated High	-10.8200	0.988	-46.3969	24.7569
	Weathered Low	-59.3533*	0.000	-94.9302	-23.7764
Soap	Control	63.0300*	0.000	27.4531	98.6069
	Ionic Low	66.5100*	0.000	30.9331	102.0869
	Ionic High	40.9667*	0.015	5.3898	76.5436
	PVP Low	69.8333*	0.000	34.2564	105.4102
	PVP High	69.5200*	0.000	33.9431	105.0969
	Sulphidized Low	58.9000*	0.000	23.3231	94.4769
	Sulphidized High	63.3667*	0.000	27.7898	98.9436
	Uncoated Low	61.9333*	0.000	26.3564	97.5102
	Uncoated High	58.7000*	0.000	23.1231	94.2769
	Weathered Low	10.1667	0.992	-25.4102	45.7436
Sulphidized Low	Control	4.1300	1.000	-31.4469	39.7069
	Ionic Low	7.6100	0.999	-27.9669	43.1869
	Ionic High	-17.9333	0.767	-53.5102	17.6436
	PVP Low	10.9333	0.987	-24.6436	46.5102
	PVP High	10.6200	0.989	-24.9569	46.1969
	Soap	-58.9000*	0.000	-94.4769	-23.3231
	Sulphidized High	4.4667	1.000	-31.1102	40.0436
	Uncoated Low	3.0333	1.000	-32.5436	38.6102
	Uncoated High	-0.2000	1.000	-35.7769	35.3769
	Weathered Low	-48.7333*	0.003	-84.3102	-13.1564
Sulphidized High	Control	-0.3367	1.000	-35.9136	35.2402
	Ionic Low	3.1433	1.000	-32.4336	38.7202
	Ionic High	-22.4000	0.496	-57.9769	13.1769
	PVP Low	6.4667	1.000	-29.1102	42.0436
	PVP High	6.1533	1.000	-29.4236	41.7302
	Soap	-63.3667*	0.000	-98.9436	-27.7898
	Sulphidized Low	-4.4667	1.000	-40.0436	31.1102
	Uncoated Low	-1.4333	1.000	-37.0102	34.1436
	Uncoated High	-4.6667	1.000	-40.2436	30.9102
	Weathered Low	-53.2000*	0.001	-88.7769	-17.6231
Uncoated Low	Control	1.0967	1.000	-34.4802	36.6736
	Ionic Low	4.5767	1.000	-31.0002	40.1536
	Ionic High	-20.9667	0.585	-56.5436	14.6102
	PVP Low	7.9000	0.999	-27.6769	43.4769
	PVP High	7.5867	0.999	-27.9902	43.1636
	Soap	-61.9333*	0.000	-97.5102	-26.3564
	Sulphidized Low	-3.0333	1.000	-38.6102	32.5436
	Sulphidized High	1.4333	1.000	-34.1436	37.0102

	Uncoated High	-3.2333	1.000	-38.8102	32.3436
	Weathered Low	-51.7667*	0.001	-87.3436	-16.1898
Uncoated High	Control	4.3300	1.000	-31.2469	39.9069
	Ionic Low	7.8100	0.999	-27.7669	43.3869
	Ionic High	-17.7333	0.778	-53.3102	17.8436
	PVP Low	11.1333	0.985	-24.4436	46.7102
	PVP High	10.8200	0.988	-24.7569	46.3969
	Soap	-58.7000*	0.000	-94.2769	-23.1231
	Sulphidized Low	0.2000	1.000	-35.3769	35.7769
	Sulphidized High	4.6667	1.000	-30.9102	40.2436
	Uncoated Low	3.2333	1.000	-32.3436	38.8102
	Weathered Low	-48.5333*	0.003	-84.1102	-12.9564
Weathered Low	Control	52.8633*	0.001	17.2864	88.4402
	Ionic Low	56.3433*	0.000	20.7664	91.9202
	Ionic High	30.8000	0.130	-4.7769	66.3769
	PVP Low	59.6667*	0.000	24.0898	95.2436
	PVP High	59.3533*	0.000	23.7764	94.9302
	Soap	-10.1667	0.992	-45.7436	25.4102
	Sulphidized Low	48.7333*	0.003	13.1564	84.3102
	Sulphidized High	53.2000*	0.001	17.6231	88.7769
	Uncoated Low	51.7667*	0.001	16.1898	87.3436
	Uncoated High	48.5333*	0.003	12.9564	84.1102

Table C.23: Treatment group subsets from Tukey test of conductivity

Treatment	N	Subset		
		1	2	3
PVP Low	3	101.1667		
PVP High	3	101.4800		
Ionic Low	3	104.4900		
Sulphidized High	3	107.6333		
Control	3	107.9700		
Uncoated Low	3	109.0667		
Sulphidized Low	3	112.1000		
Uncoated High	3	112.3000		
Ionic High	3	130.0333	130.0333	
Weathered Low	3		160.8333	160.8333
Soap Control	3			171.0000
Significance		0.186	0.130	0.992

Table C.24: Repeated Measure ANOVA for AWCD treatment measures each month

Effect	Wilk's Lambda Statistic Value	F	Hypothesis DOF	Error DOF	Significance
Time	0.380	17.103	2	21	0.000
Time*Treatment	0.392	1.254	20	42	0.262

Table C.25: Mauchly's test of sphericity for AWCD measures

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	DOF	Significance
Time	0.844	3.574	2	0.167

Table C.26: Test of within-subject effects for AWCD with sphericity assumed

Effect	Sum of Squares	DOF	Mean Square	F	Significance
Time	0.271	2	0.135	14.695	0.000
Time * Treatment	0.210	20	0.010	1.139	0.349

Table C.27: One-way ANOVA for AWCD measures of each month

		Sum of Squares	DOF	Mean Square	F	Significance
Month 1	Between Groups	0.152	10	0.015	2.035	0.079
	Within Groups	0.165	22	0.007		
	Total	0.317	32			
Month 2	Between Groups	0.269	10	0.027	3.766	0.005
	Within Groups	0.157	22	0.007		
	Total	0.426	32			
Month 3	Between Groups	0.317	10	0.032	2.102	0.070
	Within Groups	0.332	22	0.015		
	Total	0.649	32			

Table C.28: Multiple comparisons for AWCD Month 2 treatments using Tukey's test

Treatment (I)	Treatment (J)	Mean Difference (I-J)	Significance	95% Confidence Interval	
				Lower Bound	Upper Bound
Control	Soap Control	-0.07667	0.986	-0.3234	0.1701
	Weathered Low	-0.13000	0.721	-0.3767	0.1167
	Sulphidized Low	0.00000	1.000	-0.2467	0.2467
	Sulphidized High	0.01333	1.000	-0.2334	0.2601
	Ionic Low	0.10333	0.906	-0.1434	0.3501
	Ionic High	0.23000	0.082	-0.0167	0.4767
	PVP Low	0.02000	1.000	-0.2267	0.2667
	PVP High	0.07667	0.986	-0.1701	0.3234
	Uncoated Low	-0.02667	1.000	-0.2734	0.2201
	Uncoated High	0.04667	1.000	-0.2001	0.2934
Soap Control	Control	0.07667	0.986	-0.1701	0.3234
	Weathered Low	-0.05333	0.999	-0.3001	0.1934
	Sulphidized Low	0.07667	0.986	-0.1701	0.3234
	Sulphidized High	0.09000	0.958	-0.1567	0.3367

	Ionic Low	0.18000	0.302	-0.0667	0.4267
	Ionic High	0.30667*	0.007	0.0599	0.5534
	PVP Low	0.09667	0.936	-0.1501	0.3434
	PVP High	0.15333	0.514	-0.0934	0.4001
	Uncoated Low	0.05000	1.000	-0.1967	0.2967
	Uncoated High	0.12333	0.775	-0.1234	0.3701
Weathered Low	Control	0.13000	0.721	-0.1167	0.3767
	Soap Control	0.05333	0.999	-0.1934	0.3001
	Sulphidized Low	0.13000	0.721	-0.1167	0.3767
	Sulphidized High	0.14333	0.603	-0.1034	0.3901
	Ionic Low	0.23333	0.075	-0.0134	0.4801
	Ionic High	0.36000*	0.001	0.1133	0.6067
	PVP Low	0.15000	0.543	-0.0967	0.3967
	PVP High	0.20667	0.157	-0.0401	0.4534
	Uncoated Low	0.10333	0.906	-0.1434	0.3501
	Uncoated High	0.17667	0.325	-0.0701	0.4234
Sulphidized Low	Control	0.00000	1.000	-0.2467	0.2467
	Soap Control	-0.07667	0.986	-0.3234	0.1701
	Weathered Low	-0.13000	0.721	-0.3767	0.1167
	Sulphidized High	0.01333	1.000	-0.2334	0.2601
	Ionic Low	0.10333	0.906	-0.1434	0.3501
	Ionic High	0.23000	0.082	-0.0167	0.4767
	PVP Low	0.02000	1.000	-0.2267	0.2667
	PVP High	0.07667	0.986	-0.1701	0.3234
	Uncoated Low	-0.02667	1.000	-0.2734	0.2201
	Uncoated High	0.04667	1.000	-0.2001	0.2934
Sulphidized High	Control	-0.01333	1.000	-0.2601	0.2334
	Soap Control	-0.09000	0.958	-0.3367	0.1567
	Weathered Low	-.14333	0.603	-0.3901	0.1034
	Sulphidized Low	-0.01333	1.000	-0.2601	0.2334
	Ionic Low	0.09000	0.958	-0.1567	0.3367
	Ionic High	0.21667	0.120	-0.0301	0.4634
	PVP Low	0.00667	1.000	-0.2401	0.2534
	PVP High	0.06333	0.997	-0.1834	0.3101
	Uncoated Low	-0.04000	1.000	-0.2867	0.2067
	Uncoated High	0.03333	1.000	-0.2134	0.2801
Ionic Low	Control	-0.10333	0.906	-0.3501	0.1434
	Soap Control	-0.18000	0.302	-0.4267	0.0667
	Weathered Low	-0.23333	0.075	-0.4801	0.0134
	Sulphidized Low	-0.10333	0.906	-0.3501	0.1434
	Sulphidized High	-0.09000	0.958	-0.3367	0.1567
	Ionic High	0.12667	0.749	-0.1201	0.3734
	PVP Low	-0.08333	0.975	-0.3301	0.1634
	PVP High	-0.02667	1.000	-0.2734	0.2201
	Uncoated Low	-0.13000	0.721	-0.3767	0.1167
	Uncoated High	-0.05667	0.999	-0.3034	0.1901

Ionic High	Control	-0.23000	0.082	-0.4767	0.0167
	Soap Control	-0.30667*	.007	-0.5534	-0.0599
	Weathered Low	-0.36000*	0.001	-0.6067	-0.1133
	Sulphidized Low	-0.23000	0.082	-0.4767	0.0167
	Sulphidized High	-0.21667	0.120	-0.4634	0.0301
	Ionic Low	-0.12667	0.749	-0.3734	0.1201
	PVP Low	-0.21000	0.144	-0.4567	0.0367
	PVP High	-0.15333	0.514	-0.4001	0.0934
	Uncoated Low	-0.25667*	0.037	-0.5034	-0.0099
	Uncoated High	-0.18333	0.280	-0.4301	0.0634
PVP Low	Control	-0.02000	1.000	-0.2667	0.2267
	Soap Control	-0.09667	0.936	-0.3434	0.1501
	Weathered Low	-0.15000	0.543	-0.3967	0.0967
	Sulphidized Low	-0.02000	1.000	-0.2667	0.2267
	Sulphidized High	-0.00667	1.000	-0.2534	0.2401
	Ionic Low	0.08333	0.975	-0.1634	0.3301
	Ionic High	0.21000	0.144	-0.0367	0.4567
	PVP High	0.05667	0.999	-0.1901	0.3034
	Uncoated Low	-0.04667	1.000	-0.2934	0.2001
	Uncoated High	0.02667	1.000	-0.2201	0.2734
PVP High	Control	-0.07667	0.986	-0.3234	0.1701
	Soap Control	-0.15333	0.514	-0.4001	0.0934
	Weathered Low	-0.20667	0.157	-0.4534	0.0401
	Sulphidized Low	-0.07667	0.986	-0.3234	0.1701
	Sulphidized High	-0.06333	0.997	-0.3101	0.1834
	Ionic Low	0.02667	1.000	-0.2201	0.2734
	Ionic High	0.15333	0.514	-0.0934	0.4001
	PVP Low	-0.05667	0.999	-0.3034	0.1901
	Uncoated Low	-0.10333	0.906	-0.3501	0.1434
	Uncoated High	-0.03000	1.000	-0.2767	0.2167
Uncoated Low	Control	0.02667	1.000	-0.2201	0.2734
	Soap Control	-0.05000	1.000	-0.2967	0.1967
	Weathered Low	-0.10333	0.906	-0.3501	0.1434
	Sulphidized Low	0.02667	1.000	-0.2201	0.2734
	Sulphidized High	0.04000	1.000	-0.2067	0.2867
	Ionic Low	0.13000	0.721	-0.1167	0.3767
	Ionic High	0.25667*	0.037	0.0099	0.5034
	PVP Low	0.04667	1.000	-0.2001	0.2934
	PVP High	0.10333	0.906	-0.1434	0.3501
	Uncoated High	0.07333	0.990	-0.1734	0.3201
Uncoated High	Control	-0.04667	1.000	-0.2934	0.2001
	Soap Control	-0.012333	0.775	-0.3701	0.1234
	Weathered Low	-0.17667	0.325	-0.4234	0.0701
	Sulphidized Low	-0.04667	1.000	-0.2934	0.2001
	Sulphidized High	-0.03333	1.000	-0.2801	0.2134
	Ionic Low	0.05667	0.999	-0.1901	0.3034

	Ionic High	0.18333	0.280	-0.0634	0.4301
	PVP Low	-0.02667	1.000	-0.2734	0.2201
	PVP High	0.03000	1.000	-0.2167	0.2767
	Uncoated Low	-0.07333	0.990	-0.3201	0.1734

Table C.29: Tukey test subset treatment groups for Month 2 AWCD

Treatment	N	Subsets	
		1	2
Ionic High	3	0.2867	
Ionic Low	3	0.4133	0.4133
PVP High	3	0.4400	0.4400
Uncoated High	3	0.4700	0.4700
PVP Low	3	0.4967	0.4967
Sulphidized High	3	0.5033	0.5033
Control	3	0.5167	0.5167
Sulphidized Low	3	0.5167	0.5167
Uncoated Low	3		0.5433
Soap Control	3		0.5933
Weathered Low	3		0.6467
Significance		0.082	0.075

Table C.30: One-way ANOVA for richness measures of each month

		Sum of Squares	DOF	Mean Square	F	Significance
Month 1	Between Groups	104.468	10	10.447	1.786	0.123
	Within Groups	128.659	22	5.848		
	Total	233.126	32			
Month 2	Between Groups	147.629	10	14.763	3.020	0.015
	Within Groups	107.529	22	4.888		
	Total	255.158	32			
Month 3	Between Groups	220.321	10	22.032	1.887	0.103
	Within Groups	256.876	22	11.676		
	Total	477.196	32			

Table C.31: Multiple comparisons for richness Month 2 treatments using Tukey's test

Treatment (I)	Treatment (J)	Mean Difference (I-J)	Significance	95% Confidence Interval	
				Lower Bound	Upper Bound
Control	Soap Control	-2.22000	0.971	-8.6730	4.2330
	Weathered Low	-2.55667	0.931	-9.0096	3.8963
	Sulphidized Low	0.00000	1.000	-6.4530	6.4530
	Sulphidized High	0.44333	1.000	-6.0096	6.8963

	Ionic Low	2.66667	0.913	-3.7863	9.1196
	Ionic High	5.55667	0.134	-0.8963	12.0096
	PVP Low	0.55667	1.000	-5.8963	7.0096
	PVP High	1.66667	0.996	-4.7863	8.1196
	Uncoated Low	-0.11000	1.000	-6.5630	6.3430
	Uncoated High	0.33333	1.000	-6.1196	6.7863
Soap Control	Control	2.22000	0.971	-4.2330	8.6730
	Weathered Low	-0.33667	1.000	-6.7896	6.1163
	Sulphidized Low	2.22000	0.971	-4.2330	8.6730
	Sulphidized High	2.66333	0.913	-3.7896	9.1163
	Ionic Low	4.88667	0.258	-1.5663	11.3396
	Ionic High	7.77667*	0.010	1.3237	14.2296
	PVP Low	2.77667	0.891	-3.6763	9.2296
	PVP High	3.88667	0.556	-2.5663	10.3396
	Uncoated Low	2.11000	0.980	-4.3430	8.5630
	Uncoated High	2.55333	0.932	-3.8996	9.0063
Weathered Low	Control	2.55667	0.931	-3.8963	9.0096
	Soap Control	0.33667	1.000	-6.1163	6.7896
	Sulphidized Low	2.55667	0.931	-3.8963	9.0096
	Sulphidized High	3.00000	0.839	-3.4530	9.4530
	Ionic Low	5.22333	0.188	-1.2296	11.6763
	Ionic High	8.11333*	0.007	1.6604	14.5663
	PVP Low	3.11333	0.808	-3.3396	9.5663
	PVP High	4.22333	0.443	-2.2296	10.6763
	Uncoated Low	2.44667	0.947	-4.0063	8.8996
	Uncoated High	2.89000	0.866	-3.5630	9.3430
Sulphidized Low	Control	0.00000	1.000	-6.4530	6.4530
	Soap Control	-2.22000	0.971	-8.6730	4.2330
	Weathered Low	-2.55667	0.931	-9.0096	3.8963
	Sulphidized High	0.44333	1.000	-6.0096	6.8963
	Ionic Low	2.66667	0.913	-3.7863	9.1196
	Ionic High	5.55667	0.134	-0.8963	12.0096
	PVP Low	0.55667	1.000	-5.8963	7.0096
	PVP High	1.66667	0.996	-4.7863	8.1196
	Uncoated Low	-0.11000	1.000	-6.5630	6.3430
	Uncoated High	0.33333	1.000	-6.1196	6.7863
Sulphidized High	Control	-0.44333	1.000	-6.8963	6.0096
	Soap Control	-2.66333	0.913	-9.1163	3.7896
	Weathered Low	-3.00000	0.839	-9.4530	3.4530
	Sulphidized Low	-0.44333	1.000	-6.8963	6.0096
	Ionic Low	2.22333	0.971	-4.2296	8.6763
	Ionic High	5.11333	0.209	-1.3396	11.5663
	PVP Low	0.11333	1.000	-6.3396	6.5663
	PVP High	1.22333	1.000	-5.2296	7.6763
	Uncoated Low	-0.55333	1.000	-7.0063	5.8996
	Uncoated High	-0.11000	1.000	-6.5630	6.3430

Ionic Low	Control	-2.66667	0.913	-9.1196	3.7863
	Soap Control	-4.88667	0.258	-11.3396	1.5663
	Weathered Low	-5.22333	0.188	-11.6763	1.2296
	Sulphidized Low	-2.66667	0.913	-9.1196	3.7863
	Sulphidized High	-2.22333	0.971	-8.6763	4.2296
	Ionic High	2.89000	0.866	-3.5630	9.3430
	PVP Low	-2.11000	0.980	-8.5630	4.3430
	PVP High	-1.00000	1.000	-7.4530	5.4530
	Uncoated Low	-2.77667	0.891	-9.2296	3.6763
	Uncoated High	-2.33333	0.961	-8.7863	4.1196
Ionic High	Control	-5.55667	0.134	-12.0096	0.8963
	Soap Control	-7.77667*	0.010	-14.2296	-1.3237
	Weathered Low	-8.11333*	0.007	-14.5663	-1.6604
	Sulphidized Low	-5.55667	0.134	-12.0096	0.8963
	Sulphidized High	-5.11333	0.209	-11.5663	1.3396
	Ionic Low	-2.89000	0.866	-9.3430	3.5630
	PVP Low	-5.00000	0.233	-11.4530	1.4530
	PVP High	-3.89000	0.555	-10.3430	2.5630
	Uncoated Low	-5.66667	0.120	-12.1196	0.7863
	Uncoated High	-5.22333	0.188	-11.6763	1.2296
PVP Low	Control	-0.55667	1.000	-7.0096	5.8963
	Soap Control	-2.77667	0.891	-9.2296	3.6763
	Weathered Low	-3.11333	0.808	-9.5663	3.3396
	Sulphidized Low	-0.55667	1.000	-7.0096	5.8963
	Sulphidized High	-0.11333	1.000	-6.5663	6.3396
	Ionic Low	2.11000	0.980	-4.3430	8.5630
	Ionic High	5.00000	0.233	-1.4530	11.4530
	PVP High	1.11000	1.000	-5.3430	7.5630
	Uncoated Low	-0.66667	1.000	-7.1196	5.7863
	Uncoated High	-0.22333	1.000	-6.6763	6.2296
PVP High	Control	-1.66667	0.996	-8.1196	4.7863
	Soap Control	-3.88667	0.556	-10.3396	2.5663
	Weathered Low	-4.22333	0.443	-10.6763	2.2296
	Sulphidized Low	-1.66667	0.996	-8.1196	4.7863
	Sulphidized High	-1.22333	1.000	-7.6763	5.2296
	Ionic Low	1.00000	1.000	-5.4530	7.4530
	Ionic High	3.89000	0.555	-2.5630	10.3430
	PVP Low	-1.11000	1.000	-7.5630	5.3430
	Uncoated Low	-1.77667	0.994	-8.2296	4.6763
	Uncoated High	-1.33333	0.999	-7.7863	5.1196
Uncoated Low	Control	0.11000	1.000	-6.3430	6.5630
	Soap Control	-2.11000	0.980	-8.5630	4.3430
	Weathered Low	-2.44667	0.947	-8.8996	4.0063
	Sulphidized Low	0.11000	1.000	-6.3430	6.5630
	Sulphidized High	0.55333	1.000	-5.8996	7.0063
	Ionic Low	2.77667	0.891	-3.6763	9.2296

	Ionic High	5.66667	0.120	-0.7863	12.1196
	PVP Low	0.66667	1.000	-5.7863	7.1196
	PVP High	1.77667	0.994	-4.6763	8.2296
	Uncoated High	0.44333	1.000	-6.0096	6.8963
Uncoated High	Control	-0.33333	1.000	-6.7863	6.1196
	Soap Control	-2.55333	0.932	-9.0063	3.8996
	Weathered Low	-2.89000	0.866	-9.3430	3.5630
	Sulphidized Low	-0.33333	1.000	-6.7863	6.1196
	Sulphidized High	0.11000	1.000	-6.3430	6.5630
	Ionic Low	2.33333	0.961	-4.1196	8.7863
	Ionic High	5.22333	0.188	-1.2296	11.6763
	PVP Low	0.22333	1.000	-6.2296	6.6763
	PVP High	1.33333	0.999	-5.1196	7.7863
	Uncoated Low	-0.44333	1.000	-6.8963	6.0096

Table C.32: Tukey test subset treatment groups for Month 2 richness

Treatment	N	Subsets	
		1	2
Ionic High	3	9.4433	
Ionic Low	3	12.3333	12.3333
PVP High	3	13.3333	13.3333
PVP Low	3	14.4433	14.4433
Sulphidized High	3	14.5567	14.5567
Uncoated High	3	14.6667	14.6667
Control	3	15.0000	15.0000
Sulphidized Low	3	15.0000	15.0000
Uncoated Low	3	15.1100	15.1100
Soap Control	3		17.2200
Weathered Low	3		17.5567
Significance		0.120	0.188

Table C.33: One-way ANOVA for Month 1 Guilds

		Sum of Squares	DOF	Mean Square	F	Significance
Carbohydrates	Between Groups	0.025	10	0.002	1.385	0.251
	Within Groups	0.039	22	0.002		
	Total	0.064	32			
Polymers	Between Groups	0.002	10	0.000	1.459	0.220
	Within Groups	0.003	22	0.000		
	Total	0.005	32			
Carboxylic acids	Between Groups	0.043	10	0.004	3.239	0.010
	Within Groups	0.029	22	0.001		
	Total	0.072	32			
Amino acids	Between Groups	0.015	10	0.001	2.823	0.020
	Within Groups	0.011	22	0.001		
	Total	0.026	32			
Amides/ amides	Between Groups	0.001	10	0.000	1.904	0.100
	Within Groups	0.001	22	0.000		
	Total	0.002	32			
Root Exudates	Between Groups	0.032	10	0.003	3.502	0.007
	Within Groups	0.020	22	0.001		
	Total	0.052	32			

Table C.34: Multiple comparisons for carboxylic and acetic acids Month 1 treatments using Tukey's test

Treatment (I)	Treatment (J)	Mean Difference (I-J)	Significance	95% Confidence Interval	
				Lower Bound	Upper Bound
Control	Soap Control	-0.051	0.817	-0.156	0.055
	Weathered Low	-0.083	0.219	-0.189	0.023
	Sulphidized Low	0.022	0.999	-0.084	0.128
	Sulphidized High	-0.004	1.000	-0.110	0.102
	Ionic Low	0.033	0.986	-0.073	0.139
	Ionic High	0.006	1.000	-0.100	0.112
	PVP Low	0.028	0.996	-0.078	0.134
	PVP High	0.007	1.000	-0.099	0.113
	Uncoated Low	0.024	0.999	-0.082	0.130
	Uncoated High	0.042	0.928	-0.064	0.148
Soap Control	Control	0.051	0.817	-0.055	0.156
	Weathered Low	-0.033	0.987	-0.138	0.073
	Sulphidized Low	0.073	0.376	-0.033	0.179
	Sulphidized High	0.047	0.874	-0.059	0.153
	Ionic Low	0.084	0.214	-0.022	0.189
	Ionic High	0.056	0.707	-0.049	0.162
	PVP Low	0.078	0.288	-0.028	0.184
	PVP High	0.058	0.687	-0.048	0.163

	Uncoated Low	0.075	0.347	-0.031	0.180
	Uncoated High	0.093	0.121	-0.013	0.199
Weathered Low	Control	0.083	0.219	-0.023	0.189
	Soap Control	0.033	0.987	-0.073	0.138
	Sulphidized Low	0.105	0.052	-0.001	0.211
	Sulphidized High	0.079	0.269	-0.027	0.185
	Ionic Low	0.11602*	0.024	0.010	0.222
	Ionic High	0.089	0.154	-0.017	0.195
	PVP Low	0.11067*	0.035	0.005	0.217
	PVP High	0.090	0.145	-0.016	0.196
	Uncoated Low	0.10704*	0.046	0.001	0.213
	Uncoated High	0.12535*	0.012	0.020	0.231
Sulphidized Low	Control	-0.022	0.999	-0.128	0.084
	Soap Control	-0.073	0.376	-0.179	0.033
	Weathered Low	-0.105	0.052	-0.211	0.001
	Sulphidized High	-0.026	0.998	-0.132	0.080
	Ionic Low	0.011	1.000	-0.095	0.117
	Ionic High	-0.016	1.000	-0.122	0.090
	PVP Low	0.005	1.000	-0.101	0.111
	PVP High	-0.015	1.000	-0.121	0.091
	Uncoated Low	0.002	1.000	-0.104	0.108
	Uncoated High	0.020	1.000	-0.086	0.126
Sulphidized High	Control	0.004	1.000	-0.102	0.110
	Soap Control	-0.047	0.874	-0.153	0.059
	Weathered Low	-0.079	0.269	-0.185	0.027
	Sulphidized Low	0.026	0.998	-0.080	0.132
	Ionic Low	0.037	0.970	-0.069	0.143
	Ionic High	0.010	1.000	-0.096	0.116
	PVP Low	0.031	0.990	-0.075	0.137
	PVP High	0.011	1.000	-0.095	0.117
	Uncoated Low	0.028	0.996	-0.078	0.134
	Uncoated High	0.046	0.886	-0.060	0.152
Ionic Low	Control	-0.033	0.986	-0.139	0.073
	Soap Control	-0.084	0.214	-0.189	0.022
	Weathered Low	-0.11602*	0.024	-0.222	-0.010
	Sulphidized Low	-0.011	1.000	-0.117	0.095
	Sulphidized High	-0.037	0.970	-0.143	0.069
	Ionic High	-0.027	0.997	-0.133	0.079
	PVP Low	-0.005	1.000	-0.111	0.101
	PVP High	-0.026	0.998	-0.132	0.080
	Uncoated Low	-0.009	1.000	-0.115	0.097
	Uncoated High	0.009	1.000	-0.097	0.115
Ionic High	Control	-0.006	1.000	-0.112	0.100
	Soap Control	-0.056	0.707	-0.162	0.049
	Weathered Low	-0.089	0.154	-0.195	0.017
	Sulphidized Low	0.016	1.000	-0.090	0.122

	Sulphidized High	-0.010	1.000	-0.116	0.096
	Ionic Low	0.027	0.997	-0.079	0.133
	PVP Low	0.022	0.999	-0.084	0.128
	PVP High	0.001	1.000	-0.105	0.107
	Uncoated Low	0.018	1.000	-0.088	0.124
	Uncoated High	0.036	0.972	-0.070	0.142
PVP Low	Control	-0.028	0.996	-0.134	0.078
	Soap Control	-0.078	0.288	-0.184	0.028
	Weathered Low	-0.11067*	0.035	-0.217	-0.005
	Sulphidized Low	-0.005	1.000	-0.111	0.101
	Sulphidized High	-0.031	0.990	-0.137	0.075
	Ionic Low	0.005	1.000	-0.101	0.111
	Ionic High	-0.022	0.999	-0.128	0.084
	PVP High	-0.021	1.000	-0.127	0.085
	Uncoated Low	-0.004	1.000	-0.110	0.102
	Uncoated High	0.015	1.000	-0.091	0.121
PVP High	Control	-0.007	1.000	-0.113	0.099
	Soap Control	-0.058	0.687	-0.163	0.048
	Weathered Low	-0.090	0.145	-0.196	0.016
	Sulphidized Low	0.015	1.000	-0.091	0.121
	Sulphidized High	-0.011	1.000	-0.117	0.095
	Ionic Low	0.026	0.998	-0.080	0.132
	Ionic High	-0.001	1.000	-0.107	0.105
	PVP Low	0.021	1.000	-0.085	0.127
	Uncoated Low	0.017	1.000	-0.089	0.123
	Uncoated High	0.035	0.977	-0.071	0.141
Uncoated Low	Control	-0.024	0.999	-0.130	0.082
	Soap Control	-0.075	0.347	-0.180	0.031
	Weathered Low	-0.10704*	0.046	-0.213	-0.001
	Sulphidized Low	-0.002	1.000	-0.108	0.104
	Sulphidized High	-0.028	0.996	-0.134	0.078
	Ionic Low	0.009	1.000	-0.097	0.115
	Ionic High	-0.018	1.000	-0.124	0.088
	PVP Low	0.004	1.000	-0.102	0.110
	PVP High	-0.017	1.000	-0.123	0.089
	Uncoated High	0.018	1.000	-0.088	0.124
Uncoated High	Control	-0.042	0.928	-0.148	0.064
	Soap Control	-0.093	0.121	-0.199	0.013
	Weathered Low	-0.12535*	0.012	-0.231	-0.020
	Sulphidized Low	-0.020	1.000	-0.126	0.086
	Sulphidized High	-0.046	0.886	-0.152	0.060
	Ionic Low	-0.009	1.000	-0.115	0.097
	Ionic High	-0.036	0.972	-0.142	0.070
	PVP Low	-0.015	1.000	-0.121	0.091
	PVP High	-0.035	0.977	-0.141	0.071
	Uncoated Low	-0.018	1.000	-0.124	0.088

Table C.35: Tukey test subset treatment groups for carboxylic and acetic acids in Month 1

Treatment	N	Subsets	
		1	2
Uncoated High	3	0.0716	
Ionic Low	3	0.0809	
PVP Low	3	0.0863	
Uncoated Low	3	0.0899	
Sulphidized Low	3	0.0916	0.0916
PVP High	3	0.1069	0.1069
Ionic High	3	0.1080	0.1080
Control	3	0.1139	0.1139
Sulphidized High	3	0.1176	0.1176
Soap Control	3	0.1644	0.1644
Weathered Low	3		0.1969
Significance		0.121	0.052

Table C.36: Multiple comparisons for amino acids Month 1 treatments using Tukey's test

Treatment (I)	Treatment (J)	Mean Difference (I-J)	Significance	95% Confidence Interval	
				Lower Bound	Upper Bound
Control	Soap Control	-0.040	0.558	-0.106	0.027
	Weathered Low	-0.063	0.072	-0.130	0.003
	Sulphidized Low	-0.001	1.000	-0.067	0.065
	Sulphidized High	0.001	1.000	-0.065	0.068
	Ionic Low	-0.005	1.000	-0.072	0.061
	Ionic High	-0.022	0.980	-0.088	0.045
	PVP Low	-0.005	1.000	-0.071	0.062
	PVP High	-0.041	0.514	-0.108	0.025
	Uncoated Low	0.004	1.000	-0.063	0.070
	Uncoated High	-0.019	0.992	-0.086	0.047
Soap Control	Control	0.040	0.558	-0.027	0.106
	Weathered Low	-0.023	0.969	-0.090	0.043
	Sulphidized Low	0.039	0.591	-0.027	0.105
	Sulphidized High	0.041	0.520	-0.025	0.107
	Ionic Low	0.035	0.726	-0.032	0.101
	Ionic High	0.018	0.994	-0.048	0.085
	PVP Low	0.035	0.710	-0.031	0.102
	PVP High	-0.001	1.000	-0.068	0.065
	Uncoated Low	0.044	0.434	-0.023	0.110
	Uncoated High	0.021	0.985	-0.046	0.087
Weathered Low	Control	0.063	0.072	-0.003	0.130
	Soap Control	0.023	0.969	-0.043	0.090
	Sulphidized Low	0.062	0.080	-0.004	0.129
	Sulphidized High	0.064	0.063	-0.002	0.131

	Ionic Low	0.058	0.124	-0.008	0.124
	Ionic High	0.041	0.509	-0.025	0.108
	PVP Low	0.058	0.117	-0.008	0.125
	PVP High	0.022	0.979	-0.045	0.088
	Uncoated Low	0.06690*	0.047	0.001	0.133
	Uncoated High	0.044	0.429	-0.023	0.110
Sulphidized Low	Control	0.001	1.000	-0.065	0.067
	Soap Control	-0.039	0.591	-0.105	0.027
	Weathered Low	-0.062	0.080	-0.129	0.004
	Sulphidized High	0.002	1.000	-0.064	0.069
	Ionic Low	-0.004	1.000	-0.071	0.062
	Ionic High	-0.021	0.985	-0.087	0.046
	PVP Low	-0.004	1.000	-0.070	0.063
	PVP High	-0.040	0.547	-0.107	0.026
	Uncoated Low	0.005	1.000	-0.062	0.071
	Uncoated High	-0.018	0.994	-0.085	0.048
Sulphidized High	Control	-0.001	1.000	-0.068	0.065
	Soap Control	-0.041	0.520	-0.107	0.025
	Weathered Low	-0.064	0.063	-0.131	0.002
	Sulphidized Low	-0.002	1.000	-0.069	0.064
	Ionic Low	-0.006	1.000	-0.073	0.060
	Ionic High	-0.023	0.971	-0.089	0.044
	PVP Low	-0.006	1.000	-0.072	0.061
	PVP High	-0.042	0.476	-0.109	0.024
	Uncoated Low	0.003	1.000	-0.064	0.069
	Uncoated High	-0.020	0.987	-0.087	0.046
Ionic Low	Control	0.005	1.000	-0.061	0.072
	Soap Control	-0.035	0.726	-0.101	0.032
	Weathered Low	-0.058	0.124	-0.124	0.008
	Sulphidized Low	0.004	1.000	-0.062	0.071
	Sulphidized High	0.006	1.000	-0.060	0.073
	Ionic High	-0.017	0.997	-0.083	0.050
	PVP Low	0.001	1.000	-0.066	0.067
	PVP High	-0.036	0.684	-0.103	0.030
	Uncoated Low	0.009	1.000	-0.057	0.075
	Uncoated High	-0.014	0.999	-0.080	0.052
Ionic High	Control	0.022	0.980	-0.045	0.088
	Soap Control	-0.018	0.994	-0.085	0.048
	Weathered Low	-0.041	0.509	-0.108	0.025
	Sulphidized Low	0.021	0.985	-0.046	0.087
	Sulphidized High	0.023	0.971	-0.044	0.089
	Ionic Low	0.017	0.997	-0.050	0.083
	PVP Low	0.017	0.997	-0.049	0.084
	PVP High	-0.020	0.990	-0.086	0.047
	Uncoated Low	0.026	0.942	-0.041	0.092
	Uncoated High	0.003	1.000	-0.064	0.069

PVP Low	Control	0.005	1.000	-0.062	0.071
	Soap Control	-0.035	0.710	-0.102	0.031
	Weathered Low	-0.058	0.117	-0.125	0.008
	Sulphidized Low	0.004	1.000	-0.063	0.070
	Sulphidized High	0.006	1.000	-0.061	0.072
	Ionic Low	-0.001	1.000	-0.067	0.066
	Ionic High	-0.017	0.997	-0.084	0.049
	PVP High	-0.037	0.667	-0.103	0.030
	Uncoated Low	0.008	1.000	-0.058	0.075
	Uncoated High	-0.015	0.999	-0.081	0.052
PVP High	Control	0.041	0.514	-0.025	0.108
	Soap Control	0.001	1.000	-0.065	0.068
	Weathered Low	-0.022	0.979	-0.088	0.045
	Sulphidized Low	0.040	0.547	-0.026	0.107
	Sulphidized High	0.042	0.476	-0.024	0.109
	Ionic Low	0.036	0.684	-0.030	0.103
	Ionic High	0.020	0.990	-0.047	0.086
	PVP Low	0.037	0.667	-0.030	0.103
	Uncoated Low	0.045	0.394	-0.021	0.111
	Uncoated High	0.022	0.977	-0.044	0.089
Uncoated Low	Control	-0.004	1.000	-0.070	0.063
	Soap Control	-0.044	0.434	-0.110	0.023
	Weathered Low	-0.06690*	0.047	-0.133	-0.001
	Sulphidized Low	-0.005	1.000	-0.071	0.062
	Sulphidized High	-0.003	1.000	-0.069	0.064
	Ionic Low	-0.009	1.000	-0.075	0.057
	Ionic High	-0.026	0.942	-0.092	0.041
	PVP Low	-0.008	1.000	-0.075	0.058
	PVP High	-0.045	0.394	-0.111	0.021
	Uncoated High	-0.023	0.970	-0.089	0.043
Uncoated High	Control	0.019	0.992	-0.047	0.086
	Soap Control	-0.021	0.985	-0.087	0.046
	Weathered Low	-0.044	0.429	-0.110	0.023
	Sulphidized Low	0.018	0.994	-0.048	0.085
	Sulphidized High	0.020	0.987	-0.046	0.087
	Ionic Low	0.014	0.999	-0.052	0.080
	Ionic High	-0.003	1.000	-0.069	0.064
	PVP Low	0.015	0.999	-0.052	0.081
	PVP High	-0.022	0.977	-0.089	0.044
	Uncoated Low	0.023	0.970	-0.043	0.089

Table C.37: Tukey test subset treatment groups for amino acids in Month 1

Treatment	N	Subsets	
		1	2
Uncoated Low	3	0.0648	
Sulphidized High	3	0.0675	0.0675
Control	3	0.0687	0.0687
Sulphidized Low	3	0.0697	0.0697
PVP Low	3	0.0733	0.0733
Ionic Low	3	0.0738	0.0738
Uncoated High	3	0.0878	0.0878
Ionic High	3	0.0904	0.0904
Soap Control	3	0.1086	0.1086
PVP High	3	0.1099	0.1099
Weathered Low	3		0.1317
Significance		0.394	0.063

Table C.38: Multiple comparisons for root exudates Month 1 treatments using Tukey's test

Treatment (I)	Treatment (J)	Mean Difference (I-J)	Significance	95% Confidence Interval	
				Lower Bound	Upper Bound
Control	Soap Control	-0.050	0.641	-0.138	0.039
	Weathered Low	-0.088	0.053	-0.176	0.001
	Sulphidized Low	0.006	1.000	-0.083	0.094
	Sulphidized High	-0.010	1.000	-0.098	0.079
	Ionic Low	0.019	0.999	-0.069	0.108
	Ionic High	-0.012	1.000	-0.100	0.077
	PVP Low	0.004	1.000	-0.084	0.093
	PVP High	-0.035	0.935	-0.123	0.054
	Uncoated Low	0.022	0.997	-0.066	0.111
	Uncoated High	0.002	1.000	-0.087	0.090
Soap Control	Control	0.050	0.641	-0.039	0.138
	Weathered Low	-0.038	0.894	-0.126	0.051
	Sulphidized Low	0.055	0.502	-0.033	0.144
	Sulphidized High	0.040	0.859	-0.048	0.128
	Ionic Low	0.069	0.224	-0.019	0.158
	Ionic High	0.038	0.892	-0.051	0.126
	PVP Low	0.054	0.539	-0.035	0.142
	PVP High	0.015	1.000	-0.073	0.104
	Uncoated Low	0.072	0.182	-0.016	0.161
	Uncoated High	0.051	0.601	-0.037	0.140
Weathered Low	Control	0.088	0.053	-0.001	0.176
	Soap Control	0.038	0.894	-0.051	0.126
	Sulphidized Low	0.09328*	0.033	0.005	0.182
	Sulphidized High	0.078	0.118	-0.011	0.166
	Ionic Low	0.10694*	0.010	0.019	0.195

	Ionic High	0.076	0.138	-0.013	0.164
	PVP Low	0.09178*	0.038	0.003	0.180
	PVP High	0.053	0.562	-0.035	0.141
	Uncoated Low	0.10985*	0.007	0.021	0.198
	Uncoated High	0.08932*	0.046	0.001	0.178
Sulphidized Low	Control	-0.006	1.000	-0.094	0.083
	Soap Control	-0.055	0.502	-0.144	0.033
	Weathered Low	-0.09328*	0.033	-0.182	-0.005
	Sulphidized High	-0.015	1.000	-0.104	0.073
	Ionic Low	0.014	1.000	-0.075	0.102
	Ionic High	-0.017	1.000	-0.106	0.071
	PVP Low	-0.002	1.000	-0.090	0.087
	PVP High	-0.040	0.854	-0.129	0.048
	Uncoated Low	0.017	1.000	-0.072	0.105
	Uncoated High	-0.004	1.000	-0.092	0.085
Sulphidized High	Control	0.010	1.000	-0.079	0.098
	Soap Control	-0.040	0.859	-0.128	0.048
	Weathered Low	-0.078	0.118	-0.166	0.011
	Sulphidized Low	0.015	1.000	-0.073	0.104
	Ionic Low	0.029	0.979	-0.059	0.118
	Ionic High	-0.002	1.000	-0.091	0.086
	PVP Low	0.014	1.000	-0.075	0.102
	PVP High	-0.025	0.993	-0.113	0.064
	Uncoated Low	0.032	0.960	-0.056	0.121
	Uncoated High	0.011	1.000	-0.077	0.100
Ionic Low	Control	-0.019	0.999	-0.108	0.069
	Soap Control	-0.069	0.224	-0.158	0.019
	Weathered Low	-0.10694*	0.010	-0.195	-0.019
	Sulphidized Low	-0.014	1.000	-0.102	0.075
	Sulphidized High	-0.029	0.979	-0.118	0.059
	Ionic High	-0.031	0.967	-0.120	0.057
	PVP Low	-0.015	1.000	-0.104	0.073
	PVP High	-0.054	0.539	-0.142	0.035
	Uncoated Low	0.003	1.000	-0.086	0.091
	Uncoated High	-0.018	1.000	-0.106	0.071
Ionic High	Control	0.012	1.000	-0.077	0.100
	Soap Control	-0.038	0.892	-0.126	0.051
	Weathered Low	-0.076	0.138	-0.164	0.013
	Sulphidized Low	0.017	1.000	-0.071	0.106
	Sulphidized High	0.002	1.000	-0.086	0.091
	Ionic Low	0.031	0.967	-0.057	0.120
	PVP Low	0.016	1.000	-0.072	0.104
	PVP High	-0.023	0.997	-0.111	0.066
	Uncoated Low	0.034	0.942	-0.054	0.123
	Uncoated High	0.014	1.000	-0.075	0.102
PVP Low	Control	-0.004	1.000	-0.093	0.084

	Soap Control	-0.054	0.539	-0.142	0.035
	Weathered Low	-0.09178*	0.038	-0.180	-0.003
	Sulphidized Low	0.002	1.000	-0.087	0.090
	Sulphidized High	-0.014	1.000	-0.102	0.075
	Ionic Low	0.015	1.000	-0.073	0.104
	Ionic High	-0.016	1.000	-0.104	0.072
	PVP High	-0.039	0.880	-0.127	0.050
	Uncoated Low	0.018	0.999	-0.070	0.107
	Uncoated High	-0.002	1.000	-0.091	0.086
PVP High	Control	0.035	0.935	-0.054	0.123
	Soap Control	-0.015	1.000	-0.104	0.073
	Weathered Low	-0.053	0.562	-0.141	0.035
	Sulphidized Low	0.040	0.854	-0.048	0.129
	Sulphidized High	0.025	0.993	-0.064	0.113
	Ionic Low	0.054	0.539	-0.035	0.142
	Ionic High	0.023	0.997	-0.066	0.111
	PVP Low	0.039	0.880	-0.050	0.127
	Uncoated Low	0.057	0.468	-0.032	0.145
	Uncoated High	0.036	0.916	-0.052	0.125
Uncoated Low	Control	-0.022	0.997	-0.111	0.066
	Soap Control	-0.072	0.182	-0.161	0.016
	Weathered Low	-0.10985*	0.007	-0.198	-0.021
	Sulphidized Low	-0.017	1.000	-0.105	0.072
	Sulphidized High	-0.032	0.960	-0.121	0.056
	Ionic Low	-0.003	1.000	-0.091	0.086
	Ionic High	-0.034	0.942	-0.123	0.054
	PVP Low	-0.018	0.999	-0.107	0.070
	PVP High	-0.057	0.468	-0.145	0.032
	Uncoated High	-0.021	0.999	-0.109	0.068
Uncoated High	Control	-0.002	1.000	-0.090	0.087
	Soap Control	-0.051	0.601	-0.140	0.037
	Weathered Low	-0.08932*	0.046	-0.178	-0.001
	Sulphidized Low	0.004	1.000	-0.085	0.092
	Sulphidized High	-0.011	1.000	-0.100	0.077
	Ionic Low	0.018	1.000	-0.071	0.106
	Ionic High	-0.014	1.000	-0.102	0.075
	PVP Low	0.002	1.000	-0.086	0.091
	PVP High	-0.036	0.916	-0.125	0.052
	Uncoated Low	0.021	0.999	-0.068	0.109

Table C.39: Tukey test subset treatment groups for root exudates in Month 1

Treatment	N	Subsets 1	Subsets 2
Uncoated Low	3	0.0903	
Ionic Low	3	0.0932	
Sulphidized Low	3	0.1069	
PVP Low	3	0.1084	
Uncoated High	3	0.1108	
Control	3	0.1124	0.1124
Sulphidized High	3	0.1223	0.1223
Ionic High	3	0.1244	0.1244
PVP High	3	0.1471	0.1471
Soap Control	3	0.1623	0.1623
Weathered Low	3		0.2001
Significance		0.182	0.053

Table C.40: One-way ANOVA for Month 2 Guilds

		Sum of Squares	DOF	Mean Square	F	Significance
Carbohydrates	Between Groups	0.031	10	0.003	4.564	0.001
	Within Groups	0.015	22	0.001		
	Total	0.045	32			
Polymers	Between Groups	0.003	10	0.000	1.867	0.107
	Within Groups	0.003	22	0.000		
	Total	0.006	32			
Carboxylic acids	Between Groups	0.038	10	0.004	2.465	0.037
	Within Groups	0.034	22	0.002		
	Total	0.071	32			
Amino acids	Between Groups	0.008	10	0.001	2.378	0.043
	Within Groups	0.007	22	0.000		
	Total	0.015	32			
Amides/ amides	Between Groups	0.003	10	0.000	1.478	0.213
	Within Groups	0.004	22	0.000		
	Total	0.007	32			
Root Exudates	Between Groups	0.030	10	0.003	3.046	0.014
	Within Groups	0.022	22	0.001		
	Total	0.052	32			

Table C.41: Multiple comparisons for carbohydrates Month 2 treatments using Tukey's test

Treatment (I)	Treatment (J)	Mean Difference (I-J)	Significance	95% Confidence Interval	
				Lower Bound	Upper Bound
Control	Soap Control	-0.039	0.751	-0.114	0.037
	Weathered Low	-0.056	0.274	-0.132	0.019
	Sulphidized Low	0.008	1.000	-0.067	0.084
	Sulphidized High	0.008	1.000	-0.067	0.084
	Ionic Low	0.034	0.871	-0.042	0.109
	Ionic High	0.055	0.306	-0.021	0.130
	PVP Low	0.005	1.000	-0.071	0.081
	PVP High	0.037	0.802	-0.039	0.112
	Uncoated Low	-0.006	1.000	-0.081	0.070
	Uncoated High	0.010	1.000	-0.065	0.086
Soap Control	Control	0.039	0.751	-0.037	0.114
	Weathered Low	-0.018	0.998	-0.093	0.058
	Sulphidized Low	0.047	0.516	-0.029	0.122
	Sulphidized High	0.047	0.517	-0.029	0.122
	Ionic Low	0.072	0.069	-0.003	0.148
	Ionic High	0.09356*	0.008	0.018	0.169
	PVP Low	0.044	0.609	-0.032	0.119
	PVP High	0.075	0.051	0.000	0.151
	Uncoated Low	0.033	0.886	-0.043	0.108
	Uncoated High	0.049	0.457	-0.027	0.124
Weathered Low	Control	0.056	0.274	-0.019	0.132
	Soap Control	0.018	0.998	-0.058	0.093
	Sulphidized Low	0.065	0.140	-0.011	0.140
	Sulphidized High	0.065	0.140	-0.011	0.140
	Ionic Low	0.08997*	0.011	0.015	0.166
	Ionic High	0.11130*	0.001	0.036	0.187
	PVP Low	0.061	0.183	-0.014	0.137
	PVP High	0.09308*	0.008	0.018	0.169
	Uncoated Low	0.050	0.414	-0.025	0.126
	Uncoated High	0.067	0.116	-0.009	0.142
Sulphidized Low	Control	-0.008	1.000	-0.084	0.067
	Soap Control	-0.047	0.516	-0.122	0.029
	Weathered Low	-0.065	0.140	-0.140	0.011
	Sulphidized High	0.000	1.000	-0.076	0.076
	Ionic Low	0.025	0.975	-0.050	0.101
	Ionic High	0.047	0.519	-0.029	0.122
	PVP Low	-0.003	1.000	-0.079	0.072
	PVP High	0.029	0.948	-0.047	0.104
	Uncoated Low	-0.014	1.000	-0.090	0.061
	Uncoated High	0.002	1.000	-0.073	0.078
Sulphidized High	Control	-0.008	1.000	-0.084	0.067
	Soap Control	-0.047	0.517	-0.122	0.029

	Weathered Low	-0.065	0.140	-0.140	0.011
	Sulphidized Low	0.000	1.000	-0.076	0.076
	Ionic Low	0.025	0.975	-0.050	0.101
	Ionic High	0.047	0.518	-0.029	0.122
	PVP Low	-0.003	1.000	-0.079	0.072
	PVP High	0.029	0.948	-0.047	0.104
	Uncoated Low	-0.014	1.000	-0.090	0.061
	Uncoated High	0.002	1.000	-0.073	0.078
Ionic Low	Control	-0.034	0.871	-0.109	0.042
	Soap Control	-0.072	0.069	-0.148	0.003
	Weathered Low	-0.08997*	0.011	-0.166	-0.015
	Sulphidized Low	-0.025	0.975	-0.101	0.050
	Sulphidized High	-0.025	0.975	-0.101	0.050
	Ionic High	0.021	0.993	-0.054	0.097
	PVP Low	-0.029	0.948	-0.104	0.047
	PVP High	0.003	1.000	-0.072	0.079
	Uncoated Low	-0.039	0.729	-0.115	0.036
	Uncoated High	-0.023	0.986	-0.099	0.052
Ionic High	Control	-0.055	0.306	-0.130	0.021
	Soap Control	-0.09356*	0.008	-0.169	-0.018
	Weathered Low	-0.11130*	0.001	-0.187	-0.036
	Sulphidized Low	-0.047	0.519	-0.122	0.029
	Sulphidized High	-0.047	0.518	-0.122	0.029
	Ionic Low	-0.021	0.993	-0.097	0.054
	PVP Low	-0.050	0.430	-0.125	0.026
	PVP High	-0.018	0.998	-0.094	0.057
	Uncoated Low	-0.061	0.193	-0.136	0.015
	Uncoated High	-0.045	0.580	-0.120	0.031
PVP Low	Control	-0.005	1.000	-0.081	0.071
	Soap Control	-0.044	0.609	-0.119	0.032
	Weathered Low	-0.061	0.183	-0.137	0.014
	Sulphidized Low	0.003	1.000	-0.072	0.079
	Sulphidized High	0.003	1.000	-0.072	0.079
	Ionic Low	0.029	0.948	-0.047	0.104
	Ionic High	0.050	0.430	-0.026	0.125
	PVP High	0.032	0.905	-0.044	0.107
	Uncoated Low	-0.011	1.000	-0.086	0.065
	Uncoated High	0.005	1.000	-0.070	0.081
PVP High	Control	-0.037	0.802	-0.112	0.039
	Soap Control	-0.075	0.051	-0.151	0.000
	Weathered Low	-0.09308*	0.008	-0.169	-0.018
	Sulphidized Low	-0.029	0.948	-0.104	0.047
	Sulphidized High	-0.029	0.948	-0.104	0.047
	Ionic Low	-0.003	1.000	-0.079	0.072
	Ionic High	0.018	0.998	-0.057	0.094
	PVP Low	-0.032	0.905	-0.107	0.044

	Uncoated Low	-0.043	0.641	-0.118	0.033
	Uncoated High	-0.026	0.968	-0.102	0.049
Uncoated Low	Control	0.006	1.000	-0.070	0.081
	Soap Control	-0.033	0.886	-0.108	0.043
	Weathered Low	-0.050	0.414	-0.126	0.025
	Sulphidized Low	0.014	1.000	-0.061	0.090
	Sulphidized High	0.014	1.000	-0.061	0.090
	Ionic Low	0.039	0.729	-0.036	0.115
	Ionic High	0.061	0.193	-0.015	0.136
	PVP Low	0.011	1.000	-0.065	0.086
	PVP High	0.043	0.641	-0.033	0.118
	Uncoated High	0.016	0.999	-0.059	0.092
Uncoated High	Control	-0.010	1.000	-0.086	0.065
	Soap Control	-0.049	0.457	-0.124	0.027
	Weathered Low	-0.067	0.116	-0.142	0.009
	Sulphidized Low	-0.002	1.000	-0.078	0.073
	Sulphidized High	-0.002	1.000	-0.078	0.073
	Ionic Low	0.023	0.986	-0.052	0.099
	Ionic High	0.045	0.580	-0.031	0.120
	PVP Low	-0.005	1.000	-0.081	0.070
	PVP High	0.026	0.968	-0.049	0.102
	Uncoated Low	-0.016	0.999	-0.092	0.059

Table C.42: Tukey test subset treatment groups for carbohydrates in Month 2

Treatment	N	Subsets		
		1	2	3
Ionic High	3	0.0904		
PVP High	3	0.1086	0.1086	
Ionic Low	3	0.1117	0.1117	
Uncoated High	3	0.1350	0.1350	0.1350
Sulphidized Low	3	0.1371	0.1371	0.1371
Sulphidized High	3	0.1371	0.1371	0.1371
PVP Low	3	0.1403	0.1403	0.1403
Control	3	0.1453	0.1453	0.1453
Uncoated Low	3	0.1512	0.1512	0.1512
Soap Control	3		0.1839	0.1839
Weathered Low	3			0.2017
Significance		0.193	0.051	0.116

Table C.43: Multiple comparisons for carboxylic and acetic acids Month 2 treatments using Tukey's test

Treatment (I)	Treatment (J)	Mean Difference (I-J)	Significance	95% Confidence Interval	
				Lower Bound	Upper Bound
Control	Soap Control	0.003	1.000	-0.111	0.117
	Weathered Low	-0.043	0.952	-0.157	0.072
	Sulphidized Low	0.007	1.000	-0.107	0.121
	Sulphidized High	0.011	1.000	-0.103	0.125
	Ionic Low	0.041	0.963	-0.073	0.155
	Ionic High	0.098	0.138	-0.016	0.212
	PVP Low	0.004	1.000	-0.111	0.118
	PVP High	0.030	0.995	-0.084	0.145
	Uncoated Low	-0.012	1.000	-0.127	0.102
	Uncoated High	0.027	0.998	-0.087	0.141
Soap Control	Control	-0.003	1.000	-0.117	0.111
	Weathered Low	-0.046	0.927	-0.160	0.069
	Sulphidized Low	0.004	1.000	-0.110	0.118
	Sulphidized High	0.008	1.000	-0.106	0.122
	Ionic Low	0.038	0.977	-0.076	0.152
	Ionic High	0.095	0.164	-0.019	0.209
	PVP Low	0.001	1.000	-0.114	0.115
	PVP High	0.027	0.998	-0.087	0.142
	Uncoated Low	-0.015	1.000	-0.130	0.099
	Uncoated High	0.024	0.999	-0.090	0.138
Weathered Low	Control	0.043	0.952	-0.072	0.157
	Soap Control	0.046	0.927	-0.069	0.160
	Sulphidized Low	0.049	0.887	-0.065	0.164
	Sulphidized High	0.054	0.826	-0.060	0.168
	Ionic Low	0.084	0.298	-0.031	0.198
	Ionic High	0.14039*	0.008	0.026	0.255
	PVP Low	0.046	0.922	-0.068	0.160
	PVP High	0.073	0.473	-0.041	0.187
	Uncoated Low	0.030	0.996	-0.084	0.144
	Uncoated High	0.069	0.545	-0.045	0.183
Sulphidized Low	Control	-0.007	1.000	-0.121	0.107
	Soap Control	-0.004	1.000	-0.118	0.110
	Weathered Low	-0.049	0.887	-0.164	0.065
	Sulphidized High	0.004	1.000	-0.110	0.119
	Ionic Low	0.034	0.989	-0.080	0.148
	Ionic High	0.091	0.203	-0.023	0.205
	PVP Low	-0.003	1.000	-0.117	0.111
	PVP High	0.024	0.999	-0.091	0.138
	Uncoated Low	-0.019	1.000	-0.133	0.095
	Uncoated High	0.020	1.000	-0.094	0.134
Sulphidized High	Control	-0.011	1.000	-0.125	0.103
	Soap Control	-0.008	1.000	-0.122	0.106

	Weathered Low	-0.054	0.826	-0.168	0.060
	Sulphidized Low	-0.004	1.000	-0.119	0.110
	Ionic Low	0.030	0.996	-0.084	0.144
	Ionic High	0.087	0.256	-0.028	0.201
	PVP Low	-0.008	1.000	-0.122	0.106
	PVP High	0.019	1.000	-0.095	0.133
	Uncoated Low	-0.024	0.999	-0.138	0.090
	Uncoated High	0.015	1.000	-0.099	0.130
Ionic Low	Control	-0.041	0.963	-0.155	0.073
	Soap Control	-0.038	0.977	-0.152	0.076
	Weathered Low	-0.084	0.298	-0.198	0.031
	Sulphidized Low	-0.034	0.989	-0.148	0.080
	Sulphidized High	-0.030	0.996	-0.144	0.084
	Ionic High	0.057	0.778	-0.057	0.171
	PVP Low	-0.037	0.980	-0.151	0.077
	PVP High	-0.010	1.000	-0.125	0.104
	Uncoated Low	-0.053	0.835	-0.167	0.061
	Uncoated High	-0.014	1.000	-0.128	0.100
Ionic High	Control	-0.098	0.138	-0.212	0.016
	Soap Control	-0.095	0.164	-0.209	0.019
	Weathered Low	-0.14039*	0.008	-0.255	-0.026
	Sulphidized Low	-0.091	0.203	-0.205	0.023
	Sulphidized High	-0.087	0.256	-0.201	0.028
	Ionic Low	-0.057	0.778	-0.171	0.057
	PVP Low	-0.094	0.169	-0.208	0.020
	PVP High	-0.067	0.582	-0.181	0.047
	Uncoated Low	-0.110	0.064	-0.224	0.004
	Uncoated High	-0.071	0.509	-0.185	0.043
PVP Low	Control	-0.004	1.000	-0.118	0.111
	Soap Control	-0.001	1.000	-0.115	0.114
	Weathered Low	-0.046	0.922	-0.160	0.068
	Sulphidized Low	0.003	1.000	-0.111	0.117
	Sulphidized High	0.008	1.000	-0.106	0.122
	Ionic Low	0.037	0.980	-0.077	0.151
	Ionic High	0.094	0.169	-0.020	0.208
	PVP High	0.027	0.998	-0.087	0.141
	Uncoated Low	-0.016	1.000	-0.130	0.098
	Uncoated High	0.023	1.000	-0.091	0.137
PVP High	Control	-0.030	0.995	-0.145	0.084
	Soap Control	-0.027	0.998	-0.142	0.087
	Weathered Low	-0.073	0.473	-0.187	0.041
	Sulphidized Low	-0.024	0.999	-0.138	0.091
	Sulphidized High	-0.019	1.000	-0.133	0.095
	Ionic Low	0.010	1.000	-0.104	0.125
	Ionic High	0.067	0.582	-0.047	0.181
	PVP Low	-0.027	0.998	-0.141	0.087

	Uncoated Low	-0.043	0.950	-0.157	0.071
	Uncoated High	-0.004	1.000	-0.118	0.110
Uncoated Low	Control	0.012	1.000	-0.102	0.127
	Soap Control	0.015	1.000	-0.099	0.130
	Weathered Low	-0.030	0.996	-0.144	0.084
	Sulphidized Low	0.019	1.000	-0.095	0.133
	Sulphidized High	0.024	0.999	-0.090	0.138
	Ionic Low	0.053	0.835	-0.061	0.167
	Ionic High	0.110	0.064	-0.004	0.224
	PVP Low	0.016	1.000	-0.098	0.130
	PVP High	0.043	0.950	-0.071	0.157
	Uncoated High	0.039	0.972	-0.075	0.153
Uncoated High	Control	-0.027	0.998	-0.141	0.087
	Soap Control	-0.024	0.999	-0.138	0.090
	Weathered Low	-0.069	0.545	-0.183	0.045
	Sulphidized Low	-0.020	1.000	-0.134	0.094
	Sulphidized High	-0.015	1.000	-0.130	0.099
	Ionic Low	0.014	1.000	-0.100	0.128
	Ionic High	0.071	0.509	-0.043	0.185
	PVP Low	-0.023	1.000	-0.137	0.091
	PVP High	0.004	1.000	-0.110	0.118
	Uncoated Low	-0.039	0.972	-0.153	0.075

Table C.44: Tukey test subset treatment groups for carboxylic and acetic acids in Month 2

Treatment	N	Subsets	
		1	2
Ionic High	3	0.0713	
Ionic Low	3	0.1282	0.1282
PVP High	3	0.1386	0.1386
Uncoated High	3	0.1424	0.1424
Sulphidized High	3	0.1578	0.1578
Sulphidized Low	3	0.1622	0.1622
PVP Low	3	0.1655	0.1655
Soap Control	3	0.1661	0.1661
Control	3	0.1691	0.1691
Uncoated Low	3	0.1815	0.1815
Weathered Low	3		0.2117
Significance		0.064	0.298

Table C.45: Multiple comparisons for amino acids Month 2 treatments using Tukey's test

Treatment (I)	Treatment (J)	Mean Difference (I-J)	Significance	95% Confidence Interval	
				Lower Bound	Upper Bound
Control	Soap Control	-0.005	1.000	-0.058	0.048
	Weathered Low	-0.006	1.000	-0.059	0.047
	Sulphidized Low	-0.009	1.000	-0.062	0.044
	Sulphidized High	-0.005	1.000	-0.058	0.048
	Ionic Low	0.019	0.968	-0.034	0.071
	Ionic High	0.045	0.136	-0.007	0.098
	PVP Low	0.009	1.000	-0.044	0.062
	PVP High	0.002	1.000	-0.051	0.055
	Uncoated Low	-0.009	1.000	-0.062	0.044
	Uncoated High	0.008	1.000	-0.045	0.061
Soap Control	Control	0.005	1.000	-0.048	0.058
	Weathered Low	-0.002	1.000	-0.055	0.051
	Sulphidized Low	-0.004	1.000	-0.057	0.049
	Sulphidized High	0.000	1.000	-0.053	0.053
	Ionic Low	0.023	0.875	-0.030	0.076
	Ionic High	0.050	0.071	-0.003	0.103
	PVP Low	0.014	0.996	-0.039	0.067
	PVP High	0.007	1.000	-0.046	0.060
	Uncoated Low	-0.004	1.000	-0.057	0.049
	Uncoated High	0.013	0.998	-0.040	0.066
Weathered Low	Control	0.006	1.000	-0.047	0.059
	Soap Control	0.002	1.000	-0.051	0.055
	Sulphidized Low	-0.003	1.000	-0.055	0.050
	Sulphidized High	0.001	1.000	-0.052	0.054
	Ionic Low	0.025	0.827	-0.028	0.078
	Ionic High	0.052	0.057	-0.001	0.105
	PVP Low	0.016	0.991	-0.037	0.068
	PVP High	0.008	1.000	-0.045	0.061
	Uncoated Low	-0.003	1.000	-0.056	0.050
	Uncoated High	0.014	0.994	-0.038	0.067
Sulphidized Low	Control	0.009	1.000	-0.044	0.062
	Soap Control	0.004	1.000	-0.049	0.057
	Weathered Low	0.003	1.000	-0.050	0.055
	Sulphidized High	0.004	1.000	-0.049	0.057
	Ionic Low	0.027	0.737	-0.025	0.080
	Ionic High	0.05444*	0.040	0.002	0.107
	PVP Low	0.018	0.973	-0.035	0.071
	PVP High	0.011	1.000	-0.042	0.064
	Uncoated Low	0.000	1.000	-0.053	0.053
	Uncoated High	0.017	0.982	-0.036	0.070
Sulphidized High	Control	0.005	1.000	-0.048	0.058
	Soap Control	0.000	1.000	-0.053	0.053

	Weathered Low	-0.001	1.000	-0.054	0.052
	Sulphidized Low	-0.004	1.000	-0.057	0.049
	Ionic Low	0.024	0.867	-0.029	0.077
	Ionic High	0.051	0.069	-0.002	0.104
	PVP Low	0.014	0.995	-0.039	0.067
	PVP High	0.007	1.000	-0.046	0.060
	Uncoated Low	-0.004	1.000	-0.057	0.049
	Uncoated High	0.013	0.997	-0.040	0.066
Ionic Low	Control	-0.019	0.968	-0.071	0.034
	Soap Control	-0.023	0.875	-0.076	0.030
	Weathered Low	-0.025	0.827	-0.078	0.028
	Sulphidized Low	-0.027	0.737	-0.080	0.025
	Sulphidized High	-0.024	0.867	-0.077	0.029
	Ionic High	0.027	0.756	-0.026	0.080
	PVP Low	-0.009	1.000	-0.062	0.044
	PVP High	-0.017	0.984	-0.070	0.036
	Uncoated Low	-0.028	0.730	-0.081	0.025
	Uncoated High	-0.010	1.000	-0.063	0.042
Ionic High	Control	-0.045	0.136	-0.098	0.007
	Soap Control	-0.050	0.071	-0.103	0.003
	Weathered Low	-0.052	0.057	-0.105	0.001
	Sulphidized Low	-0.05444*	0.040	-0.107	-0.002
	Sulphidized High	-0.051	0.069	-0.104	0.002
	Ionic Low	-0.027	0.756	-0.080	0.026
	PVP Low	-0.036	0.377	-0.089	0.017
	PVP High	-0.044	0.169	-0.097	0.009
	Uncoated Low	-0.05462*	0.039	-0.108	-0.002
	Uncoated High	-0.037	0.340	-0.090	0.016
PVP Low	Control	-0.009	1.000	-0.062	0.044
	Soap Control	-0.014	0.996	-0.067	0.039
	Weathered Low	-0.016	0.991	-0.068	0.037
	Sulphidized Low	-0.018	0.973	-0.071	0.035
	Sulphidized High	-0.014	0.995	-0.067	0.039
	Ionic Low	0.009	1.000	-0.044	0.062
	Ionic High	0.036	0.377	-0.017	0.089
	PVP High	-0.007	1.000	-0.060	0.046
	Uncoated Low	-0.018	0.971	-0.071	0.035
	Uncoated High	-0.001	1.000	-0.054	0.052
PVP High	Control	-0.002	1.000	-0.055	0.051
	Soap Control	-0.007	1.000	-0.060	0.046
	Weathered Low	-0.008	1.000	-0.061	0.045
	Sulphidized Low	-0.011	1.000	-0.064	0.042
	Sulphidized High	-0.007	1.000	-0.060	0.046
	Ionic Low	0.017	0.984	-0.036	0.070
	Ionic High	0.044	0.169	-0.009	0.097
	PVP Low	0.007	1.000	-0.046	0.060

	Uncoated Low	-0.011	0.999	-0.064	0.042
	Uncoated High	0.006	1.000	-0.047	0.059
Uncoated Low	Control	0.009	1.000	-0.044	0.062
	Soap Control	0.004	1.000	-0.049	0.057
	Weathered Low	0.003	1.000	-0.050	0.056
	Sulphidized Low	0.000	1.000	-0.053	0.053
	Sulphidized High	0.004	1.000	-0.049	0.057
	Ionic Low	0.028	0.730	-0.025	0.081
	Ionic High	0.05462*	0.039	0.002	0.108
	PVP Low	0.018	0.971	-0.035	0.071
	PVP High	0.011	0.999	-0.042	0.064
	Uncoated High	0.017	0.981	-0.036	0.070
Uncoated High	Control	-0.008	1.000	-0.061	0.045
	Soap Control	-0.013	0.998	-0.066	0.040
	Weathered Low	-0.014	0.994	-0.067	0.038
	Sulphidized Low	-0.017	0.982	-0.070	0.036
	Sulphidized High	-0.013	0.997	-0.066	0.040
	Ionic Low	0.010	1.000	-0.042	0.063
	Ionic High	0.037	0.340	-0.016	0.090
	PVP Low	0.001	1.000	-0.052	0.054
	PVP High	-0.006	1.000	-0.059	0.047
	Uncoated Low	-0.017	0.981	-0.070	0.036

Table C.46: Tukey test subset treatment groups for amino acids in Month 2

Treatment	N	Subsets	
		1	2
Ionic High	3	0.0494	
Ionic Low	3	0.0764	0.0764
PVP Low	3	0.0858	0.0858
Uncoated High	3	0.0869	0.0869
PVP High	3	0.0932	0.0932
Control	3	0.0949	0.0949
Soap Control	3	0.0998	0.0998
Sulphidized High	3	0.1001	0.1001
Weathered Low	3	0.1014	0.1014
Sulphidized Low	3		0.1039
Uncoated Low	3		0.1041
Significance		0.057	0.73

Table C.47: Multiple comparisons for root exudates in Month 2 treatments using Tukey's test

Treatment (I)	Treatment (J)	Mean Difference (I-J)	Significance	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Control	Soap Control	-0.023	0.997	-0.116	0.069
	Weathered Low	-0.034	0.960	-0.126	0.059
	Sulphidized Low	0.008	1.000	-0.084	0.100
	Sulphidized High	0.010	1.000	-0.082	0.103
	Ionic Low	0.044	0.816	-0.048	0.137
	Ionic High	0.080	0.127	-0.012	0.173
	PVP Low	0.016	1.000	-0.077	0.108
	PVP High	0.023	0.998	-0.070	0.115
	Uncoated Low	-0.010	1.000	-0.102	0.083
	Uncoated High	0.032	0.971	-0.060	0.124
Soap Control	Control	0.023	0.997	-0.069	0.116
	Weathered Low	-0.010	1.000	-0.102	0.082
	Sulphidized Low	0.032	0.973	-0.061	0.124
	Sulphidized High	0.034	0.958	-0.059	0.126
	Ionic Low	0.068	0.297	-0.025	0.160
	Ionic High	0.10379*	0.019	0.012	0.196
	PVP Low	0.039	0.900	-0.053	0.131
	PVP High	0.046	0.779	-0.046	0.138
	Uncoated Low	0.014	1.000	-0.078	0.106
	Uncoated High	0.055	0.561	-0.037	0.148
Weathered Low	Control	0.034	0.960	-0.059	0.126
	Soap Control	0.010	1.000	-0.082	0.102
	Sulphidized Low	0.042	0.862	-0.051	0.134
	Sulphidized High	0.044	0.823	-0.049	0.136
	Ionic Low	0.078	0.153	-0.015	0.170
	Ionic High	0.11382*	0.008	0.022	0.206
	PVP Low	0.049	0.711	-0.043	0.141
	PVP High	0.056	0.546	-0.036	0.148
	Uncoated Low	0.024	0.996	-0.068	0.116
	Uncoated High	0.065	0.339	-0.027	0.158
Sulphidized Low	Control	-0.008	1.000	-0.100	0.084
	Soap Control	-0.032	0.973	-0.124	0.061
	Weathered Low	-0.042	0.862	-0.134	0.051
	Sulphidized High	0.002	1.000	-0.090	0.095
	Ionic Low	0.036	0.937	-0.056	0.128
	Ionic High	0.072	0.222	-0.020	0.165
	PVP Low	0.007	1.000	-0.085	0.100
	PVP High	0.014	1.000	-0.078	0.107
	Uncoated Low	-0.018	1.000	-0.110	0.075
	Uncoated High	0.024	0.997	-0.069	0.116
Sulphidized High	Control	-0.010	1.000	-0.103	0.082
	Soap Control	-0.034	0.958	-0.126	0.059

	Weathered Low	-0.044	0.823	-0.136	0.049
	Sulphidized Low	-0.002	1.000	-0.095	0.090
	Ionic Low	0.034	0.957	-0.058	0.126
	Ionic High	0.070	0.256	-0.022	0.162
	PVP Low	0.005	1.000	-0.087	0.098
	PVP High	0.012	1.000	-0.080	0.105
	Uncoated Low	-0.020	0.999	-0.112	0.073
	Uncoated High	0.022	0.998	-0.071	0.114
Ionic Low	Control	-0.044	0.816	-0.137	0.048
	Soap Control	-0.068	0.297	-0.160	0.025
	Weathered Low	-0.078	0.153	-0.170	0.015
	Sulphidized Low	-0.036	0.937	-0.128	0.056
	Sulphidized High	-0.034	0.957	-0.126	0.058
	Ionic High	0.036	0.936	-0.056	0.129
	PVP Low	-0.029	0.986	-0.121	0.064
	PVP High	-0.022	0.998	-0.114	0.071
	Uncoated Low	-0.054	0.602	-0.146	0.039
	Uncoated High	-0.012	1.000	-0.105	0.080
Ionic High	Control	-0.080	0.127	-0.173	0.012
	Soap Control	-0.10379*	0.019	-0.196	-0.012
	Weathered Low	-0.11382*	0.008	-0.206	-0.022
	Sulphidized Low	-0.072	0.222	-0.165	0.020
	Sulphidized High	-0.070	0.256	-0.162	0.022
	Ionic Low	-0.036	0.936	-0.129	0.056
	PVP Low	-0.065	0.352	-0.157	0.028
	PVP High	-0.058	0.504	-0.150	0.035
	Uncoated Low	-0.090	0.061	-0.182	0.003
	Uncoated High	-0.048	0.726	-0.141	0.044
PVP Low	Control	-0.016	1.000	-0.108	0.077
	Soap Control	-0.039	0.900	-0.131	0.053
	Weathered Low	-0.049	0.711	-0.141	0.043
	Sulphidized Low	-0.007	1.000	-0.100	0.085
	Sulphidized High	-0.005	1.000	-0.098	0.087
	Ionic Low	0.029	0.986	-0.064	0.121
	Ionic High	0.065	0.352	-0.028	0.157
	PVP High	0.007	1.000	-0.085	0.099
	Uncoated Low	-0.025	0.995	-0.117	0.067
	Uncoated High	0.016	1.000	-0.076	0.109
PVP High	Control	-0.023	0.998	-0.115	0.070
	Soap Control	-0.046	0.779	-0.138	0.046
	Weathered Low	-0.056	0.546	-0.148	0.036
	Sulphidized Low	-0.014	1.000	-0.107	0.078
	Sulphidized High	-0.012	1.000	-0.105	0.080
	Ionic Low	0.022	0.998	-0.071	0.114
	Ionic High	0.058	0.504	-0.035	0.150
	PVP Low	-0.007	1.000	-0.099	0.085

	Uncoated Low	-0.032	0.970	-0.124	0.060
	Uncoated High	0.009	1.000	-0.083	0.102
Uncoated Low	Control	0.010	1.000	-0.083	0.102
	Soap Control	-0.014	1.000	-0.106	0.078
	Weathered Low	-0.024	0.996	-0.116	0.068
	Sulphidized Low	0.018	1.000	-0.075	0.110
	Sulphidized High	0.020	0.999	-0.073	0.112
	Ionic Low	0.054	0.602	-0.039	0.146
	Ionic High	0.090	0.061	-0.003	0.182
	PVP Low	0.025	0.995	-0.067	0.117
	PVP High	0.032	0.970	-0.060	0.124
	Uncoated High	0.041	0.864	-0.051	0.134
Uncoated High	Control	-0.032	0.971	-0.124	0.060
	Soap Control	-0.055	0.561	-0.148	0.037
	Weathered Low	-0.065	0.339	-0.158	0.027
	Sulphidized Low	-0.024	0.997	-0.116	0.069
	Sulphidized High	-0.022	0.998	-0.114	0.071
	Ionic Low	0.012	1.000	-0.080	0.105
	Ionic High	0.048	0.726	-0.044	0.141
	PVP Low	-0.016	1.000	-0.109	0.076
	PVP High	-0.009	1.000	-0.102	0.083
	Uncoated Low	-0.041	0.864	-0.134	0.051

Table C.48: Tukey test subset treatment groups for root exudates in Month 2

Treatment	N	Subsets	
		1	2
Ionic High	3	0.0962	
Ionic Low	3	0.1324	0.1324
Uncoated High	3	0.1446	0.1446
PVP High	3	0.1540	0.1540
PVP Low	3	0.1609	0.1609
Sulphidized High	3	0.1662	0.1662
Sulphidized Low	3	0.1684	0.1684
Control	3	0.1765	0.1765
Uncoated Low	3	0.1860	0.1860
Soap Control	3		0.2000
Weathered Low	3		0.2100
Significance		0.061	0.153

Table C.49: One-way ANOVA for Month 3 Guilds

		Sum of Squares	DOF	Mean Square	F	Significance
Carbohydrates	Between Groups	0.019	10	0.002	1.892	0.102
	Within Groups	0.022	22	0.001		
	Total	0.041	32			
Polymers	Between Groups	0.004	10	0	1.612	0.168
	Within Groups	0.005	22	0		
	Total	0.009	32			
Carboxylic acids	Between Groups	0.038	10	0.004	1.305	0.288
	Within Groups	0.064	22	0.003		
	Total	0.102	32			
Amino acids	Between Groups	0.021	10	0.002	2.292	0.050
	Within Groups	0.02	22	0.001		
	Total	0.041	32			
Amides/ amides	Between Groups	0.005	10	0.001	3.192	0.011
	Within Groups	0.003	22	0		
	Total	0.009	32			
Root Exudates	Between Groups	0.055	10	0.006	2.302	0.050
	Within Groups	0.053	22	0.002		
	Total	0.108	32			

Table C.50: Multiple comparisons for amino acids in Month 3 treatments using Tukey's test

Treatment (I)	Treatment (J)	Mean Difference (I-J)	Significance	95% Confidence Interval	
				Lower Bound	Upper Bound
Control	Soap Control	-0.052	0.585	-0.140	0.036
	Weathered Low	-0.029	0.980	-0.117	0.060
	Sulphidized Low	0.046	0.726	-0.042	0.135
	Sulphidized High	-0.025	0.993	-0.113	0.064
	Ionic Low	0.007	1.000	-0.081	0.095
	Ionic High	0.022	0.997	-0.066	0.111
	PVP Low	-0.002	1.000	-0.091	0.086
	PVP High	-0.001	1.000	-0.089	0.088
	Uncoated Low	-0.020	0.999	-0.108	0.068
	Uncoated High	-0.011	1.000	-0.099	0.078
Soap Control	Control	0.052	0.585	-0.036	0.140
	Weathered Low	0.023	0.996	-0.065	0.112
	Sulphidized Low	0.09837*	0.021	0.010	0.187
	Sulphidized High	0.027	0.987	-0.061	0.116
	Ionic Low	0.059	0.412	-0.029	0.148
	Ionic High	0.074	0.155	-0.014	0.163
	PVP Low	0.050	0.640	-0.039	0.138
	PVP High	0.051	0.599	-0.037	0.140

	Uncoated Low	0.032	0.961	-0.056	0.120
	Uncoated High	0.041	0.831	-0.047	0.130
Weathered Low	Control	0.029	0.980	-0.060	0.117
	Soap Control	-0.023	0.996	-0.112	0.065
	Sulphidized Low	0.075	0.144	-0.013	0.163
	Sulphidized High	0.004	1.000	-0.084	0.092
	Ionic Low	0.036	0.920	-0.052	0.124
	Ionic High	0.051	0.613	-0.037	0.139
	PVP Low	0.027	0.989	-0.062	0.115
	PVP High	0.028	0.983	-0.060	0.117
	Uncoated Low	0.009	1.000	-0.080	0.097
	Uncoated High	0.018	0.999	-0.070	0.107
Sulphidized Low	Control	-0.046	0.726	-0.135	0.042
	Soap Control	-0.09837*	0.021	-0.187	-0.010
	Weathered Low	-0.075	0.144	-0.163	0.013
	Sulphidized High	-0.071	0.193	-0.160	0.017
	Ionic Low	-0.039	0.871	-0.128	0.049
	Ionic High	-0.024	0.994	-0.113	0.064
	PVP Low	-0.049	0.673	-0.137	0.040
	PVP High	-0.047	0.712	-0.135	0.041
	Uncoated Low	-0.066	0.266	-0.155	0.022
	Uncoated High	-0.057	0.465	-0.145	0.031
Sulphidized High	Control	0.025	0.993	-0.064	0.113
	Soap Control	-0.027	0.987	-0.116	0.061
	Weathered Low	-0.004	1.000	-0.092	0.084
	Sulphidized Low	0.071	0.193	-0.017	0.160
	Ionic Low	0.032	0.961	-0.056	0.120
	Ionic High	0.047	0.711	-0.041	0.135
	PVP Low	0.023	0.997	-0.066	0.111
	PVP High	0.024	0.994	-0.064	0.113
	Uncoated Low	0.005	1.000	-0.084	0.093
	Uncoated High	0.014	1.000	-0.074	0.103
Ionic Low	Control	-0.007	1.000	-0.095	0.081
	Soap Control	-0.059	0.412	-0.148	0.029
	Weathered Low	-0.036	0.920	-0.124	0.052
	Sulphidized Low	0.039	0.871	-0.049	0.128
	Sulphidized High	-0.032	0.961	-0.120	0.056
	Ionic High	0.015	1.000	-0.073	0.103
	PVP Low	-0.009	1.000	-0.098	0.079
	PVP High	-0.008	1.000	-0.096	0.081
	Uncoated Low	-0.027	0.987	-0.116	0.061
	Uncoated High	-0.018	1.000	-0.106	0.071
Ionic High	Control	-0.022	0.997	-0.111	0.066
	Soap Control	-0.074	0.155	-0.163	0.014
	Weathered Low	-0.051	0.613	-0.139	0.037
	Sulphidized Low	0.024	0.994	-0.064	0.113

	Sulphidized High	-0.047	0.711	-0.135	0.041
	Ionic Low	-0.015	1.000	-0.103	0.073
	PVP Low	-0.024	0.994	-0.113	0.064
	PVP High	-0.023	0.997	-0.111	0.066
	Uncoated Low	-0.042	0.816	-0.131	0.046
	Uncoated High	-0.033	0.954	-0.121	0.056
PVP Low	Control	0.002	1.000	-0.086	0.091
	Soap Control	-0.050	0.640	-0.138	0.039
	Weathered Low	-0.027	0.989	-0.115	0.062
	Sulphidized Low	0.049	0.673	-0.040	0.137
	Sulphidized High	-0.023	0.997	-0.111	0.066
	Ionic Low	0.009	1.000	-0.079	0.098
	Ionic High	0.024	0.994	-0.064	0.113
	PVP High	0.002	1.000	-0.087	0.090
	Uncoated Low	-0.018	1.000	-0.106	0.070
	Uncoated High	-0.008	1.000	-0.097	0.080
PVP High	Control	0.001	1.000	-0.088	0.089
	Soap Control	-0.051	0.599	-0.140	0.037
	Weathered Low	-0.028	0.983	-0.117	0.060
	Sulphidized Low	0.047	0.712	-0.041	0.135
	Sulphidized High	-0.024	0.994	-0.113	0.064
	Ionic Low	0.008	1.000	-0.081	0.096
	Ionic High	0.023	0.997	-0.066	0.111
	PVP Low	-0.002	1.000	-0.090	0.087
	Uncoated Low	-0.020	0.999	-0.108	0.069
	Uncoated High	-0.010	1.000	-0.098	0.078
Uncoated Low	Control	0.020	0.999	-0.068	0.108
	Soap Control	-0.032	0.961	-0.120	0.056
	Weathered Low	-0.009	1.000	-0.097	0.080
	Sulphidized Low	0.066	0.266	-0.022	0.155
	Sulphidized High	-0.005	1.000	-0.093	0.084
	Ionic Low	0.027	0.987	-0.061	0.116
	Ionic High	0.042	0.816	-0.046	0.131
	PVP Low	0.018	1.000	-0.070	0.106
	PVP High	0.020	0.999	-0.069	0.108
	Uncoated High	0.010	1.000	-0.079	0.098
Uncoated High	Control	0.011	1.000	-0.078	0.099
	Soap Control	-0.041	0.831	-0.130	0.047
	Weathered Low	-0.018	0.999	-0.107	0.070
	Sulphidized Low	0.057	0.465	-0.031	0.145
	Sulphidized High	-0.014	1.000	-0.103	0.074
	Ionic Low	0.018	1.000	-0.071	0.106
	Ionic High	0.033	0.954	-0.056	0.121
	PVP Low	0.008	1.000	-0.080	0.097
	PVP High	0.010	1.000	-0.078	0.098
	Uncoated Low	-0.010	1.000	-0.098	0.079

Table C.51: Tukey test subset treatment groups for amino acids in Month 3

Treatment	N	Subsets	
		1	2
Sulphidized Low	3	0.0178	
Ionic High	3	0.042	0.042
Ionic Low	3	0.057	0.057
Control	3	0.0641	0.0641
PVP High	3	0.0647	0.0647
PVP Low	3	0.0663	0.0663
Uncoated High	3	0.0747	0.0747
Uncoated Low	3	0.0842	0.0842
Sulphidized High	3	0.0889	0.0889
Weathered Low	3	0.0929	0.0929
Soap Control	3		0.1162
Significance		0.144	0.155

Table C.52: Multiple comparisons for amine/amides in Month 3 treatments using Tukey's test

Treatment (I)	Treatment (J)	Mean Difference (I-J)	Significance	95% Confidence Interval	
				Lower Bound	Upper Bound
Control	Soap Control	-0.03772*	0.041	-0.075	-0.001
	Weathered Low	-0.026	0.359	-0.063	0.011
	Sulphidized Low	-0.002	1.000	-0.039	0.035
	Sulphidized High	0.000	1.000	-0.036	0.037
	Ionic Low	-0.006	1.000	-0.042	0.031
	Ionic High	0.004	1.000	-0.033	0.041
	PVP Low	-0.006	1.000	-0.043	0.031
	PVP High	0.001	1.000	-0.036	0.038
	Uncoated Low	-0.008	0.999	-0.045	0.029
	Uncoated High	-0.018	0.801	-0.055	0.019
Soap Control	Control	0.03772*	0.041	0.001	0.075
	Weathered Low	0.012	0.980	-0.025	0.049
	Sulphidized Low	0.036	0.064	-0.001	0.072
	Sulphidized High	0.03819*	0.038	0.001	0.075
	Ionic Low	0.032	0.125	-0.005	0.069
	Ionic High	0.04165*	0.018	0.005	0.079
	PVP Low	0.031	0.141	-0.005	0.068
	PVP High	0.03901*	0.032	0.002	0.076
	Uncoated Low	0.029	0.199	-0.007	0.066
	Uncoated High	0.020	0.696	-0.017	0.057
Weathered Low	Control	0.026	0.359	-0.011	0.063
	Soap Control	-0.012	0.980	-0.049	0.025
	Sulphidized Low	0.024	0.475	-0.013	0.060
	Sulphidized High	0.026	0.336	-0.011	0.063

	Ionic Low	0.020	0.683	-0.017	0.057
	Ionic High	0.030	0.194	-0.007	0.066
	PVP Low	0.019	0.722	-0.017	0.056
	PVP High	0.027	0.297	-0.010	0.064
	Uncoated Low	0.017	0.826	-0.019	0.054
	Uncoated High	0.008	0.999	-0.029	0.045
Sulphidized Low	Control	0.002	1.000	-0.035	0.039
	Soap Control	-0.036	0.064	-0.072	0.001
	Weathered Low	-0.024	0.475	-0.060	0.013
	Sulphidized High	0.003	1.000	-0.034	0.039
	Ionic Low	-0.003	1.000	-0.040	0.033
	Ionic High	0.006	1.000	-0.031	0.043
	PVP Low	-0.004	1.000	-0.041	0.033
	PVP High	0.003	1.000	-0.033	0.040
	Uncoated Low	-0.006	1.000	-0.043	0.031
	Uncoated High	-0.016	0.894	-0.053	0.021
Sulphidized High	Control	0.000	1.000	-0.037	0.036
	Soap Control	-0.03819*	0.038	-0.075	-0.001
	Weathered Low	-0.026	0.336	-0.063	0.011
	Sulphidized Low	-0.003	1.000	-0.039	0.034
	Ionic Low	-0.006	1.000	-0.043	0.031
	Ionic High	0.003	1.000	-0.033	0.040
	PVP Low	-0.007	1.000	-0.044	0.030
	PVP High	0.001	1.000	-0.036	0.038
	Uncoated Low	-0.009	0.998	-0.046	0.028
	Uncoated High	-0.018	0.777	-0.055	0.018
Ionic Low	Control	0.006	1.000	-0.031	0.042
	Soap Control	-0.032	0.125	-0.069	0.005
	Weathered Low	-0.020	0.683	-0.057	0.017
	Sulphidized Low	0.003	1.000	-0.033	0.040
	Sulphidized High	0.006	1.000	-0.031	0.043
	Ionic High	0.010	0.996	-0.027	0.046
	PVP Low	-0.001	1.000	-0.038	0.036
	PVP High	0.007	1.000	-0.030	0.044
	Uncoated Low	-0.003	1.000	-0.040	0.034
	Uncoated High	-0.012	0.977	-0.049	0.025
Ionic High	Control	-0.004	1.000	-0.041	0.033
	Soap Control	-0.04165*	0.018	-0.079	-0.005
	Weathered Low	-0.030	0.194	-0.066	0.007
	Sulphidized Low	-0.006	1.000	-0.043	0.031
	Sulphidized High	-0.003	1.000	-0.040	0.033
	Ionic Low	-0.010	0.996	-0.046	0.027
	PVP Low	-0.010	0.994	-0.047	0.027
	PVP High	-0.003	1.000	-0.039	0.034
	Uncoated Low	-0.012	0.978	-0.049	0.025
	Uncoated High	-0.022	0.576	-0.059	0.015

PVP Low	Control	0.006	1.000	-0.031	0.043
	Soap Control	-0.031	0.141	-0.068	0.005
	Weathered Low	-0.019	0.722	-0.056	0.017
	Sulphidized Low	0.004	1.000	-0.033	0.041
	Sulphidized High	0.007	1.000	-0.030	0.044
	Ionic Low	0.001	1.000	-0.036	0.038
	Ionic High	0.010	0.994	-0.027	0.047
	PVP High	0.008	0.999	-0.029	0.044
	Uncoated Low	-0.002	1.000	-0.039	0.035
	Uncoated High	-0.012	0.984	-0.048	0.025
PVP High	Control	-0.001	1.000	-0.038	0.036
	Soap Control	-0.03901*	0.032	-0.076	-0.002
	Weathered Low	-0.027	0.297	-0.064	0.010
	Sulphidized Low	-0.003	1.000	-0.040	0.033
	Sulphidized High	-0.001	1.000	-0.038	0.036
	Ionic Low	-0.007	1.000	-0.044	0.030
	Ionic High	0.003	1.000	-0.034	0.039
	PVP Low	-0.008	0.999	-0.044	0.029
	Uncoated Low	-0.010	0.996	-0.046	0.027
	Uncoated High	-0.019	0.732	-0.056	0.018
Uncoated Low	Control	0.008	0.999	-0.029	0.045
	Soap Control	-0.029	0.199	-0.066	0.007
	Weathered Low	-0.017	0.826	-0.054	0.019
	Sulphidized Low	0.006	1.000	-0.031	0.043
	Sulphidized High	0.009	0.998	-0.028	0.046
	Ionic Low	0.003	1.000	-0.034	0.040
	Ionic High	0.012	0.978	-0.025	0.049
	PVP Low	0.002	1.000	-0.035	0.039
	PVP High	0.010	0.996	-0.027	0.046
	Uncoated High	-0.010	0.996	-0.046	0.027
Uncoated High	Control	0.018	0.801	-0.019	0.055
	Soap Control	-0.020	0.696	-0.057	0.017
	Weathered Low	-0.008	0.999	-0.045	0.029
	Sulphidized Low	0.016	0.894	-0.021	0.053
	Sulphidized High	0.018	0.777	-0.018	0.055
	Ionic Low	0.012	0.977	-0.025	0.049
	Ionic High	0.022	0.576	-0.015	0.059
	PVP Low	0.012	0.984	-0.025	0.048
	PVP High	0.019	0.732	-0.018	0.056
	Uncoated Low	0.010	0.996	-0.027	0.046

Table C.53: Tukey test subset treatment groups for amides/amines in Month 3

Treatment	N	Subsets	
		1	2
Ionic High	3	0.0017	
PVP High	3	0.0043	
Sulphidized High	3	0.0051	
Control	3	0.0056	
Sulphidized Low	3	0.0077	0.0077
Ionic Low	3	0.0112	0.0112
PVP Low	3	0.0119	0.0119
Uncoated Low	3	0.0139	0.0139
Uncoated High	3	0.0235	0.0235
Weathered Low	3	0.0313	0.0313
Soap Control	3		0.0433
Significance		0.194	0.064

Table C.54: Multiple comparisons for root exudates in Month 3 treatments using Tukey's test

Treatment (I)	Treatment (J)	Mean Difference (I-J)	Significance	95% Confidence Interval	
				Lower Bound	Upper Bound
Control	Soap Control	-0.079	0.659	-0.223	0.064
	Weathered Low	-0.045	0.984	-0.188	0.098
	Sulphidized Low	0.077	0.703	-0.066	0.220
	Sulphidized High	-0.014	1.000	-0.157	0.129
	Ionic Low	0.026	1.000	-0.117	0.169
	Ionic High	0.054	0.951	-0.089	0.197
	PVP Low	0.012	1.000	-0.131	0.155
	PVP High	0.006	1.000	-0.137	0.149
	Uncoated Low	-0.014	1.000	-0.157	0.129
	Uncoated High	-0.010	1.000	-0.153	0.133
Soap Control	Control	0.079	0.659	-0.064	0.223
	Weathered Low	0.034	0.998	-0.109	0.177
	Sulphidized Low	0.15604*	0.025	0.013	0.299
	Sulphidized High	0.065	0.851	-0.078	0.208
	Ionic Low	0.105	0.290	-0.038	0.248
	Ionic High	0.133	0.083	-0.010	0.276
	PVP Low	0.092	0.474	-0.052	0.235
	PVP High	0.086	0.559	-0.057	0.229
	Uncoated Low	0.065	0.852	-0.078	0.208
	Uncoated High	0.069	0.802	-0.074	0.213
Weathered Low	Control	0.045	0.984	-0.098	0.188
	Soap Control	-0.034	0.998	-0.177	0.109
	Sulphidized Low	0.122	0.144	-0.021	0.265
	Sulphidized High	0.031	0.999	-0.112	0.174

	Ionic Low	0.071	0.783	-0.072	0.214
	Ionic High	0.099	0.374	-0.044	0.242
	PVP Low	0.057	0.928	-0.086	0.200
	PVP High	0.051	0.962	-0.092	0.195
	Uncoated Low	0.031	0.999	-0.112	0.174
	Uncoated High	0.035	0.998	-0.108	0.178
Sulphidized Low	Control	-0.077	0.703	-0.220	0.066
	Soap Control	-0.15604*	0.025	-0.299	-0.013
	Weathered Low	-0.122	0.144	-0.265	0.021
	Sulphidized High	-0.091	0.488	-0.234	0.052
	Ionic Low	-0.051	0.965	-0.194	0.092
	Ionic High	-0.023	1.000	-0.166	0.120
	PVP Low	-0.065	0.861	-0.208	0.079
	PVP High	-0.070	0.793	-0.213	0.073
	Uncoated Low	-0.091	0.486	-0.234	0.052
	Uncoated High	-0.087	0.549	-0.230	0.057
Sulphidized High	Control	0.014	1.000	-0.129	0.157
	Soap Control	-0.065	0.851	-0.208	0.078
	Weathered Low	-0.031	0.999	-0.174	0.112
	Sulphidized Low	0.091	0.488	-0.052	0.234
	Ionic Low	0.040	0.994	-0.103	0.183
	Ionic High	0.068	0.825	-0.075	0.211
	PVP Low	0.026	1.000	-0.117	0.169
	PVP High	0.020	1.000	-0.123	0.164
	Uncoated Low	0.000	1.000	-0.143	0.143
	Uncoated High	0.004	1.000	-0.139	0.147
Ionic Low	Control	-0.026	1.000	-0.169	0.117
	Soap Control	-0.105	0.290	-0.248	0.038
	Weathered Low	-0.071	0.783	-0.214	0.072
	Sulphidized Low	0.051	0.965	-0.092	0.194
	Sulphidized High	-0.040	0.994	-0.183	0.103
	Ionic High	0.028	1.000	-0.115	0.171
	PVP Low	-0.014	1.000	-0.157	0.129
	PVP High	-0.019	1.000	-0.162	0.124
	Uncoated Low	-0.040	0.993	-0.183	0.103
	Uncoated High	-0.036	0.997	-0.179	0.107
Ionic High	Control	-0.054	0.951	-0.197	0.089
	Soap Control	-0.133	0.083	-0.276	0.010
	Weathered Low	-0.099	0.374	-0.242	0.044
	Sulphidized Low	0.023	1.000	-0.120	0.166
	Sulphidized High	-0.068	0.825	-0.211	0.075
	Ionic Low	-0.028	1.000	-0.171	0.115
	PVP Low	-0.042	0.991	-0.185	0.102
	PVP High	-0.047	0.979	-0.190	0.096
	Uncoated Low	-0.068	0.824	-0.211	0.075
	Uncoated High	-0.064	0.871	-0.207	0.079

PVP Low	Control	-0.012	1.000	-0.155	0.131
	Soap Control	-0.092	0.474	-0.235	0.052
	Weathered Low	-0.057	0.928	-0.200	0.086
	Sulphidized Low	0.065	0.861	-0.079	0.208
	Sulphidized High	-0.026	1.000	-0.169	0.117
	Ionic Low	0.014	1.000	-0.129	0.157
	Ionic High	0.042	0.991	-0.102	0.185
	PVP High	-0.006	1.000	-0.149	0.137
	Uncoated Low	-0.026	1.000	-0.169	0.117
	Uncoated High	-0.022	1.000	-0.165	0.121
PVP High	Control	-0.006	1.000	-0.149	0.137
	Soap Control	-0.086	0.559	-0.229	0.057
	Weathered Low	-0.051	0.962	-0.195	0.092
	Sulphidized Low	0.070	0.793	-0.073	0.213
	Sulphidized High	-0.020	1.000	-0.164	0.123
	Ionic Low	0.019	1.000	-0.124	0.162
	Ionic High	0.047	0.979	-0.096	0.190
	PVP Low	0.006	1.000	-0.137	0.149
	Uncoated Low	-0.021	1.000	-0.164	0.122
	Uncoated High	-0.016	1.000	-0.159	0.127
Uncoated Low	Control	0.014	1.000	-0.129	0.157
	Soap Control	-0.065	0.852	-0.208	0.078
	Weathered Low	-0.031	0.999	-0.174	0.112
	Sulphidized Low	0.091	0.486	-0.052	0.234
	Sulphidized High	0.000	1.000	-0.143	0.143
	Ionic Low	0.040	0.993	-0.103	0.183
	Ionic High	0.068	0.824	-0.075	0.211
	PVP Low	0.026	1.000	-0.117	0.169
	PVP High	0.021	1.000	-0.122	0.164
	Uncoated High	0.004	1.000	-0.139	0.147
Uncoated High	Control	0.010	1.000	-0.133	0.153
	Soap Control	-0.069	0.802	-0.213	0.074
	Weathered Low	-0.035	0.998	-0.178	0.108
	Sulphidized Low	0.087	0.549	-0.057	0.230
	Sulphidized High	-0.004	1.000	-0.147	0.139
	Ionic Low	0.036	0.997	-0.107	0.179
	Ionic High	0.064	0.871	-0.079	0.207
	PVP Low	0.022	1.000	-0.121	0.165
	PVP High	0.016	1.000	-0.127	0.159
	Uncoated Low	-0.004	1.000	-0.147	0.139

Table C.55: Tukey test subset treatment groups for root exudates in Month 3

Treatment	N	Subsets	
		1	2
Sulphidized Low	3	0.0463	
Ionic High	3	0.0693	0.0693
Ionic Low	3	0.097	0.097
Control	3	0.1108	0.1108
PVP High	3	0.1164	0.1164
PVP Low	3	0.1228	0.1228
Uncoated High	3	0.1328	0.1328
Uncoated Low	3	0.1369	0.1369
Sulphidized High	3	0.137	0.137
Weathered Low	3	0.1679	0.1679
Soap Control	3		0.2023
Significance		0.144	0.083

Table C.56: One-way ANOVA for Month 1 enzyme assays

		Sum of Squares	DOF	Mean Square	F	Significance
β- glucosidase	Between Groups	31395.966	10	3139.597	1.750	0.132
	Within Groups	39480.020	22	1794.546		
	Total	70875.986	32			
α- glucosidase	Between Groups	871.090	10	87.109	1.269	0.305
	Within Groups	1509.695	22	68.622		
	Total	2380.784	32			
Xylosidase	Between Groups	759.005	10	75.901	1.249	0.316
	Within Groups	1336.724	22	60.760		
	Total	2095.730	32			
Cellobiosidase	Between Groups	213.445	10	21.344	0.823	0.611
	Within Groups	570.549	22	25.934		
	Total	783.993	32			
n-acetylglucosaminase	Between Groups	1036.936	10	103.694	1.469	0.216
	Within Groups	1552.489	22	70.568		
	Total	2589.425	32			
Phosphatase	Between Groups	292450.405	10	29245.041	0.976	0.490
	Within Groups	659196.938	22	29963.497		
	Total	951647.343	32			
Leucine aminopeptidase	Between Groups	2180.300	10	218.030	0.910	0.541
	Within Groups	5268.234	22	239.465		
	Total	7448.534	32			

Table C.57: One-way ANOVA for Month 2 enzyme assays

		Sum of Squares	DOF	Mean Square	F	Significance
β- glucosidase	Between Groups	12910.238	10	1291.024	0.805	0.626
	Within Groups	35292.456	22	1604.203		
	Total	48202.694	32			
α- glucosidase	Between Groups	296.363	10	29.636	0.791	0.638
	Within Groups	824.447	22	37.475		
	Total	1120.810	32			
Xylosidase	Between Groups	359.105	10	35.911	0.734	0.686
	Within Groups	1075.860	22	48.903		
	Total	1434.966	32			
Cellobiosidase	Between Groups	486.837	10	48.684	0.510	0.865
	Within Groups	2099.090	22	95.413		
	Total	2585.927	32			
n-acetylglucosaminase	Between Groups	1194.158	10	119.416	0.756	0.667
	Within Groups	3475.468	22	157.976		
	Total	4669.626	32			
Phosphatase	Between Groups	6072.186	10	607.219	0.712	0.704
	Within Groups	18755.731	22	852.533		
	Total	24827.917	32			
Leucine aminopeptidase	Between Groups	2397.525	10	239.752	0.542	0.842
	Within Groups	9735.646	22			
	Total	12133.171	32			

Table C.58: One-way ANOVA for Month 3 enzyme assays

		Sum of Squares	DOF	Mean Square	F	Significance
β- glucosidase	Between Groups	229276.967	10	22927.697	0.848	0.590
	Within Groups	594533.957	22	27024.271		
	Total	823810.924	32			
Xylosidase	Between Groups	4000.779	10	400.078	0.964	0.499
	Within Groups	9126.061	22	414.821		
	Total	13126.839	32			
Cellobiosidase	Between Groups	1800.347	10	180.035	0.835	0.601
	Within Groups	4741.793	22	215.536		
	Total	6542.141	32			
n-acetylglucosaminase	Between Groups	6789.507	10	678.951	0.861	0.580
	Within Groups	17351.435	22	788.702		
	Total	24140.942	32			
Phosphatase	Between Groups	3388684.823	10	338868.482	0.950	0.510
	Within Groups	7847445.086	22	356702.049		
	Total	11236129.908	32			
Leucine aminopeptidase	Between Groups	3110.400	10	311.040	1.026	0.455
	Within Groups	6672.425	22			
	Total	9782.825	32			

Table C.59: Enzymatic activity (nmol/ g d.w. soil h) of various enzymes in soil treatments over three months of exposure

	Enzyme Activity (nmol/ g d.w. soil h)											
	β-glucosidase			α-glucosidase			Xylosidase			Cellobiosidase		
Treatment	Month 1	Month 2	Month 3	Month 1	Month 2	Month 3	Month 1	Month 2	Month 3	Month 1	Month 2	Month 3
Control	30.0±27.5	10.0±10.0	11.1±14.3	0.0±0.0	7.4±8.0	0.0±0.0	0.0±0.0	7.9±8.2	0.0±0.0	5.2±6.3	13.7±16.3	0.0±0.0
Soap Control	14.3±20.2	27.4±5.9	1.8±2.6	0.0±0.0	4.4±3.7	0.0±0.0	0.0±0.0	5.4±1.5	0.0±0.0	0.0±0.0	7.1±5.0	0.0±0.0
Weathered Low	122.0±70.5	51.7±5.8	15.9±15.9	17.9±22.4	2.1±3.0	0.0±0.0	16.8±19.7	2.8±3.7	0.0±0.0	8.1±5.7	6.9±5.7	0.0±0.0
Sulphidized Low	14.0±14.4	25.1±13.4	16.1±9.3	0.0±0.1	0.0±0.0	0.0±0.0	1.6±2.2	0.0±0.0	0.0±0.0	3.7±5.3	0.0±0.0	0.0±0.0
Uncoated Low	3.8±4.7	76.2±59.3	0.9±1.3	0.0±0.0	2.9±4.1	0.0±0.0	0.0±0.0	3.3±4.7	0.0±0.0	0.0±0.0	3.7±5.2	0.0±0.0
PVP Low	32.2±22.8	50.0±42.2	10.7±15.1	0.7±1.0	3.7±0.0	0.0±0.0	4.8±6.8	4.9±1.0	0.0±0.0	2.4±3.3	5.7±0.5	0.0±0.0
Ionic Low	54.4±54.5	32.1±22.8	22.9±18.5	0.0±0.0	6.1±2.0	0.0±0.0	0.2±0.3	7.8±2.5	1.8±0.0	2.2±2.2	9.2±5.1	0.4±0.0
Sulphidized High	39.3±45.6	55.4±30.6	9.5±9.0	0.0±0.0	5.1±3.8	0.0±0.0	0.0±0.0	7.0±5.0	0.0±0.0	0.0±0.0	8.0±5.6	0.0±0.0
Uncoated High	28.8±20.9	90.8±47.8	90.3±120.5	0.0±0.0	0.0±0.0	0.0±0.0	0.1±0.1	0.0±0.0	0.4±0.5	4.6±6.5	6.5±6.8	4.9±6.9
PVP High	29.3±21.0	52.1±18.7	9.4±1.1	0.0±0.0	0.2±0.1	0.0±0.0	0.2±0.3	1.9±0.7	0.0±0.0	4.8±5.7	3.5±2.5	1.4±2.8
Ionic High	11.4±9.0	51.0±31.8	26.9±12.1	0.0±0.0	9.5±10.5	0.0±0.0	1.5±2.5	10.7±11.1	0.0±0.0	0.4±0.6	13.0±11.9	0.2±0.2

	Enzyme Activity (nmol/ g d.w. soil h)								
	N-acetylglucosaminadase			Phosphatase			Leucine aminopeptidase		
Treatment	Month 1	Month 2	Month 3	Month 1	Month 2	Month 3	Month 1	Month 2	Month 3
Control	0.1±0.1	24.1±17.1	0.0±0.0	23.2±5.5	52.3±14.6	74.9±78.4	22.7±16.7	25.5±22.6	31.4±17.3
Soap Control	0.0±0.0	8.5±6.3	3.0±4.3	22.6±28.5	48.6±3.6	22.7±10.1	15.1±10.7	14.9±10.4	21.0±8.6
Weathered Low	19.6±17.7	9.9±7.1	1.0±1.0	46.9±9.4	86.9±11.1	55.0±50.2	25.0±6.1	25.2±27.1	3.4±3.4
Sulphidized Low	6.7±5.6	0.0±0.0	0.0±0.0	35.2±30.3	70.1±12.7	29.7±17.1	10.7±12.5	31.5±27.0	31.9±3.8
Uncoated Low	0.0±0.0	4.7±6.7	0.0±0.0	23.7±19.0	48.5±12.7	28.4±24.2	27.8±10.7	26.3±23.3	32.0±1.2
PVP Low	5.2±4.9	9.7±3.6	0.0±0.0	41.6±19.0	76.4±22.2	18.1±3.3	2.3±2.6	9.5±1.2	14.6±1.1
Ionic Low	8.1±7.3	14.1±4.3	1.0±4.3	60.0±45.2	71.3±23.6	39.4±16.1	6.0±2.2	14.5±18.9	16.7±23.5
Sulphidized High	0.0±0.0	14.9±12.0	0.0±0.0	24.5±13.6	84.7±40.7	30.5±28.8	16.4±2.2	6.8±7.4	33.0±18.0
Uncoated High	6.5±9.2	8.0±6.3	8.4±11.9	41.2±36.7	79.5±19.3	106.9±101.1	28.3±9.6	33.3±15.7	9.0±3.5
PVP High	3.5±3.0	9.4±4.9	0.4±0.4	43.3±30.4	55.5±11.9	37.2±4.3	18.9±21.2	28.9±6.3	27.2±1.2
Ionic High	0.8±1.1	15.5±13.3	1.2±1.7	33.2±24.2	70.7±26.1	52.0±7.9	19.3±17.2	22.9±2.8	30.1±26.0

Table C.60: One-way ANOVA for heterotrophic plate count

	Sum of Squares	DOF	Mean Square	F	Significance
Between Groups	2.32E+17	10	2.32E+16	1.177	0.356
Within Groups	4.34E+17	22	1.97E+16		
Total	6.67E+17	32			

Table C.61: One-way ANOVA for substrate-induced respiration

	Sum of Squares	DOF	Mean Square	F	Significance
Between Groups	33.667	10	3.367	0.704	0.711
Within Groups	105.161	22	4.780		
Total	138.828	32			

Table C.62: One-way ANOVA of DNA extracted from treatments after three month's exposure

	Sum of squares	DOF	Mean square	F	Significance
Between Groups	103064.813	10	10306.481	0.993	0.478
Within Groups	228375.366	22	10380.698		
Total	331440.179	32			

Table C.63: One-way ANOVA of Shannon diversity index, species richness and evenness from metagenomic sequencing after three month's exposure

		Sum of Squares	DOF	Mean Square	F	Significance
Shannon Diversity Index (H)	Between Groups	0.050	11	0.005	1.918	0.088
	Within Groups	0.056	24	0.002		
	Total	0.106	35			
Species Richness (S)	Between Groups	238708.972	11	21700.816	1.342	0.262
	Within Groups	388106.000	24	16171.083		
	Total	626814.972	35			
Evenness (E)	Between Groups	0.001	11	9.597E-05	2.988	0.012
	Within Groups	0.001	24	3.213E-05		
	Total	0.002	35			

Table C.64: Multiple comparisons for species evenness using Tukey's test

Treatment (I)	Treatment (J)	Mean Difference (I-J)	Significance	95% Confidence Interval	
				Lower Bound	Upper Bound
Control	Ionic Low	0.0051	0.991	-0.0116	0.0218
	Ionic High	-0.0075	0.888	-0.0242	0.0092
	Ionic Maximum	-0.0010	1.000	-0.0176	0.0157
	PVP Low	0.0036	1.000	-0.0131	0.0203
	PVP High	0.0038	0.999	-0.0129	0.0205
	Soap Control	-0.0090	0.721	-0.0257	0.0077
	Sulphidized Low	0.0102	0.566	-0.0065	0.0269
	Sulphidized High	0.0073	0.904	-0.0094	0.0239
	Uncoated Low	0.0029	1.000	-0.0138	0.0196
	Uncoated High	0.0034	1.000	-0.0132	0.0201
	Weathered Low	-0.0025	1.000	-0.0192	0.0142
Ionic Low	Control	-0.0051	0.991	-0.0218	0.0116
	Ionic High	-0.0126	0.276	-0.0293	0.0041
	Ionic Maximum	-0.0061	0.969	-0.0228	0.0106
	PVP Low	-0.0015	1.000	-0.0182	0.0152
	PVP High	-0.0013	1.000	-0.0180	0.0154
	Soap Control	-0.0141	0.154	-0.0308	0.0026
	Sulphidized Low	0.0051	0.992	-0.0116	0.0217
	Sulphidized High	0.0021	1.000	-0.0145	0.0188
	Uncoated Low	-0.0022	1.000	-0.0189	0.0145
	Uncoated High	-0.0017	1.000	-0.0183	0.0150
	Weathered Low	-0.0076	0.878	-0.0243	0.0091
Ionic High	Control	0.0075	0.888	-0.0092	0.0242
	Ionic Low	0.0126	0.276	-0.0041	0.0293
	Ionic Maximum	0.0065	0.951	-0.0102	0.0232
	PVP Low	0.0111	0.443	-0.0056	0.0278
	PVP High	0.0113	0.418	-0.0054	0.0280
	Soap Control	-0.0015	1.000	-0.0182	0.0151
	Sulphidized Low	0.0176*	0.032	0.0009	0.0343
	Sulphidized High	0.0147	0.120	-0.0020	0.0314
	Uncoated Low	0.0104	0.535	-0.0063	0.0271
	Uncoated High	0.0109	0.467	-0.0058	0.0276
	Weathered Low	0.0050	0.993	-0.0117	0.0217

Ionic Maximum	Control	0.0010	1.000	-0.0157	0.0176
	Ionic Low	0.0061	0.969	-0.0106	0.0228
	Ionic High	-0.0065	0.951	-0.0232	0.0102
	PVP Low	0.0046	0.996	-0.0121	0.0213
	PVP High	0.0048	0.995	-0.0119	0.0215
	Soap Control	-0.0081	0.833	-0.0247	0.0086
	Sulphidized Low	0.0111	0.439	-0.0056	0.0278
	Sulphidized High	0.0082	0.815	-0.0085	0.0249
	Uncoated Low	0.0039	0.999	-0.0128	0.0206
	Uncoated High	0.0044	0.997	-0.0123	0.0211
	Weathered Low	-0.0015	1.000	-0.0182	0.0152
PVP Low	Control	-0.0036	1.000	-0.0203	0.0131
	Ionic Low	0.0015	1.000	-0.0152	0.0182
	Ionic High	-0.0111	0.443	-0.0278	0.0056
	Ionic Maximum	-0.0046	0.996	-0.0213	0.0121
	PVP High	0.0002	1.000	-0.0165	0.0169
	Soap Control	-0.0126	0.269	-0.0293	0.0040
	Sulphidized Low	0.0065	0.949	-0.0101	0.0232
	Sulphidized High	0.0036	1.000	-0.0131	0.0203
	Uncoated Low	-0.0007	1.000	-0.0174	0.0160
	Uncoated High	-0.0002	1.000	-0.0169	0.0165
	Weathered Low	-0.0061	0.968	-0.0228	0.0106
PVP High	Control	-0.0038	0.999	-0.0205	0.0129
	Ionic Low	0.0013	1.000	-0.0154	0.0180
	Ionic High	-0.0113	0.418	-0.0280	0.0054
	Ionic Maximum	-0.0048	0.995	-0.0215	0.0119
	PVP Low	-0.0002	1.000	-0.0169	0.0165
	Soap Control	-0.0128	0.251	-0.0295	0.0038
	Sulphidized Low	0.0063	0.959	-0.0103	0.0230
	Sulphidized High	0.0034	1.000	-0.0133	0.0201
	Uncoated Low	-0.0009	1.000	-0.0176	0.0158
	Uncoated High	-0.0004	1.000	-0.0171	0.0163
	Weathered Low	-0.0063	0.961	-0.0230	0.0104
Soap Control	Control	0.0090	0.721	-0.0077	0.0257
	Ionic Low	0.0141	0.154	-0.0026	0.0308
	Ionic High	0.0015	1.000	-0.0151	0.0182
	Ionic Maximum	0.0081	0.833	-0.0086	0.0247
	PVP Low	0.0126	0.269	-0.0040	0.0293

	PVP High	0.0128	0.251	-0.0038	0.0295
	Sulphidized Low	0.0192*	0.015	0.0025	0.0359
	Sulphidized High	0.0163	0.061	-0.0004	0.0330
	Uncoated Low	0.0119	0.342	-0.0047	0.0286
	Uncoated High	0.0125	0.287	-0.0042	0.0291
	Weathered Low	0.0065	0.949	-0.0101	0.0232
Sulphidized Low	Control	-0.0102	0.566	-0.0269	0.0065
	Ionic Low	-0.0051	0.992	-0.0217	0.0116
	Ionic High	-0.0176*	0.032	-0.0343	-0.0009
	Ionic Maximum	-0.0111	0.439	-0.0278	0.0056
	PVP Low	-0.0065	0.949	-0.0232	0.0101
	PVP High	-0.0063	0.959	-0.0230	0.0103
	Soap Control	-0.0192*	0.015	-0.0359	-0.0025
	Sulphidized High	-0.0029	1.000	-0.0196	0.0138
	Uncoated Low	-0.0072	0.906	-0.0239	0.0094
	Uncoated High	-0.0067	0.940	-0.0234	0.0100
	Weathered Low	-0.0126	0.270	-0.0293	0.0040
Sulphidized High	Control	-0.0073	0.904	-0.0239	0.0094
	Ionic Low	-0.0021	1.000	-0.0188	0.0145
	Ionic High	-0.0147	0.120	-0.0314	0.0020
	Ionic Maximum	-0.0082	0.815	-0.0249	0.0085
	PVP Low	-0.0036	1.000	-0.0203	0.0131
	PVP High	-0.0034	1.000	-0.0201	0.0133
	Soap Control	-0.0163	0.061	-0.0330	0.0004
	Sulphidized Low	0.0029	1.000	-0.0138	0.0196
	Uncoated Low	-0.0043	0.998	-0.0210	0.0124
	Uncoated High	-0.0038	0.999	-0.0205	0.0129
	Weathered Low	-0.0097	0.627	-0.0264	0.0070
Uncoated Low	Control	-0.0029	1.000	-0.0196	0.0138
	Ionic Low	0.0022	1.000	-0.0145	0.0189
	Ionic High	-0.0104	0.535	-0.0271	0.0063
	Ionic Maximum	-0.0039	0.999	-0.0206	0.0128
	PVP Low	0.0007	1.000	-0.0160	0.0174
	PVP High	0.0009	1.000	-0.0158	0.0176
	Soap Control	-0.0119	0.342	-0.0286	0.0047
	Sulphidized Low	0.0072	0.906	-0.0094	0.0239
	Sulphidized High	0.0043	0.998	-0.0124	0.0210
	Uncoated High	0.0005	1.000	-0.0162	0.0172

	Weathered Low	-0.0054	0.987	-0.0221	0.0113
Uncoated High	Control	-0.0034	1.000	-0.0201	0.0132
	Ionic Low	0.0017	1.000	-0.0150	0.0183
	Ionic High	-0.0109	0.467	-0.0276	0.0058
	Ionic Maximum	-0.0044	0.997	-0.0211	0.0123
	PVP Low	0.0002	1.000	-0.0165	0.0169
	PVP High	0.0004	1.000	-0.0163	0.0171
	Soap Control	-0.0125	0.287	-0.0291	0.0042
	Sulphidized Low	0.0067	0.940	-0.0100	0.0234
	Sulphidized High	0.0038	0.999	-0.0129	0.0205
	Uncoated Low	-0.0005	1.000	-0.0172	0.0162
	Weathered Low	-0.0059	0.974	-0.0226	0.0108
Weathered Low	Control	0.0025	1.000	-0.0142	0.0192
	Ionic Low	0.0076	0.878	-0.0091	0.0243
	Ionic High	-0.0050	0.993	-0.0217	0.0117
	Ionic Maximum	0.0015	1.000	-0.0152	0.0182
	PVP Low	0.0061	0.968	-0.0106	0.0228
	PVP High	0.0063	0.961	-0.0104	0.0230
	Soap Control	-0.0065	0.949	-0.0232	0.0101
	Sulphidized Low	0.0126	0.270	-0.0040	0.0293
	Sulphidized High	0.0097	0.627	-0.0070	0.0264
	Uncoated Low	0.0054	0.987	-0.0113	0.0221
	Uncoated High	0.0059	0.974	-0.0108	0.0226

Table C.65: Tukey test subset treatment groups for species evenness

Treatment	N	Subsets	
		1	2
Sulphidized Low	3	0.3446	
Sulphidized High	3	0.3475	0.3475
Ionic Low	3	0.3497	0.3497
PVP High	3	0.3509	0.3509
PVP Low	3	0.3511	0.3511
Uncoated High	3	0.3513	0.3513
Uncoated Low	3	0.3518	0.3518
Control	3	0.3548	0.3548
Ionic Maximum	3	0.3557	0.3557
Weathered Low	3	0.3572	0.3572
Ionic High	3		0.3622
Soap Control	3		0.3638
Significance		0.270	0.061

Table C.66: One-way ANOVA of relative abundance of *R. limosa*, *F. alni*, *A. malthae* and *X. oryzae* from DNA sequencing after three month's exposure

		Sum of Squares	DOF	Mean Square	F	Significance
R. limosa	Between Groups	23.283	11	2.117	31.934	0.000
	Within Groups	1.591	24	0.066		
	Total	24.873	35			
F. alni	Between Groups	1.095	11	0.100	15.597	0.000
	Within Groups	0.153	24	0.006		
	Total	1.248	35			
A. malthae	Between Groups	2.846	11	0.259	11.038	0.000
	Within Groups	0.563	24	0.023		
	Total	3.408	35			
X. oryzae	Between Groups	0.007	11	0.001	7.903	0.000
	Within Groups	0.002	24	0.000		
	Total	0.009	35			

Table C.67: Tukey test subset treatment groups for *R. limosa* abundance

Treatment	N	Subsets	
		1	2
Control	3	0.1761	
Sulphidized Low	3	0.2351	
Weathered Low	3	0.2586	
Sulphidized High	3	0.2932	
PVP High	3	0.3145	
Ionic Low	3	0.3223	
Soap Control	3	0.3253	
Uncoated Low	3	0.3345	
PVP Low	3	0.3415	
Uncoated High	3	0.4180	
Ionic High	3	0.6059	
Ionic Maximum	3		3.2160
Significance		0.662	1.000

Table C.68: Tukey test subset treatment groups for *F. alni* abundance

Treatment	N	Subsets	
		1	2
Weathered Low	3	0.1283	
PVP High	3	0.1330	
Ionic Low	3	0.1509	
Uncoated High	3	0.1526	
PVP Low	3	0.1691	
Sulphidized High	3	0.1824	
Control	3	0.1954	
Uncoated Low	3	0.2021	
Soap Control	3	0.2114	
Sulphidized Low	3	0.2147	
Ionic High	3		0.5311
Ionic Maximum	3		0.7180
Significance		0.967	0.215

Table C.69: Tukey test subset treatment groups for *A. malthae* abundance

Treatment	N	Subsets		
		1	2	3
Sulphidized High	3	0.0722		
Control	3	0.0795		
Uncoated Low	3	0.0798		
Sulphidized Low	3	0.0808		
PVP High	3	0.0860		
Weathered Low	3	0.0887		
PVP Low	3	0.0923		
Soap Control	3	0.0934		
Ionic Low	3	0.1022	0.1022	
Uncoated High	3	0.1051	0.1051	
Ionic High	3		0.5510	
Ionic Maximum	3			1.0363
Significance		1.000	0.052	1.000

Table C.70: Tukey test subset treatment groups for *X. oryzae* abundance

Treatment	N	Subsets		
		1	2	3
PVP High	3	0.0018		
Ionic Low	3	0.0025		
Uncoated High	3	0.0029	0.0029	
PVP Low	3	0.0029	0.0029	
Uncoated Low	3	0.0029	0.0029	
Control	3	0.0034	0.0034	
Sulphidized Low	3	0.0035	0.0035	
Weathered Low	3	0.0035	0.0035	
Soap Control	3	0.0047	0.0047	
Sulphidized High	3	0.0050	0.0050	
Ionic High	3		0.0294	0.0294
Ionic Maximum	3			0.0493
Significance		1.000	0.051	0.288

Table C.71: Multiple comparisons to the control for specified variables from post-hoc Dunnett's 2-sided test

Dependent Variable		Mean Difference (I-J)	Significance	95% Confidence Interval	
				Lower Bound	Upper Bound
pH	Weathered Low	-0.115333	0.980	-0.55513	0.32446
	Uncoated High	-0.011333	1.000	-0.45113	0.42846
	Ionic High	-0.065667	1.000	-0.50546	0.37413
	Sulphidized High	0.098333	0.993	-0.34146	0.53813
	Uncoated Low	0.116333	0.979	-0.32346	0.55613
	Sulphidized Low	0.073667	0.999	-0.36613	0.51346
	PVP High	0.119667	0.975	-0.32013	0.55946
	Ionic Low	0.082000	0.998	-0.35780	0.52180
	PVP Low	0.101667	0.991	-0.33813	0.54146
	Soap Control	0.091333	0.996	-0.34846	0.53113
Conductivity	Weathered Low	52.863333*	0.000	23.43335	82.29331
	Uncoated High	4.330000	1.000	-25.09998	33.75998
	Ionic High	22.063333	0.212	-7.36665	51.49331

	Sulphidized High	-0.336667	1.000	-29.76665	29.09331
	Uncoated Low	1.096667	1.000	-28.33331	30.52665
	Sulphidized Low	4.130000	1.000	-25.29998	33.55998
	PVP High	-6.490000	0.994	-35.91998	22.93998
	Ionic Low	-3.480000	1.000	-32.90998	25.94998
	PVP Low	-6.803333	0.991	-36.23331	22.62665
	Soap Control	63.030000*	0.000	33.60002	92.45998
Moisture Content	Weathered Low	4.077935	0.050	-0.00241	8.15828
	Uncoated High	0.484620	1.000	-3.59573	4.56497
	Ionic High	1.063391	0.981	-3.01696	5.14374
	Sulphidized High	-2.257494	0.518	-6.33784	1.82286
	Uncoated Low	-0.750506	0.998	-4.83085	3.32984
	Sulphidized Low	-0.723473	0.999	-4.80382	3.35688
	PVP High	-0.561115	1.000	-4.64146	3.51923
	Ionic Low	0.334063	1.000	-3.74629	4.41441
	PVP Low	-1.269005	0.946	-5.34935	2.81134
	Soap Control	1.237708	0.953	-2.84264	5.31806
Organic Matter	Weathered Low	-2.457370	0.063	-5.00904	0.09430
	Uncoated High	-2.434055	0.066	-4.98572	0.11761
	Ionic High	-2.697211*	0.035	-5.24888	-0.14554
	Sulphidized High	-2.856685*	0.023	-5.40835	-0.30502
	Uncoated Low	-3.263002*	0.008	-5.81467	-0.71134
	Sulphidized Low	-2.951636*	0.018	-5.50330	-0.39997
	PVP High	-2.943050*	0.019	-5.49472	-0.39138
	Ionic Low	-3.015805*	0.015	-5.56747	-0.46414
	PVP Low	-2.606105	0.085	-5.45896	0.24675
	Soap Control	-2.284751	0.094	-4.83642	0.26692

AWCD Month 1	Weathered Low	0.146667	0.270	-0.06227	0.35560
	Uncoated High	0.010000	1.000	-0.19893	0.21893
	Ionic High	-0.006667	1.000	-0.21560	0.20227
	Sulphidized High	0.033333	1.000	-0.17560	0.24227
	Uncoated Low	-0.106667	0.605	-0.31560	0.10227
	Sulphidized Low	0.006667	1.000	-0.20227	0.21560
	PVP High	0.036667	0.999	-0.17227	0.24560
	Ionic Low	-0.053333	0.983	-0.26227	0.15560
	PVP Low	0.030000	1.000	-0.17893	0.23893
	Soap Control	0.126667	0.418	-0.08227	0.33560
AWCD Month 2	Weathered Low	0.130	0.366	-0.07	0.33
	Uncoated High	-0.047	0.992	-0.25	0.16
	Ionic High	-0.230*	0.022	-0.43	-0.03
	Sulphidized High	-0.013	1.000	-0.22	0.19
	Uncoated Low	0.027	1.000	-0.18	0.23
	Sulphidized Low	0.000	1.000	-0.20	0.20
	PVP High	-0.077	0.864	-0.28	0.13
	Ionic Low	-0.103	0.613	-0.31	0.10
	PVP Low	-0.020	1.000	-0.22	0.18
	Soap Control	0.077	0.864	-0.13	0.28
AWCD Month 3	Weathered Low	0.156667	0.569	-0.13997	0.45331
	Uncoated High	0.056667	0.998	-0.23997	0.35331
	Ionic High	-0.110000	0.872	-0.40664	0.18664
	Sulphidized High	-0.013333	1.000	-0.30997	0.28331
	Uncoated Low	0.043333	1.000	-0.25331	0.33997
	Sulphidized Low	-0.150000	0.615	-0.44664	0.14664
	PVP High	-0.030000	1.000	-0.32664	0.26664

	Ionic Low	-0.006667	1.000	-0.30331	0.28997
	PVP Low	0.010000	1.000	-0.28664	0.30664
	Soap Control	0.206667	0.277	-0.08997	0.50331
Richness Month 1	Weathered Low	3.553333	0.414	-2.28566	9.39232
	Uncoated High	-0.223333	1.000	-6.06232	5.61566
	Ionic High	-1.553333	0.978	-7.39232	4.28566
	Sulphidized High	-0.556667	1.000	-6.39566	5.28232
	Uncoated Low	-3.223333	0.521	-9.06232	2.61566
	Sulphidized Low	0.220000	1.000	-5.61899	6.05899
	PVP High	0.110000	1.000	-5.72899	5.94899
	Ionic Low	-1.666667	0.967	-7.50566	4.17232
	PVP Low	-0.670000	1.000	-6.50899	5.16899
	Soap Control	2.443333	0.790	-3.39566	8.28232
Richness Month 2	Weathered Low	2.556667	0.670	-2.78137	7.89470
	Uncoated High	-0.333333	1.000	-5.67137	5.00470
	Ionic High	-5.556667*	0.039	-10.89470	-0.21863
	Sulphidized High	-0.443333	1.000	-5.78137	4.89470
	Uncoated Low	0.110000	1.000	-5.22803	5.44803
	Sulphidized Low	0.000000	1.000	-5.33803	5.33803
	PVP High	-1.666667	0.945	-7.00470	3.67137
	Ionic Low	-2.666667	0.627	-8.00470	2.67137
	PVP Low	-0.556667	1.000	-5.89470	4.78137
	Soap Control	2.220000	0.795	-3.11803	7.55803
Richness Month 3	Weathered Low	5.776667	0.272	-2.47382	14.02716
	Uncoated High	2.556667	0.947	-5.69382	10.80716
	Ionic High	-1.220000	1.000	-9.47049	7.03049
	Sulphidized High	0.223333	1.000	-8.02716	8.47382

	Uncoated Low	1.780000	0.995	-6.47049	10.03049
	Sulphidized Low	-3.110000	0.862	-11.36049	5.14049
	PVP High	0.446667	1.000	-7.80382	8.69716
	Ionic Low	0.330000	1.000	-7.92049	8.58049
	PVP Low	0.780000	1.000	-7.47049	9.03049
	Soap Control	5.890000	0.254	-2.36049	14.14049
Carbohydrates Month 1	Weathered Low	0.002369	1.000	-0.09963	0.10437
	Uncoated High	0.020473	0.997	-0.08153	0.12248
	Ionic High	-0.018978	0.998	-0.12098	0.08302
	Sulphidized High	0.019416	0.998	-0.08259	0.12142
	Uncoated Low	-0.058229	0.484	-0.16023	0.04377
	Sulphidized Low	0.025892	0.984	-0.07611	0.12790
	PVP High	-0.000039	1.000	-0.10204	0.10196
	Ionic Low	-0.015047	1.000	-0.11705	0.08696
	PVP Low	0.044090	0.764	-0.05791	0.14609
	Soap Control	0.033194	0.931	-0.06881	0.13520
Polymers Month 1	Weathered Low	0.005631	0.998	-0.02367	0.03493
	Uncoated High	0.000993	1.000	-0.02831	0.03030
	Ionic High	-0.005939	0.997	-0.03524	0.02336
	Sulphidized High	0.009749	0.923	-0.01955	0.03905
	Uncoated Low	-0.015007	0.601	-0.04431	0.01430
	Sulphidized Low	-0.006290	0.995	-0.03559	0.02301
	PVP High	-0.004434	1.000	-0.03374	0.02487
	Ionic Low	-0.015272	0.583	-0.04458	0.01403
	PVP Low	0.004434	1.000	-0.02487	0.03374
	Soap Control	0.007014	0.989	-0.02229	0.03632
	Weathered Low	0.083061	0.069	-0.00453	0.17065

Carboxylic and acetic acids Month 1	Uncoated High	-0.042294	0.662	-0.12988	0.04529
	Ionic High	-0.005925	1.000	-0.09351	0.08166
	Sulphidized High	0.003677	1.000	-0.08391	0.09126
	Uncoated Low	-0.023982	0.974	-0.11157	0.06360
	Sulphidized Low	-0.022319	0.984	-0.10991	0.06527
	PVP High	-0.006953	1.000	-0.09454	0.08063
	Ionic Low	-0.032957	0.863	-0.12054	0.05463
	PVP Low	-0.027613	0.942	-0.11520	0.05997
	Soap Control	0.050548	0.472	-0.03704	0.13813
Amino acids Month 1	Weathered Low	0.063090*	0.019	0.00818	0.11800
	Uncoated High	0.019172	0.903	-0.03573	0.07408
	Ionic High	0.021706	0.832	-0.03320	0.07661
	Sulphidized High	-0.001151	1.000	-0.05606	0.05376
	Uncoated Low	-0.003814	1.000	-0.05872	0.05109
	Sulphidized Low	0.000993	1.000	-0.05391	0.05590
	PVP High	0.041251	0.210	-0.01366	0.09616
	Ionic Low	0.005118	1.000	-0.04979	0.06003
	PVP Low	0.004609	1.000	-0.05030	0.05952
	Soap Control	0.039921	0.238	-0.01499	0.09483
Amines/amides Month 1	Weathered Low	-0.009480	0.545	-0.02705	0.00809
	Uncoated High	0.008455	0.665	-0.00911	0.02602
	Ionic High	0.001774	1.000	-0.01580	0.01934
	Sulphidized High	0.001194	1.000	-0.01638	0.01876
	Uncoated Low	-0.008186	0.697	-0.02576	0.00938
	Sulphidized Low	0.003376	0.998	-0.01419	0.02095
	PVP High	0.007172	0.809	-0.01040	0.02474
	Ionic Low	0.003412	0.998	-0.01416	0.02098
	PVP Low	0.003326	0.998	-0.01424	0.02090

	Soap Control	-0.004609	0.980	-0.02218	0.01296
Root exudates Month 1	Weathered Low	0.087717*	0.014	0.01456	0.16087
	Uncoated High	-0.001602	1.000	-0.07476	0.07155
	Ionic High	0.011928	0.999	-0.06123	0.08508
	Sulphidized High	0.009892	1.000	-0.06326	0.08305
	Uncoated Low	-0.022133	0.954	-0.09529	0.05102
	Sulphidized Low	-0.005566	1.000	-0.07872	0.06759
	PVP High	0.034710	0.679	-0.03845	0.10787
	Ionic Low	-0.019222	0.980	-0.09238	0.05393
	PVP Low	-0.004061	1.000	-0.07722	0.06910
	Soap Control	0.049882	0.297	-0.02327	0.12304
Carbohydrates Month 2	Weathered Low	0.056401	0.090	-0.00605	0.11885
	Uncoated High	-0.010247	0.999	-0.07270	0.05220
	Ionic High	-0.054903	0.104	-0.11735	0.00755
	Sulphidized High	-0.008140	1.000	-0.07059	0.05431
	Uncoated Low	0.005910	1.000	-0.05654	0.06836
	Sulphidized Low	-0.008172	1.000	-0.07062	0.05428
	PVP High	-0.036674	0.453	-0.09912	0.02578
	Ionic Low	-0.033573	0.549	-0.09602	0.02888
	PVP Low	-0.005011	1.000	-0.06746	0.05744
	Soap Control	0.038659	0.396	-0.02379	0.10111
Polymers Month 2	Weathered Low	0.014344	0.666	-0.01549	0.04418
	Uncoated High	-0.001832	1.000	-0.03166	0.02800
	Ionic High	-0.020219	0.303	-0.05005	0.00961
	Sulphidized High	0.001943	1.000	-0.02789	0.03178
	Uncoated Low	0.000746	1.000	-0.02909	0.03058

	Sulphidized Low	-0.006025	0.997	-0.03586	0.02381
	PVP High	-0.010498	0.899	-0.04033	0.01933
	Ionic Low	-0.011305	0.859	-0.04114	0.01853
	PVP Low	-0.000803	1.000	-0.03064	0.02903
	Soap Control	0.010151	0.914	-0.01968	0.03998
Carboxylic and acetic acids Month 2	Weathered Low	0.042616	0.726	-0.05174	0.13698
	Uncoated High	-0.026645	0.969	-0.12100	0.06771
	Ionic High	-0.097774*	0.040	-0.19213	-0.00342
	Sulphidized High	-0.011258	1.000	-0.10562	0.08310
	Uncoated Low	0.012401	1.000	-0.08196	0.10676
	Sulphidized Low	-0.006835	1.000	-0.10119	0.08752
	PVP High	-0.030437	0.934	-0.12480	0.06392
	Ionic Low	-0.040892	0.762	-0.13525	0.05347
	PVP Low	-0.003538	1.000	-0.09790	0.09082
	Soap Control	-0.002993	1.000	-0.09735	0.09137
Amino acids Month 2	Weathered Low	0.006441	1.000	-0.03732	0.05020
	Uncoated High	-0.008036	0.998	-0.05180	0.03573
	Ionic High	-0.045477*	0.039	-0.08924	-0.00172
	Sulphidized High	0.005140	1.000	-0.03862	0.04890
	Uncoated Low	0.009143	0.996	-0.03462	0.05290
	Sulphidized Low	0.008968	0.996	-0.03479	0.05273
	PVP High	-0.001749	1.000	-0.04551	0.04201
	Ionic Low	-0.018505	0.782	-0.06227	0.02526
	PVP Low	-0.009090	0.996	-0.05285	0.03467
	Soap Control	0.004860	1.000	-0.03890	0.04862
Amines/amides Month 2	Weathered Low	0.009921	0.950	-0.02246	0.04230
	Uncoated High	0.000792	1.000	-0.03159	0.03318

	Ionic High	-0.013434	0.797	-0.04582	0.01895
	Sulphidized High	0.001495	1.000	-0.03089	0.03388
	Uncoated Low	0.001065	1.000	-0.03132	0.03345
	Sulphidized Low	0.010946	0.917	-0.02144	0.04333
	PVP High	-0.000165	1.000	-0.03255	0.03222
	Ionic Low	-0.000262	1.000	-0.03265	0.03212
	PVP Low	-0.003918	1.000	-0.03630	0.02847
	Soap Control	0.023441	0.242	-0.00894	0.05582
Root exudates Month 2	Weathered Low	0.033498	0.751	-0.04287	0.10987
	Uncoated High	-0.031903	0.792	-0.10827	0.04446
	Ionic High	-0.080323*	0.036	-0.15669	-0.00395
	Sulphidized High	-0.010280	1.000	-0.08665	0.06609
	Uncoated Low	0.009527	1.000	-0.06684	0.08589
	Sulphidized Low	-0.008100	1.000	-0.08447	0.06827
	PVP High	-0.022527	0.960	-0.09889	0.05384
	Ionic Low	-0.044151	0.470	-0.12052	0.03222
	PVP Low	-0.015591	0.997	-0.09196	0.06078
	Soap Control	0.023470	0.949	-0.05290	0.09984
Carbohydrates Month 3	Weathered Low	0.026237	0.908	-0.04978	0.10225
	Uncoated High	0.002312	1.000	-0.07370	0.07832
	Ionic High	-0.025219	0.924	-0.10123	0.05079
	Sulphidized High	-0.028746	0.860	-0.10476	0.04727
	Uncoated Low	-0.015785	0.996	-0.09180	0.06023
	Sulphidized Low	-0.058806	0.187	-0.13482	0.01721
	PVP High	-0.016348	0.995	-0.09236	0.05966
	Ionic Low	-0.023272	0.950	-0.09928	0.05274
	PVP Low	-0.016581	0.994	-0.09259	0.05943
	Soap Control	0.028179	0.872	-0.04783	0.10419

Polymers Month 3	Weathered Low	0.027369	0.226	-0.00976	0.06450
	Uncoated High	0.013717	0.874	-0.02341	0.05085
	Ionic High	0.001900	1.000	-0.03523	0.03903
	Sulphidized High	-0.001416	1.000	-0.03854	0.03571
	Uncoated Low	0.008143	0.994	-0.02899	0.04527
	Sulphidized Low	-0.010832	0.962	-0.04796	0.02630
	PVP High	0.006333	0.999	-0.03080	0.04346
	Ionic Low	0.014674	0.832	-0.02246	0.05180
	PVP Low	0.003771	1.000	-0.03336	0.04090
	Soap Control	0.023914	0.354	-0.01322	0.06104
Carboxylic and acetic acids Month 3	Weathered Low	0.050068	0.851	-0.08029	0.18043
	Uncoated High	0.018036	1.000	-0.11232	0.14839
	Ionic High	-0.055695	0.774	-0.18605	0.07466
	Sulphidized High	-0.008616	1.000	-0.13897	0.12174
	Uncoated Low	0.025950	0.997	-0.10441	0.15631
	Sulphidized Low	-0.033677	0.982	-0.16404	0.09668
	PVP High	-0.016315	1.000	-0.14667	0.11404
	Ionic Low	0.002201	1.000	-0.12816	0.13256
	PVP Low	0.020007	1.000	-0.11035	0.15036
	Soap Control	0.068072	0.581	-0.06229	0.19843
Amino acids Month 3	Weathered Low	0.028792	0.834	-0.04427	0.10185
	Uncoated High	0.010552	1.000	-0.06251	0.08361
	Ionic High	-0.022143	0.953	-0.09520	0.05092
	Sulphidized High	0.024810	0.915	-0.04825	0.09787
	Uncoated Low	0.020097	0.973	-0.05296	0.09316
	Sulphidized Low	-0.046337	0.370	-0.11940	0.02672
	PVP High	0.000559	1.000	-0.07250	0.07362

	Ionic Low	-0.007111	1.000	-0.08017	0.06595
	PVP Low	0.002197	1.000	-0.07086	0.07526
	Soap Control	0.052036	0.256	-0.02102	0.12510
Amines/amides Month 3	Weathered Low	0.025670	0.128	-0.00478	0.05612
	Uncoated High	0.017903	0.452	-0.01254	0.04835
	Ionic High	-0.003928	1.000	-0.03438	0.02652
	Sulphidized High	-0.000466	1.000	-0.03091	0.02998
	Uncoated Low	0.008276	0.975	-0.02217	0.03872
	Sulphidized Low	0.002140	1.000	-0.02831	0.03259
	PVP High	-0.001287	1.000	-0.03173	0.02916
	Ionic Low	0.005620	0.998	-0.02483	0.03607
	PVP Low	0.006297	0.996	-0.02415	0.03674
	Soap Control	0.037724*	0.010	0.00728	0.06817
Root exudates Month 3	Weathered Low	0.045050	0.856	-0.07325	0.16335
	Uncoated High	0.009993	1.000	-0.10830	0.12829
	Ionic High	-0.053566	0.723	-0.17186	0.06473
	Sulphidized High	0.014050	1.000	-0.10425	0.13235
	Uncoated Low	0.014172	1.000	-0.10412	0.13247
	Sulphidized Low	-0.076556	0.350	-0.19485	0.04174
	PVP High	-0.006427	1.000	-0.12472	0.11187
	Ionic Low	-0.025860	0.994	-0.14416	0.09244
	PVP Low	-0.012047	1.000	-0.13034	0.10625
	Soap Control	0.079484	0.312	-0.03881	0.19778
CFU	Weathered Low	-1590000.000	0.425	-4229818.403	1049818.404
	Uncoated High	-1783333.333	0.306	-4423151.736	856485.070
	Ionic High	-1080000.000	0.807	-3719818.403	1559818.404
	Sulphidized High	615555.556	0.991	-2024262.848	3255373.959

	Uncoated Low	-564444.444	0.995	-3204262.848	2075373.959
	Sulphidized Low	-1480000.000	0.504	-4119818.403	1159818.403
	PVP High	-1312222.222	0.632	-3952040.625	1327596.181
	Ionic Low	-1103333.333	0.791	-3743151.736	1536485.070
	PVP Low	-912222.222	0.907	-3552040.625	1727596.181
	Soap Control	-434444.444	0.999	-3074262.848	2205373.959
Substrate-Induced Respiration	Weathered Low	0.225435	1.000	-5.05350	5.50437
	Uncoated High	0.321139	1.000	-4.95780	5.60007
	Ionic High	-1.920067	0.882	-7.19900	3.35887
	Sulphidized High	-0.657693	1.000	-5.93663	4.62124
	Uncoated Low	0.197051	1.000	-5.08188	5.47599
	Sulphidized Low	0.054352	1.000	-5.22458	5.33329
	PVP High	-1.109231	0.996	-6.38817	4.16970
	Ionic Low	-0.668061	1.000	-5.94700	4.61087
	PVP Low	-2.941941	0.510	-8.22088	2.33699
	Soap Control	0.287845	1.000	-4.99109	5.56678
β-Glucosidase Month 3	Weathered Low	126.741064	0.938	-270.18223	523.66436
	Uncoated High	79.181375	0.997	-317.74192	476.10467
	Ionic High	15.764949	1.000	-381.15835	412.68825
	Sulphidized High	274.663662	0.283	-122.25964	671.58696
	Uncoated Low	-10.200343	1.000	-407.12364	386.72295
	Sulphidized Low	4.955742	1.000	-391.96756	401.87904
	PVP High	-1.756720	1.000	-398.68002	395.16658
	Ionic Low	11.786333	1.000	-385.13696	408.70963
	PVP Low	-0.461463	1.000	-397.38476	396.46183
	Soap Control	-9.268523	1.000	-406.19182	387.65477

Xylosidase Month 3	Weathered Low	4.549223	1.000	-44.62754	53.72599
	Uncoated High	0.379057	1.000	-48.79771	49.55582
	Ionic High	0.000000	1.000	-49.17676	49.17676
	Sulphidized High	38.690860	0.174	-10.48590	87.86762
	Uncoated Low	0.000000	1.000	-49.17676	49.17676
	Sulphidized Low	0.000000	1.000	-49.17676	49.17676
	PVP High	0.000000	1.000	-49.17676	49.17676
	Ionic Low	1.785583	1.000	-47.39118	50.96235
	PVP Low	0.000000	1.000	-49.17676	49.17676
	Soap Control	0.000000	1.000	-49.17676	49.17676
Cellobiosidase Month 3	Weathered Low	21.606040	0.413	-13.84178	57.05386
	Uncoated High	4.895437	1.000	-30.55239	40.34326
	Ionic High	0.169863	1.000	-35.27796	35.61769
	Sulphidized High	17.243907	0.654	-18.20392	52.69173
	Uncoated Low	0.000000	1.000	-35.44782	35.44782
	Sulphidized Low	0.000000	1.000	-35.44782	35.44782
	PVP High	1.424763	1.000	-34.02306	36.87259
	Ionic Low	0.406164	1.000	-35.04166	35.85399
	PVP Low	0.000000	1.000	-35.44782	35.44782
	Soap Control	0.000000	1.000	-35.44782	35.44782
n-Acetylglucoaminidase Month 3	Weathered Low	24.940088	0.876	-42.86870	92.74887
	Uncoated High	8.442112	1.000	-59.36667	76.25090
	Ionic High	1.171354	1.000	-66.63743	68.98014
	Sulphidized High	47.269642	0.276	-20.53914	115.07843
	Uncoated Low	0.000000	1.000	-67.80878	67.80878
	Sulphidized Low	0.000000	1.000	-67.80878	67.80878
	PVP High	0.448265	1.000	-67.36052	68.25705

	Ionic Low	0.950553	1.000	-66.85823	68.75934
	PVP Low	0.000000	1.000	-67.80878	67.80878
	Soap Control	3.017004	1.000	-64.79178	70.82579
Acid phosphatase Month 3	Weathered Low	102.178170	1.000	-1339.87874	1544.23508
	Uncoated High	31.953979	1.000	-1410.10293	1474.01089
	Ionic High	-22.870133	1.000	-1464.92704	1419.18678
	Sulphidized High	1088.089131	0.207	-353.96778	2530.14604
	Uncoated Low	-46.482527	1.000	-1488.53944	1395.57438
	Sulphidized Low	-39.881549	1.000	-1481.93846	1402.17536
	PVP High	-37.674462	1.000	-1479.73137	1404.38245
	Ionic Low	-35.525660	1.000	-1477.58257	1406.53125
	PVP Low	-56.831241	1.000	-1498.88815	1385.22567
	Soap Control	-52.225762	1.000	-1494.28267	1389.83115
Leucine aminopeptidase Month 3	Weathered Low	-25.755983	0.407	-67.80541	16.29345
	Uncoated High	-22.318337	0.563	-64.36777	19.73109
	Ionic High	-1.251676	1.000	-43.30111	40.79775
	Sulphidized High	2.921082	1.000	-39.12835	44.97051
	Uncoated Low	0.676503	1.000	-41.37293	42.72593
	Sulphidized Low	0.529538	1.000	-41.51989	42.57897
	PVP High	-4.161836	1.000	-46.21127	37.88759
	Ionic Low	-14.645182	0.904	-56.69461	27.40425
	PVP Low	-16.719522	0.828	-58.76895	25.32991
	Soap Control	-10.369948	0.987	-52.41938	31.67948
DNA Extracted	Weathered Low	-75.189024	0.960	-320.69548	170.31743
	Uncoated High	-55.697967	0.995	-301.20443	189.80849
	Ionic High	-106.955935	0.776	-352.46239	138.55052
	Sulphidized High	31.375129	1.000	-214.13133	276.88159

	Uncoated Low	-94.775674	0.864	-340.28213	150.73078
	Sulphidized Low	-21.739782	1.000	-267.24624	223.76668
	PVP High	-61.291641	0.990	-306.79810	184.21482
	Ionic Low	-16.022560	1.000	-261.52902	229.48390
	PVP Low	-74.999179	0.960	-320.50564	170.50728
	Soap Control	-182.954775	0.223	-428.46123	62.55168
	Ionic Maximum	-35.880471	1.000	-281.38693	209.62599
Shannon Diversity Index	Weathered Low	0.094000	0.165	-0.02339	0.21139
	Uncoated High	0.022667	0.999	-0.09473	0.14006
	Ionic High	0.077333	0.342	-0.04006	0.19473
	Sulphidized High	0.002333	1.000	-0.11506	0.11973
	Uncoated Low	-0.006000	1.000	-0.12339	0.11139
	Sulphidized Low	-0.040667	0.920	-0.15806	0.07673
	PVP High	0.037333	0.950	-0.08006	0.15473
	Ionic Low	-0.005333	1.000	-0.12273	0.11206
	PVP Low	0.007333	1.000	-0.11006	0.12473
	Soap Control	0.054667	0.717	-0.06273	0.17206
	Ionic Maximum	0.001000	1.000	-0.11639	0.11839
Species Richness	Weathered Low	245.666667	0.169	-62.47105	553.80438
	Uncoated High	137.333333	0.757	-170.80438	445.47105
	Ionic High	72.000000	0.994	-236.13772	380.13772
	Sulphidized High	170.666667	0.534	-137.47105	478.80438
	Uncoated Low	40.000000	1.000	-268.13772	348.13772
	Sulphidized Low	92.666667	0.964	-215.47105	400.80438
	PVP High	202.333333	0.345	-105.80438	510.47105
	Ionic Low	85.000000	0.979	-223.13772	393.13772
	PVP Low	95.333333	0.957	-212.80438	403.47105

	Soap Control	-16.000000	1.000	-324.13772	292.13772
	Ionic Maximum	-18.666667	1.000	-326.80438	289.47105
Evenness	Weathered Low	0.002466	0.999	-0.01127	0.01620
	Uncoated High	-0.003449	0.989	-0.01718	0.01028
	Ionic High	0.007465	0.555	-0.00627	0.02120
	Sulphidized High	-0.007257	0.586	-0.02099	0.00648
	Uncoated Low	-0.002932	0.997	-0.01667	0.01080
	Sulphidized Low	-0.010171	0.228	-0.02390	0.00356
	PVP High	-0.003829	0.978	-0.01756	0.00990
	Ionic Low	-0.005110	0.886	-0.01884	0.00862
	PVP Low	-0.003631	0.984	-0.01737	0.01010
	Soap Control	0.009013	0.346	-0.00472	0.02275
	Ionic Maximum	0.000960	1.000	-0.01277	0.01469

8.4 Appendix D

Table D.1: Repeated measure ANOVA comparing total silver concentrations in each month's treatments

Effect	Wilk's Lambda Statistic Value	F	Hypothesis DOF	Error DOF	Significance
Time	1.000	0.007	2	43	0.993
Time*Treatment	0.563	0.682	42	86	0.914

Table D.2: T-test of treatment upper and lower region silver concentrations

Treatment	t	DOF	Significance (2-tailed)	Mean Difference	95% Confidence Interval	
					Lower	Upper
Weathered Low	-0.053	16	0.958	-0.00791	-0.32138	0.30556
Sulphidized Low, 120 nm	0.448	16	0.660	0.06394	-0.23879	0.36667
Sulphidized Low, 160 nm	-0.898	16	0.382	-0.08438	-0.28349	0.11473
Ionic Low	1.219	16	0.240	0.60486	-0.44695	1.65667
Ionic High	-0.057	16	0.955	-0.07640	-2.89846	2.74566
PVP Low	-1.157	16	0.264	-0.08191	-0.23199	0.06816
PVP High	-0.446	16	0.662	-0.21944	-1.26287	0.82399
Uncoated Low	0.481	16	0.637	0.01728	-0.05894	0.09349
Uncoated High	0.647	16	0.527	0.93953	-2.13796	4.01702
Ionic Maximum	-0.428	16	0.674	-2.63863	-15.70818	10.43093

Table D.3: One-way ANOVA for silver concentrations of low and high concentration treatments

		Sum of Squares	DOF	Mean Square	F	Significance
Low	Between Groups	14.619	4	3.655	13.725	0.000
	Within Groups	22.635	85	0.266		
	Total	37.253	89			
High	Between Groups	455.832	3	151.944	34.680	0.000
	Within Groups	297.933	68	4.381		
	Total	753.766	71			

Table D.4: Multiple comparisons for low concentration treatments using Tukey's test

Treatment (I)	Treatment (J)	Mean Difference (I-J)	Significance	95% Confidence Interval	
				Lower Bound	Upper Bound
Weathered Low	Sulphidized Low, 120 nm	0.49325*	0.041	0.0138	0.9727
	Ionic Low	0.16961	0.861	-0.3098	0.6490
	PVP Low	0.93576*	0.000	0.4563	1.4152
	Uncoated Low	1.01543*	0.000	0.5360	1.4949
Sulphidized Low, 120 nm	Weathered Low	-0.49325*	0.041	-0.9727	-0.0138
	Ionic Low	-0.32364	0.335	-0.8031	0.1558
	PVP Low	0.44251	0.085	-0.0369	0.9219
	Uncoated Low	0.52218*	0.026	0.0428	1.0016
Ionic Low	Weathered Low	-0.16961	0.861	-0.6490	0.3098
	Sulphidized Low, 120 nm	0.32364	0.335	-0.1558	0.8031
	PVP Low	0.76615*	0.000	0.2867	1.2456
	Uncoated Low	0.84582*	0.000	0.3664	1.3252
PVP Low	Weathered Low	-0.93576*	0.000	-1.4152	-0.4563
	Sulphidized Low, 120 nm	-0.44251	0.085	-0.9219	0.0369
	Ionic Low	-0.76615*	0.000	-1.2456	-0.2867
	Uncoated Low	0.07967	0.990	-0.3998	0.5591
Uncoated Low	Weathered Low	-1.01543*	0.000	-1.4949	-0.5360
	Sulphidized Low, 120 nm	-0.52218*	0.026	-1.0016	-0.0428
	Ionic Low	-0.84582*	0.000	-1.3252	-0.3664
	PVP Low	-0.07967	0.990	-0.5591	0.3998

Table D.5: Tukey test subset treatment groups for low concentration treatments

Treatment	N	Subset		
		1	2	3
Uncoated Low	18	0.1789		
PVP Low	18	0.2586	0.2586	
Sulphidized Low, 120 nm	18		0.7011	
Ionic Low	18			1.0247
Weathered Low	18			1.1944
Significance		0.990	0.085	0.861

Table D.6: Multiple comparisons for high concentration treatments using Tukey's test

Treatment (I)	Treatment (J)	Mean Difference (I-J)	Significance	95% Confidence Interval	
				Lower Bound	Upper Bound
Sulphidized Low, 160 nm	Ionic High	-7.06571*	0.000	-8.9033	-5.2281
	PVP High	-3.57278*	0.000	-5.4104	-1.7352
	Uncoated High	-4.23980*	0.000	-6.0774	-2.4022
Ionic High	Sulphidized Low, 160 nm	7.06571*	0.000	5.2281	8.9033
	PVP High	3.49293*	0.000	1.6553	5.3305
	Uncoated High	2.82591*	0.001	0.9883	4.6635
PVP High	Sulphidized Low, 160 nm	3.57278*	0.000	1.7352	5.4104
	Ionic High	-3.49293*	0.000	-5.3305	-1.6553
	Uncoated High	-0.66702	0.775	-2.5046	1.1706
Uncoated High	Sulphidized Low, 160 nm	4.23980*	0.000	2.4022	6.0774
	Ionic High	-2.82591*	0.001	-4.6635	-0.9883
	PVP High	0.66702	0.775	-1.1706	2.5046

Table D.7: Tukey test subset treatment groups for high concentration treatments

Treatment	N	Subsets		
		1	2	3
Sulphidized Low, 160 nm	18	0.6907		
PVP High	18		4.2635	
Uncoated High	18		4.9305	
Ionic High	18			7.7564
Significance		1.000	0.775	1.000

Table D.8: One-way ANOVA for moisture content

	Sum of Squares	DOF	Mean Square	F	Significance
Between Groups	397.351	10	39.735	2.200	0.059
Within Groups	397.263	22	18.057		
Total	794.615	32			

Table D.9: One-way ANOVA for organic matter

	Sum of Squares	DOF	Mean Square	F	Significance
Between Groups	49.990	10	4.999	0.985	0.484
Within Groups	111.618	22	5.074		
Total	161.608	32			

Table D.10: T-test for water holding capacity at 24 hours for controls and Ionic Maximum treatments

Levene's Test for Equality of Variances		t-test for Equality of Means					
F	Significance	t	DOF	Significance (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
						Lower	Upper
0.209	0.671	1.312	4	0.260	0.04169	-0.04653	0.12991

Table D.11: T-test for water holding capacity at 48 hours for control and Ionic Maximum treatments

Levene's Test for Equality of Variances		t-test for Equality of Means					
F	Significance	t	DOF	Significance (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
						Lower	Upper
0.012	0.919	1.459	4	0.218	0.11109	-0.10031	0.32250

Table D.12: One-way ANOVA for pH

	Sum of Squares	DOF	Mean Square	F	Significance
Between Groups	1.438	10	0.144	2.081	0.073
Within Groups	1.520	22	0.069		
Total	2.957	32			

Table D.13: One-way ANOVA for conductivity

	Sum of Squares	DOF	Mean Square	F	Significance
Between Groups	870.082	10	87.008	0.769	0.656
Within Groups	2488.300	22	113.105		
Total	3358.382	32			

Table D.14: Repeated Measure ANOVA for AWCD treatment measures each month

Effect	Wilk's Lambda Statistic Value	F	Hypothesis DOF	Error DOF	Significance
Time	0.649	5.677	2	21	0.011
Time*Treatment	0.555	0.720	20	42	0.784

Table D.15: Mauchly's test of sphericity for AWCD measures

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	DOF	Significance
Time	0.949	1.089	2	0.580

Table D.16: Test of within-subject effects for AWCD with sphericity assumed

Source	Type III Sum of Squares	DOF	Mean Square	F	Significance
Time	0.161	2	0.081	4.925	0.012
Time * Treatment	0.238	20	0.012	0.728	0.777

Table D.17: One-way ANOVA for AWCD measures of each month

		Sum of Squares	DOF	Mean Square	F	Significance
Month 1	Between Groups	0.505	10	0.051	3.163	0.012
	Within Groups	0.351	22	0.016		
	Total	0.856	32			
Month 2	Between Groups	0.505	10	0.051	2.695	0.025
	Within Groups	0.412	22	0.019		
	Total	0.917	32			
Month 3	Between Groups	0.810	10	0.081	2.655	0.027
	Within Groups	0.671	22	0.031		
	Total	1.481	32			

Table D.18: Multiple comparisons for AWCD Month 1 treatments using Tukey's test

Treatment (I)	Treatment (J)	Mean Difference (I-J)	Significance	95% Confidence Interval	
				Lower Bound	Upper Bound
Control	Ionic Maximum	0.36667	0.052	-0.0022	0.7355
	Weathered Low	-0.02667	1.000	-0.3955	0.3422
	Sulphidized Low, 120 nm	-0.07333	1.000	-0.4422	0.2955

	Sulphidized Low, 160 nm	0.13333	0.961	-0.2355	0.5022
	Ionic Low	0.09333	0.997	-0.2755	0.4622
	Ionic High	0.11667	0.984	-0.2522	0.4855
	PVP Low	0.03000	1.000	-0.3389	0.3989
	PVP High	-0.07333	1.000	-0.4422	0.2955
	Uncoated Low	0.08667	0.998	-0.2822	0.4555
	Uncoated High	-0.07000	1.000	-0.4389	0.2989
Ionic Maximum	Control	-0.36667	0.052	-0.7355	0.0022
	Weathered Low	-0.39333*	0.030	-0.7622	-0.0245
	Sulphidized Low, 120 nm	-0.44000*	0.011	-0.8089	-0.0711
	Sulphidized Low, 160 nm	-0.23333	0.490	-0.6022	0.1355
	Ionic Low	-0.27333	0.283	-0.6422	0.0955
	Ionic High	-0.25000	0.396	-0.6189	0.1189
	PVP Low	-0.33667	0.094	-0.7055	0.0322
	PVP High	-0.44000*	0.011	-0.8089	-0.0711
	Uncoated Low	-0.28000	0.255	-0.6489	0.0889
	Uncoated High	-0.43667*	0.012	-0.8055	-0.0678
Weathered Low	Control	0.02667	1.000	-0.3422	0.3955
	Ionic Maximum	0.39333*	0.030	0.0245	0.7622
	Sulphidized Low, 120 nm	-0.04667	1.000	-0.4155	0.3222
	Sulphidized Low, 160 nm	0.16000	0.886	-0.2089	0.5289
	Ionic Low	0.12000	0.980	-0.2489	0.4889
	Ionic High	0.14333	0.939	-0.2255	0.5122
	PVP Low	0.05667	1.000	-0.3122	0.4255
	PVP High	-0.04667	1.000	-0.4155	0.3222
	Uncoated Low	0.11333	0.987	-0.2555	0.4822
	Uncoated High	-0.04333	1.000	-0.4122	0.3255
Sulphidized Low, 120 nm	Control	0.07333	1.000	-0.2955	0.4422
	Ionic Maximum	0.44000*	0.011	0.0711	0.8089
	Weathered Low	0.04667	1.000	-0.3222	0.4155
	Sulphidized Low, 160 nm	0.20667	0.649	-0.1622	0.5755
	Ionic Low	0.16667	0.860	-0.2022	0.5355
	Ionic High	0.19000	0.745	-0.1789	0.5589
	PVP Low	0.10333	0.993	-0.2655	0.4722
	PVP High	0.00000	1.000	-0.3689	0.3689
	Uncoated Low	0.16000	0.886	-0.2089	0.5289
	Uncoated High	0.00333	1.000	-0.3655	0.3722
Sulphidized Low, 160 nm	Control	-0.13333	0.961	-0.5022	0.2355
	Ionic Maximum	0.23333	0.490	-0.1355	0.6022
	Weathered Low	-0.16000	0.886	-0.5289	0.2089

	Sulphidized Low, 120 nm	-0.20667	0.649	-0.5755	0.1622
	Ionic Low	-0.04000	1.000	-0.4089	0.3289
	Ionic High	-0.01667	1.000	-0.3855	0.3522
	PVP Low	-0.10333	0.993	-0.4722	0.2655
	PVP High	-0.20667	0.649	-0.5755	0.1622
	Uncoated Low	-0.04667	1.000	-0.4155	0.3222
	Uncoated High	-0.20333	0.669	-0.5722	0.1655
Ionic Low	Control	-0.09333	0.997	-0.4622	0.2755
	Ionic Maximum	0.27333	0.283	-0.0955	0.6422
	Weathered Low	-0.12000	0.980	-0.4889	0.2489
	Sulphidized Low, 120 nm	-0.16667	0.860	-0.5355	0.2022
	Sulphidized Low, 160 nm	0.04000	1.000	-0.3289	0.4089
	Ionic High	0.02333	1.000	-0.3455	0.3922
	PVP Low	-0.06333	1.000	-0.4322	0.3055
	PVP High	-0.16667	0.860	-0.5355	0.2022
	Uncoated Low	-0.00667	1.000	-0.3755	0.3622
	Uncoated High	-0.16333	0.873	-0.5322	0.2055
Ionic High	Control	-0.11667	0.984	-0.4855	0.2522
	Ionic Maximum	0.25000	0.396	-0.1189	0.6189
	Weathered Low	-0.14333	0.939	-0.5122	0.2255
	Sulphidized Low, 120 nm	-0.19000	0.745	-0.5589	0.1789
	Sulphidized Low, 160 nm	0.01667	1.000	-0.3522	0.3855
	Ionic Low	-0.02333	1.000	-0.3922	0.3455
	PVP Low	-0.08667	0.998	-0.4555	0.2822
	PVP High	-0.19000	0.745	-0.5589	0.1789
	Uncoated Low	-0.03000	1.000	-0.3989	0.3389
	Uncoated High	-0.18667	0.763	-0.5555	0.1822
PVP Low	Control	-0.03000	1.000	-0.3989	0.3389
	Ionic Maximum	0.33667	0.094	-0.0322	0.7055
	Weathered Low	-0.05667	1.000	-0.4255	0.3122
	Sulphidized Low, 120 nm	-0.10333	0.993	-0.4722	0.2655
	Sulphidized Low, 160 nm	0.10333	0.993	-0.2655	0.4722
	Ionic Low	0.06333	1.000	-0.3055	0.4322
	Ionic High	0.08667	0.998	-0.2822	0.4555
	PVP High	-0.10333	0.993	-0.4722	0.2655
	Uncoated Low	0.05667	1.000	-0.3122	0.4255
	Uncoated High	-0.10000	0.995	-0.4689	0.2689
PVP High	Control	0.07333	1.000	-0.2955	0.4422
	Ionic Maximum	0.44000*	0.011	0.0711	0.8089

	Weathered Low	0.04667	1.000	-0.3222	0.4155
	Sulphidized Low, 120 nm	0.00000	1.000	-0.3689	0.3689
	Sulphidized Low, 160 nm	0.20667	0.649	-0.1622	0.5755
	Ionic Low	0.16667	0.860	-0.2022	0.5355
	Ionic High	0.19000	0.745	-0.1789	0.5589
	PVP Low	0.10333	0.993	-0.2655	0.4722
	Uncoated Low	0.16000	0.886	-0.2089	0.5289
	Uncoated High	0.00333	1.000	-0.3655	0.3722
Uncoated Low	Control	-0.08667	0.998	-0.4555	0.2822
	Ionic Maximum	0.28000	0.255	-0.0889	0.6489
	Weathered Low	-0.11333	0.987	-0.4822	0.2555
	Sulphidized Low, 120 nm	-0.16000	0.886	-0.5289	0.2089
	Sulphidized Low, 160 nm	0.04667	1.000	-0.3222	0.4155
	Ionic Low	0.00667	1.000	-0.3622	0.3755
	Ionic High	0.03000	1.000	-0.3389	0.3989
	PVP Low	-0.05667	1.000	-0.4255	0.3122
	PVP High	-0.16000	0.886	-0.5289	0.2089
	Uncoated High	-0.15667	0.898	-0.5255	0.2122
Uncoated Low	Control	0.07000	1.000	-0.2989	0.4389
	Ionic Maximum	0.43667*	0.012	0.0678	0.8055
	Weathered Low	0.04333	1.000	-0.3255	0.4122
	Sulphidized Low, 120 nm	-0.00333	1.000	-0.3722	0.3655
	Sulphidized Low, 160 nm	0.20333	0.669	-0.1655	0.5722
	Ionic Low	0.16333	0.873	-0.2055	0.5322
	Ionic High	0.18667	0.763	-0.1822	0.5555
	PVP Low	0.10000	0.995	-0.2689	0.4689
	PVP High	-0.00333	1.000	-0.3722	0.3655
	Uncoated High	0.15667	0.898	-0.2122	0.5255

Table D.19: Tukey test subset treatment groups for Month 1 AWCD

Treatment	N	Subsets	
		1	2
Ionic Maximum	3	0.2333	
Sulphidized Low, 160 nm	3	0.4667	0.4667
Ionic High	3	0.4833	0.4833
Ionic Low	3	0.5067	0.5067
Uncoated Low	3	0.5133	0.5133
PVP Low	3	0.5700	0.5700
Control	3	0.6000	0.6000
Weathered Low	3		0.6267
Uncoated High	3		0.6700
Sulphidized Low, 120 nm	3		0.6733
PVP High	3		0.6733
Significance		0.052	0.649

Table D.20: Multiple comparisons for AWCD Month 2 treatments using Tukey's test

Treatment (I)	Treatment (J)	Mean Difference (I-J)	Significance	95% Confidence Interval	
				Lower Bound	Upper Bound
Control	Ionic Maximum	0.30667	0.243	-0.0929	0.7062
	Weathered Low	-0.15667	0.935	-0.5562	0.2429
	Sulphidized Low, 120 nm	-0.10333	0.996	-0.5029	0.2962
	Sulphidized Low, 160 nm	0.05000	1.000	-0.3495	0.4495
	Ionic Low	-0.00333	1.000	-0.4029	0.3962
	Ionic High	-0.17333	0.886	-0.5729	0.2262
	PVP Low	0.02000	1.000	-0.3795	0.4195
	PVP High	-0.01333	1.000	-0.4129	0.3862
	Uncoated Low	-0.04000	1.000	-0.4395	0.3595
	Uncoated High	-0.09000	0.999	-0.4895	0.3095
Ionic Maximum	Control	-0.30667	0.243	-0.7062	0.0929
	Weathered Low	-0.46333*	0.014	-0.8629	-0.0638
	Sulphidized Low, 120 nm	-0.41000*	0.041	-0.8095	-0.0105
	Sulphidized Low, 160 nm	-0.25667	0.469	-0.6562	0.1429
	Ionic Low	-0.31000	0.231	-0.7095	0.0895
	Ionic High	-0.48000*	0.010	-0.8795	-0.0805
	PVP Low	-0.28667	0.323	-0.6862	0.1129
	PVP High	-0.32000	0.198	-0.7195	0.0795

	Uncoated Low	-0.34667	0.129	-0.7462	0.0529
	Uncoated High	-0.39667	0.053	-0.7962	0.0029
Weathered Low	Control	0.15667	0.935	-0.2429	0.5562
	Ionic Maximum	0.46333*	0.014	0.0638	0.8629
	Sulphidized Low, 120 nm	0.05333	1.000	-0.3462	0.4529
	Sulphidized Low, 160 nm	0.20667	0.741	-0.1929	0.6062
	Ionic Low	0.15333	0.943	-0.2462	0.5529
	Ionic High	-0.01667	1.000	-0.4162	0.3829
	PVP Low	0.17667	0.874	-0.2229	0.5762
	PVP High	0.14333	0.963	-0.2562	0.5429
	Uncoated Low	0.11667	0.991	-0.2829	0.5162
	Uncoated High	0.06667	1.000	-0.3329	0.4662
Sulphidized Low, 120 nm	Control	0.10333	0.996	-0.2962	0.5029
	Ionic Maximum	0.41000*	0.041	0.0105	0.8095
	Weathered Low	-0.05333	1.000	-0.4529	0.3462
	Sulphidized Low, 160 nm	0.15333	0.943	-0.2462	0.5529
	Ionic Low	0.10000	0.997	-0.2995	0.4995
	Ionic High	-0.07000	1.000	-0.4695	0.3295
	PVP Low	0.12333	0.986	-0.2762	0.5229
	PVP High	0.09000	0.999	-0.3095	0.4895
	Uncoated Low	0.06333	1.000	-0.3362	0.4629
	Uncoated High	0.01333	1.000	-0.3862	0.4129
Sulphidized Low, 160 nm	Control	-0.05000	1.000	-0.4495	0.3495
	Ionic Maximum	0.25667	0.469	-0.1429	0.6562
	Weathered Low	-0.20667	0.741	-0.6062	0.1929
	Sulphidized Low, 120 nm	-0.15333	0.943	-0.5529	0.2462
	Ionic Low	-0.05333	1.000	-0.4529	0.3462
	Ionic High	-0.22333	0.652	-0.6229	0.1762
	PVP Low	-0.03000	1.000	-0.4295	0.3695
	PVP High	-0.06333	1.000	-0.4629	0.3362
	Uncoated Low	-0.09000	0.999	-0.4895	0.3095
	Uncoated High	-0.14000	0.968	-0.5395	0.2595
Ionic Low	Control	0.00333	1.000	-0.3962	0.4029
	Ionic Maximum	0.31000	0.231	-0.0895	0.7095
	Weathered Low	-0.15333	0.943	-0.5529	0.2462
	Sulphidized Low, 120 nm	-0.10000	0.997	-0.4995	0.2995
	Sulphidized Low, 160 nm	0.05333	1.000	-0.3462	0.4529
	Ionic High	-0.17000	0.897	-0.5695	0.2295
	PVP Low	0.02333	1.000	-0.3762	0.4229
	PVP High	-0.01000	1.000	-0.4095	0.3895

	Uncoated Low	-0.03667	1.000	-0.4362	0.3629
	Uncoated High	-0.08667	0.999	-0.4862	0.3129
Ionic High	Control	0.17333	0.886	-0.2262	0.5729
	Ionic Maximum	0.48000*	0.010	0.0805	0.8795
	Weathered Low	0.01667	1.000	-0.3829	0.4162
	Sulphidized Low, 120 nm	0.07000	1.000	-0.3295	0.4695
	Sulphidized Low, 160 nm	0.22333	0.652	-0.1762	0.6229
	Ionic Low	0.17000	0.897	-0.2295	0.5695
	PVP Low	0.19333	0.805	-0.2062	0.5929
	PVP High	0.16000	0.927	-0.2395	0.5595
	Uncoated Low	0.13333	0.977	-0.2662	0.5329
	Uncoated High	0.08333	0.999	-0.3162	0.4829
PVP Low	Control	-0.02000	1.000	-0.4195	0.3795
	Ionic Maximum	0.28667	0.323	-0.1129	0.6862
	Weathered Low	-0.17667	0.874	-0.5762	0.2229
	Sulphidized Low, 120 nm	-0.12333	0.986	-0.5229	0.2762
	Sulphidized Low, 160 nm	0.03000	1.000	-0.3695	0.4295
	Ionic Low	-0.02333	1.000	-0.4229	0.3762
	Ionic High	-0.19333	0.805	-0.5929	0.2062
	PVP High	-0.03333	1.000	-0.4329	0.3662
	Uncoated Low	-0.06000	1.000	-0.4595	0.3395
	Uncoated High	-0.11000	0.994	-0.5095	0.2895
PVP High	Control	0.01333	1.000	-0.3862	0.4129
	Ionic Maximum	0.32000	0.198	-0.0795	0.7195
	Weathered Low	-0.14333	0.963	-0.5429	0.2562
	Sulphidized Low, 120 nm	-0.09000	0.999	-0.4895	0.3095
	Sulphidized Low, 160 nm	0.06333	1.000	-0.3362	0.4629
	Ionic Low	0.01000	1.000	-0.3895	0.4095
	Ionic High	-0.16000	0.927	-0.5595	0.2395
	PVP Low	0.03333	1.000	-0.3662	0.4329
	Uncoated Low	-0.02667	1.000	-0.4262	0.3729
	Uncoated High	-0.07667	1.000	-0.4762	0.3229
Uncoated Low	Control	0.04000	1.000	-0.3595	0.4395
	Ionic Maximum	0.34667	0.129	-0.0529	0.7462
	Weathered Low	-0.11667	0.991	-0.5162	0.2829
	Sulphidized Low, 120 nm	-0.06333	1.000	-0.4629	0.3362
	Sulphidized Low, 160 nm	0.09000	0.999	-0.3095	0.4895
	Ionic Low	0.03667	1.000	-0.3629	0.4362

	Ionic High	-0.13333	0.977	-0.5329	0.2662
	PVP Low	0.06000	1.000	-0.3395	0.4595
	PVP High	0.02667	1.000	-0.3729	0.4262
	Uncoated High	-0.05000	1.000	-0.4495	0.3495
Uncoated High	Control	0.09000	0.999	-0.3095	0.4895
	Ionic Maximum	0.39667	0.053	-0.0029	0.7962
	Weathered Low	-0.06667	1.000	-0.4662	0.3329
	Sulphidized Low, 120 nm	-0.01333	1.000	-0.4129	0.3862
	Sulphidized Low, 160 nm	0.14000	0.968	-0.2595	0.5395
	Ionic Low	0.08667	0.999	-0.3129	0.4862
	Ionic High	-0.08333	0.999	-0.4829	0.3162
	PVP Low	0.11000	0.994	-0.2895	0.5095
	PVP High	0.07667	1.000	-0.3229	0.4762
	Uncoated Low	0.05000	1.000	-0.3495	0.4495

Table D.21: Post-hoc Tukey test subset treatment groups for Month 2 AWCD

Treatment	N	Subsets	
		1	2
Ionic Maximum	3	0.1233	
Sulphidized Low, 160 nm	3	0.3800	0.3800
PVP Low	3	0.4100	0.4100
Control	3	0.4300	0.4300
Ionic Low	3	0.4333	0.4333
PVP High	3	0.4433	0.4433
Uncoated Low	3	0.4700	0.4700
Uncoated High	3	0.5200	0.5200
Sulphidized Low, 120 nm	3		0.5333
Weathered Low	3		0.5867
Ionic High	3		0.6033
Significance		0.053	0.652

Table D.22: Multiple comparisons for AWCD Month 3 treatments using Tukey's test

Treatment (I)	Treatment (J)	Mean Difference (I-J)	Significance	95% Confidence Interval	
				Lower Bound	Upper Bound
Control	Ionic Maximum	0.44333	0.127	-0.0664	0.9531
	Weathered Low	-0.16000	0.985	-0.6698	0.3498
	Sulphidized Low, 120 nm	-0.11333	0.999	-0.6231	0.3964

	Sulphidized Low, 160 nm	0.23333	0.850	-0.2764	0.7431
	Ionic Low	0.11667	0.999	-0.3931	0.6264
	Ionic High	0.11667	0.999	-0.3931	0.6264
	PVP Low	0.15667	0.987	-0.3531	0.6664
	PVP High	0.03667	1.000	-0.4731	0.5464
	Uncoated Low	0.04000	1.000	-0.4698	0.5498
	Uncoated High	0.04667	1.000	-0.4631	0.5564
Ionic Maximum	Control	-0.44333	0.127	-0.9531	0.0664
	Weathered Low	-0.60333*	0.012	-1.1131	-0.0936
	Sulphidized Low, 120 nm	-0.55667*	0.025	-1.0664	-0.0469
	Sulphidized Low, 160 nm	-0.21000	0.914	-0.7198	0.2998
	Ionic Low	-0.32667	0.472	-0.8364	0.1831
	Ionic High	-0.32667	0.472	-0.8364	0.1831
	PVP Low	-0.28667	0.645	-0.7964	0.2231
	PVP High	-0.40667	0.202	-0.9164	0.1031
	Uncoated Low	-0.40333	0.211	-0.9131	0.1064
	Uncoated High	-0.39667	0.228	-0.9064	0.1131
Weathered Low	Control	0.16000	0.985	-0.3498	0.6698
	Ionic Maximum	0.60333*	0.012	0.0936	1.1131
	Sulphidized Low, 120 nm	0.04667	1.000	-0.4631	0.5564
	Sulphidized Low, 160 nm	0.39333	0.237	-0.1164	0.9031
	Ionic Low	0.27667	0.687	-0.2331	0.7864
	Ionic High	0.27667	0.687	-0.2331	0.7864
	PVP Low	0.31667	0.514	-0.1931	0.8264
	PVP High	0.19667	0.941	-0.3131	0.7064
	Uncoated Low	0.20000	0.935	-0.3098	0.7098
	Uncoated High	0.20667	0.921	-0.3031	0.7164
Sulphidized Low, 120 nm	Control	0.11333	0.999	-0.3964	0.6231
	Ionic Maximum	0.55667*	0.025	0.0469	1.0664
	Weathered Low	-0.04667	1.000	-0.5564	0.4631
	Sulphidized Low, 160 nm	0.34667	0.392	-0.1631	0.8564
	Ionic Low	0.23000	0.861	-0.2798	0.7398
	Ionic High	0.23000	0.861	-0.2798	0.7398
	PVP Low	0.27000	0.715	-0.2398	0.7798
	PVP High	0.15000	0.990	-0.3598	0.6598
	Uncoated Low	0.15333	0.989	-0.3564	0.6631
	Uncoated High	0.16000	0.985	-0.3498	0.6698
Sulphidized Low, 160 nm	Control	-0.23333	0.850	-0.7431	0.2764
	Ionic Maximum	0.21000	0.914	-0.2998	0.7198
	Weathered Low	-0.39333	0.237	-0.9031	0.1164

	Sulphidized Low, 120 nm	-0.34667	0.392	-0.8564	0.1631
	Ionic Low	-0.11667	0.999	-0.6264	0.3931
	Ionic High	-0.11667	0.999	-0.6264	0.3931
	PVP Low	-0.07667	1.000	-0.5864	0.4331
	PVP High	-0.19667	0.941	-0.7064	0.3131
	Uncoated Low	-0.19333	0.947	-0.7031	0.3164
	Uncoated High	-0.18667	0.957	-0.6964	0.3231
Ionic Low	Control	-0.11667	0.999	-0.6264	0.3931
	Ionic Maximum	0.32667	0.472	-0.1831	0.8364
	Weathered Low	-0.27667	0.687	-0.7864	0.2331
	Sulphidized Low, 120 nm	-0.23000	0.861	-0.7398	0.2798
	Sulphidized Low, 160 nm	0.11667	0.999	-0.3931	0.6264
	Ionic High	0.00000	1.000	-0.5098	0.5098
	PVP Low	0.04000	1.000	-0.4698	0.5498
	PVP High	-0.08000	1.000	-0.5898	0.4298
	Uncoated Low	-0.07667	1.000	-0.5864	0.4331
	Uncoated High	-0.07000	1.000	-0.5798	0.4398
Ionic High	Control	-0.11667	0.999	-0.6264	0.3931
	Ionic Maximum	0.32667	0.472	-0.1831	0.8364
	Weathered Low	-0.27667	0.687	-0.7864	0.2331
	Sulphidized Low, 120 nm	-0.23000	0.861	-0.7398	0.2798
	Sulphidized Low, 160 nm	0.11667	0.999	-0.3931	0.6264
	Ionic Low	0.00000	1.000	-0.5098	0.5098
	PVP Low	0.04000	1.000	-0.4698	0.5498
	PVP High	-0.08000	1.000	-0.5898	0.4298
	Uncoated Low	-0.07667	1.000	-0.5864	0.4331
	Uncoated High	-0.07000	1.000	-0.5798	0.4398
PVP Low	Control	-0.15667	0.987	-0.6664	0.3531
	Ionic Maximum	0.28667	0.645	-0.2231	0.7964
	Weathered Low	-0.31667	0.514	-0.8264	0.1931
	Sulphidized Low, 120 nm	-0.27000	0.715	-0.7798	0.2398
	Sulphidized Low, 160 nm	0.07667	1.000	-0.4331	0.5864
	Ionic Low	-0.04000	1.000	-0.5498	0.4698
	Ionic High	-0.04000	1.000	-0.5498	0.4698
	PVP High	-0.12000	0.998	-0.6298	0.3898
	Uncoated Low	-0.11667	0.999	-0.6264	0.3931
	Uncoated High	-0.11000	0.999	-0.6198	0.3998
PVP High	Control	-0.03667	1.000	-0.5464	0.4731
	Ionic Maximum	0.40667	0.202	-0.1031	0.9164

	Weathered Low	-0.19667	0.941	-0.7064	0.3131
	Sulphidized Low, 120 nm	-0.15000	0.990	-0.6598	0.3598
	Sulphidized Low, 160 nm	0.19667	0.941	-0.3131	0.7064
	Ionic Low	0.08000	1.000	-0.4298	0.5898
	Ionic High	0.08000	1.000	-0.4298	0.5898
	PVP Low	0.12000	0.998	-0.3898	0.6298
	Uncoated Low	0.00333	1.000	-0.5064	0.5131
	Uncoated High	0.01000	1.000	-0.4998	0.5198
Uncoated Low	Control	-0.04000	1.000	-0.5498	0.4698
	Ionic Maximum	0.40333	0.211	-0.1064	0.9131
	Weathered Low	-0.20000	0.935	-0.7098	0.3098
	Sulphidized Low, 120 nm	-0.15333	0.989	-0.6631	0.3564
	Sulphidized Low, 160 nm	0.19333	0.947	-0.3164	0.7031
	Ionic Low	0.07667	1.000	-0.4331	0.5864
	Ionic High	0.07667	1.000	-0.4331	0.5864
	PVP Low	0.11667	0.999	-0.3931	0.6264
	PVP High	-0.00333	1.000	-0.5131	0.5064
	Uncoated High	0.00667	1.000	-0.5031	0.5164
Uncoated High	Control	-0.04667	1.000	-0.5564	0.4631
	Ionic Maximum	0.39667	0.228	-0.1131	0.9064
	Weathered Low	-0.20667	0.921	-0.7164	0.3031
	Sulphidized Low, 120 nm	-0.16000	0.985	-0.6698	0.3498
	Sulphidized Low, 160 nm	0.18667	0.957	-0.3231	0.6964
	Ionic Low	0.07000	1.000	-0.4398	0.5798
	Ionic High	0.07000	1.000	-0.4398	0.5798
	PVP Low	0.11000	0.999	-0.3998	0.6198
	PVP High	-0.01000	1.000	-0.5198	0.4998
	Uncoated Low	-0.00667	1.000	-0.5164	0.5031

Table D.23: Post-hoc Tukey test subset treatment groups for Month 3 AWCD

Treatment	N	Subsets	
		1	2
Ionic Maximum	3	0.1300	
Sulphidized Low, 160 nm	3	0.3400	0.3400
PVP Low	3	0.4167	0.4167
Ionic Low	3	0.4567	0.4567
Ionic High	3	0.4567	0.4567
Uncoated High	3	0.5267	0.5267
Uncoated Low	3	0.5333	0.5333
PVP High	3	0.5367	0.5367
Control	3	0.5733	0.5733
Sulphidized Low, 120 nm	3		0.6867
Weathered Low	3		0.7333
Significance		0.127	0.237

Table D.24: One-way ANOVA for richness measures of each month

		Sum of Squares	DOF	Mean Square	F	Significance
Month 1	Between Groups	304.839	10	30.484	4.904	0.001
	Within Groups	136.741	22	6.215		
	Total	441.580	32			
Month 2	Between Groups	449.705	10	44.970	5.993	0.000
	Within Groups	165.085	22	7.504		
	Total	614.789	32			
Month 3	Between Groups	417.404	10	41.740	3.753	0.005
	Within Groups	244.662	22	11.121		
	Total	662.065	32			

Table D.25: Multiple comparisons for richness Month 1 treatments using Tukey's test

Treatment (I)	Treatment (J)	Mean Difference (I-J)	Significance	95% Confidence Interval	
				Lower Bound	Upper Bound
Control	Ionic Maximum	10.22000*	0.002	2.9431	17.4969
	Weathered Low	-0.44667	1.000	-7.7236	6.8302
	Sulphidized Low, 120 nm	-0.33667	1.000	-7.6136	6.9402
	Sulphidized Low, 160 nm	2.44333	0.976	-4.8336	9.7202
	Ionic Low	0.00000	1.000	-7.2769	7.2769
	Ionic High	2.77667	0.945	-4.5002	10.0536

	PVP Low	1.66667	0.999	-5.6102	8.9436
	PVP High	-1.00000	1.000	-8.2769	6.2769
	Uncoated Low	0.77667	1.000	-6.5002	8.0536
	Uncoated High	-0.66667	1.000	-7.9436	6.6102
Ionic Maximum	Control	-10.22000*	0.002	-17.4969	-2.9431
	Weathered Low	-10.66667*	0.001	-17.9436	-3.3898
	Sulphidized Low, 120 nm	-10.55667*	0.001	-17.8336	-3.2798
	Sulphidized Low, 160 nm	-7.77667*	0.030	-15.0536	-0.4998
	Ionic Low	-10.22000*	0.002	-17.4969	-2.9431
	Ionic High	-7.44333*	0.042	-14.7202	-0.1664
	PVP Low	-8.55333*	0.013	-15.8302	-1.2764
	PVP High	-11.22000*	0.001	-18.4969	-3.9431
	Uncoated Low	-9.44333*	0.005	-16.7202	-2.1664
	Uncoated High	-10.88667*	0.001	-18.1636	-3.6098
Weathered Low	Control	0.44667	1.000	-6.8302	7.7236
	Ionic Maximum	10.66667*	0.001	3.3898	17.9436
	Sulphidized Low, 120 nm	0.11000	1.000	-7.1669	7.3869
	Sulphidized Low, 160 nm	2.89000	0.930	-4.3869	10.1669
	Ionic Low	0.44667	1.000	-6.8302	7.7236
	Ionic High	3.22333	0.873	-4.0536	10.5002
	PVP Low	2.11333	0.991	-5.1636	9.3902
	PVP High	-0.55333	1.000	-7.8302	6.7236
	Uncoated Low	1.22333	1.000	-6.0536	8.5002
	Uncoated High	-0.22000	1.000	-7.4969	7.0569
Sulphidized Low, 120 nm	Control	0.33667	1.000	-6.9402	7.6136
	Ionic Maximum	10.55667*	0.001	3.2798	17.8336
	Weathered Low	-0.11000	1.000	-7.3869	7.1669
	Sulphidized Low, 160 nm	2.78000	0.945	-4.4969	10.0569
	Ionic Low	0.33667	1.000	-6.9402	7.6136
	Ionic High	3.11333	0.894	-4.1636	10.3902
	PVP Low	2.00333	0.994	-5.2736	9.2802
	PVP High	-0.66333	1.000	-7.9402	6.6136
	Uncoated Low	1.11333	1.000	-6.1636	8.3902
	Uncoated High	-0.33000	1.000	-7.6069	6.9469
Sulphidized Low, 160 nm	Control	-2.44333	0.976	-9.7202	4.8336
	Ionic Maximum	7.77667*	0.030	0.4998	15.0536
	Weathered Low	-2.89000	0.930	-10.1669	4.3869
	Sulphidized Low, 120 nm	-2.78000	0.945	-10.0569	4.4969
	Ionic Low	-2.44333	0.976	-9.7202	4.8336
	Ionic High	0.33333	1.000	-6.9436	7.6102

	PVP Low	-0.77667	1.000	-8.0536	6.5002
	PVP High	-3.44333	0.825	-10.7202	3.8336
	Uncoated Low	-1.66667	0.999	-8.9436	5.6102
	Uncoated High	-3.11000	0.895	-10.3869	4.1669
Ionic Low	Control	0.00000	1.000	-7.2769	7.2769
	Ionic Maximum	10.22000*	0.002	2.9431	17.4969
	Weathered Low	-0.44667	1.000	-7.7236	6.8302
	Sulphidized Low, 120 nm	-0.33667	1.000	-7.6136	6.9402
	Sulphidized Low, 160 nm	2.44333	0.976	-4.8336	9.7202
	Ionic High	2.77667	0.945	-4.5002	10.0536
	PVP Low	1.66667	0.999	-5.6102	8.9436
	PVP High	-1.00000	1.000	-8.2769	6.2769
	Uncoated Low	0.77667	1.000	-6.5002	8.0536
	Uncoated High	-0.66667	1.000	-7.9436	6.6102
Ionic High	Control	-2.77667	0.945	-10.0536	4.5002
	Ionic Maximum	7.44333*	0.042	0.1664	14.7202
	Weathered Low	-3.22333	0.873	-10.5002	4.0536
	Sulphidized Low, 120 nm	-3.11333	0.894	-10.3902	4.1636
	Sulphidized Low, 160 nm	-0.33333	1.000	-7.6102	6.9436
	Ionic Low	-2.77667	0.945	-10.0536	4.5002
	PVP Low	-1.11000	1.000	-8.3869	6.1669
	PVP High	-3.77667	0.737	-11.0536	3.5002
	Uncoated Low	-2.00000	0.994	-9.2769	5.2769
	Uncoated High	-3.44333	0.825	-10.7202	3.8336
PVP Low	Control	-1.66667	0.999	-8.9436	5.6102
	Ionic Maximum	8.55333*	0.013	1.2764	15.8302
	Weathered Low	-2.11333	0.991	-9.3902	5.1636
	Sulphidized Low, 120 nm	-2.00333	0.994	-9.2802	5.2736
	Sulphidized Low, 160 nm	0.77667	1.000	-6.5002	8.0536
	Ionic Low	-1.66667	0.999	-8.9436	5.6102
	Ionic High	1.11000	1.000	-6.1669	8.3869
	PVP High	-2.66667	0.957	-9.9436	4.6102
	Uncoated Low	-0.89000	1.000	-8.1669	6.3869
	Uncoated High	-2.33333	0.982	-9.6102	4.9436
PVP High	Control	1.00000	1.000	-6.2769	8.2769
	Ionic Maximum	11.22000*	0.001	3.9431	18.4969
	Weathered Low	0.55333	1.000	-6.7236	7.8302
	Sulphidized Low, 120 nm	0.66333	1.000	-6.6136	7.9402

	Sulphidized Low, 160 nm	3.44333	0.825	-3.8336	10.7202
	Ionic Low	1.00000	1.000	-6.2769	8.2769
	Ionic High	3.77667	0.737	-3.5002	11.0536
	PVP Low	2.66667	0.957	-4.6102	9.9436
	Uncoated Low	1.77667	0.998	-5.5002	9.0536
	Uncoated High	0.33333	1.000	-6.9436	7.6102
Uncoated Low	Control	-0.77667	1.000	-8.0536	6.5002
	Ionic Maximum	9.44333*	0.005	2.1664	16.7202
	Weathered Low	-1.22333	1.000	-8.5002	6.0536
	Sulphidized Low, 120 nm	-1.11333	1.000	-8.3902	6.1636
	Sulphidized Low, 160 nm	1.66667	0.999	-5.6102	8.9436
	Ionic Low	-0.77667	1.000	-8.0536	6.5002
	Ionic High	2.00000	0.994	-5.2769	9.2769
	PVP Low	0.89000	1.000	-6.3869	8.1669
	PVP High	-1.77667	0.998	-9.0536	5.5002
	Uncoated High	-1.44333	1.000	-8.7202	5.8336
Uncoated Low	Control	0.66667	1.000	-6.6102	7.9436
	Ionic Maximum	10.88667*	0.001	3.6098	18.1636
	Weathered Low	0.22000	1.000	-7.0569	7.4969
	Sulphidized Low, 120 nm	0.33000	1.000	-6.9469	7.6069
	Sulphidized Low, 160 nm	3.11000	0.895	-4.1669	10.3869
	Ionic Low	0.66667	1.000	-6.6102	7.9436
	Ionic High	3.44333	0.825	-3.8336	10.7202
	PVP Low	2.33333	0.982	-4.9436	9.6102
	PVP High	-0.33333	1.000	-7.6102	6.9436
	Uncoated High	1.44333	1.000	-5.8336	8.7202

Table D.26: Tukey test subset treatment groups for Month 1 richness

Treatment	N	Subsets	
		1	2
Ionic Maximum	3	8.5567	
Ionic High	3		16.0000
Sulphidized Low, 160 nm	3		16.3333
PVP Low	3		17.1100
Uncoated Low	3		18.0000
Control	3		18.7767
Ionic Low	3		18.7767
Sulphidized Low, 120 nm	3		19.1133
Weathered Low	3		19.2233
Uncoated High	3		19.4433
PVP High	3		19.7767
Significance		1.000	0.737

Table D.27: Multiple comparisons for richness Month 2 treatments using Tukey's test

Treatment (I)	Treatment (J)	Mean Difference (I-J)	Significance	95% Confidence Interval	
				Lower Bound	Upper Bound
Control	Ionic Maximum	9.77667*	0.009	1.7811	17.7722
	Weathered Low	-4.66667	0.597	-12.6622	3.3289
	Sulphidized Low, 120 nm	-3.11000	0.938	-11.1056	4.8856
	Sulphidized Low, 160 nm	-0.33333	1.000	-8.3289	7.6622
	Ionic Low	-2.00000	0.997	-9.9956	5.9956
	Ionic High	-2.66667	0.977	-10.6622	5.3289
	PVP Low	-1.44667	1.000	-9.4422	6.5489
	PVP High	-0.11000	1.000	-8.1056	7.8856
	Uncoated Low	-0.44667	1.000	-8.4422	7.5489
	Uncoated High	-4.11000	0.747	-12.1056	3.8856
Ionic Maximum	Control	-9.77667*	0.009	-17.7722	-1.7811
	Weathered Low	-14.44333*	0.000	-22.4389	-6.4478
	Sulphidized Low, 120 nm	-12.88667*	0.000	-20.8822	-4.8911
	Sulphidized Low, 160 nm	-10.11000*	0.006	-18.1056	-2.1144
	Ionic Low	-11.77667*	0.001	-19.7722	-3.7811
	Ionic High	-12.44333*	0.001	-20.4389	-4.4478
	PVP Low	-11.22333*	0.002	-19.2189	-3.2278
	PVP High	-9.88667*	0.008	-17.8822	-1.8911

	Uncoated Low	-10.22333*	0.006	-18.2189	-2.2278
	Uncoated High	-13.88667*	0.000	-21.8822	-5.8911
Weathered Low	Control	4.66667	0.597	-3.3289	12.6622
	Ionic Maximum	14.44333*	0.000	6.4478	22.4389
	Sulphidized Low, 120 nm	1.55667	1.000	-6.4389	9.5522
	Sulphidized Low, 160 nm	4.33333	0.689	-3.6622	12.3289
	Ionic Low	2.66667	0.977	-5.3289	10.6622
	Ionic High	2.00000	0.997	-5.9956	9.9956
	PVP Low	3.22000	0.924	-4.7756	11.2156
	PVP High	4.55667	0.628	-3.4389	12.5522
	Uncoated Low	4.22000	0.719	-3.7756	12.2156
	Uncoated High	0.55667	1.000	-7.4389	8.5522
Sulphidized Low, 120 nm	Control	3.11000	0.938	-4.8856	11.1056
	Ionic Maximum	12.88667*	0.000	4.8911	20.8822
	Weathered Low	-1.55667	1.000	-9.5522	6.4389
	Sulphidized Low, 160 nm	2.77667	0.970	-5.2189	10.7722
	Ionic Low	1.11000	1.000	-6.8856	9.1056
	Ionic High	0.44333	1.000	-7.5522	8.4389
	PVP Low	1.66333	0.999	-6.3322	9.6589
	PVP High	3.00000	0.950	-4.9956	10.9956
	Uncoated Low	2.66333	0.977	-5.3322	10.6589
	Uncoated High	-1.00000	1.000	-8.9956	6.9956
Sulphidized Low, 160 nm	Control	0.33333	1.000	-7.6622	8.3289
	Ionic Maximum	10.11000*	0.006	2.1144	18.1056
	Weathered Low	-4.33333	0.689	-12.3289	3.6622
	Sulphidized Low, 120 nm	-2.77667	0.970	-10.7722	5.2189
	Ionic Low	-1.66667	0.999	-9.6622	6.3289
	Ionic High	-2.33333	0.991	-10.3289	5.6622
	PVP Low	-1.11333	1.000	-9.1089	6.8822
	PVP High	0.22333	1.000	-7.7722	8.2189
	Uncoated Low	-0.11333	1.000	-8.1089	7.8822
	Uncoated High	-3.77667	0.826	-11.7722	4.2189
Ionic Low	Control	2.00000	0.997	-5.9956	9.9956
	Ionic Maximum	11.77667*	0.001	3.7811	19.7722
	Weathered Low	-2.66667	0.977	-10.6622	5.3289
	Sulphidized Low, 120 nm	-1.11000	1.000	-9.1056	6.8856
	Sulphidized Low, 160 nm	1.66667	0.999	-6.3289	9.6622
	Ionic High	-0.66667	1.000	-8.6622	7.3289
	PVP Low	0.55333	1.000	-7.4422	8.5489
	PVP High	1.89000	0.998	-6.1056	9.8856

	Uncoated Low	1.55333	1.000	-6.4422	9.5489
	Uncoated High	-2.11000	0.996	-10.1056	5.8856
Ionic High	Control	2.66667	0.977	-5.3289	10.6622
	Ionic Maximum	12.44333*	0.001	4.4478	20.4389
	Weathered Low	-2.00000	0.997	-9.9956	5.9956
	Sulphidized Low, 120 nm	-0.44333	1.000	-8.4389	7.5522
	Sulphidized Low, 160 nm	2.33333	0.991	-5.6622	10.3289
	Ionic Low	0.66667	1.000	-7.3289	8.6622
	PVP Low	1.22000	1.000	-6.7756	9.2156
	PVP High	2.55667	0.983	-5.4389	10.5522
	Uncoated Low	2.22000	0.994	-5.7756	10.2156
	Uncoated High	-1.44333	1.000	-9.4389	6.5522
PVP Low	Control	1.44667	1.000	-6.5489	9.4422
	Ionic Maximum	11.22333*	0.002	3.2278	19.2189
	Weathered Low	-3.22000	0.924	-11.2156	4.7756
	Sulphidized Low, 120 nm	-1.66333	0.999	-9.6589	6.3322
	Sulphidized Low, 160 nm	1.11333	1.000	-6.8822	9.1089
	Ionic Low	-0.55333	1.000	-8.5489	7.4422
	Ionic High	-1.22000	1.000	-9.2156	6.7756
	PVP High	1.33667	1.000	-6.6589	9.3322
	Uncoated Low	1.00000	1.000	-6.9956	8.9956
	Uncoated High	-2.66333	0.977	-10.6589	5.3322
PVP High	Control	0.11000	1.000	-7.8856	8.1056
	Ionic Maximum	9.88667*	0.008	1.8911	17.8822
	Weathered Low	-4.55667	0.628	-12.5522	3.4389
	Sulphidized Low, 120 nm	-3.00000	0.950	-10.9956	4.9956
	Sulphidized Low, 160 nm	-0.22333	1.000	-8.2189	7.7722
	Ionic Low	-1.89000	0.998	-9.8856	6.1056
	Ionic High	-2.55667	0.983	-10.5522	5.4389
	PVP Low	-1.33667	1.000	-9.3322	6.6589
	Uncoated Low	-0.33667	1.000	-8.3322	7.6589
	Uncoated High	-4.00000	0.775	-11.9956	3.9956
Uncoated Low	Control	0.44667	1.000	-7.5489	8.4422
	Ionic Maximum	10.22333*	0.006	2.2278	18.2189
	Weathered Low	-4.22000	0.719	-12.2156	3.7756
	Sulphidized Low, 120 nm	-2.66333	0.977	-10.6589	5.3322
	Sulphidized Low, 160 nm	0.11333	1.000	-7.8822	8.1089
	Ionic Low	-1.55333	1.000	-9.5489	6.4422

	Ionic High	-2.22000	0.994	-10.2156	5.7756
	PVP Low	-1.00000	1.000	-8.9956	6.9956
	PVP High	0.33667	1.000	-7.6589	8.3322
	Uncoated High	-3.66333	0.850	-11.6589	4.3322
Uncoated High	Control	4.11000	0.747	-3.8856	12.1056
	Ionic Maximum	13.88667*	0.000	5.8911	21.8822
	Weathered Low	-0.55667	1.000	-8.5522	7.4389
	Sulphidized Low, 120 nm	1.00000	1.000	-6.9956	8.9956
	Sulphidized Low, 160 nm	3.77667	0.826	-4.2189	11.7722
	Ionic Low	2.11000	0.996	-5.8856	10.1056
	Ionic High	1.44333	1.000	-6.5522	9.4389
	PVP Low	2.66333	0.977	-5.3322	10.6589
	PVP High	4.00000	0.775	-3.9956	11.9956
	Uncoated Low	3.66333	0.850	-4.3322	11.6589

Table D.28: Tukey test subset treatment groups for Month 2 richness

Treatment	N	Subsets	
		1	2
Ionic Maximum	3	4.8900	
Control	3		14.6667
PVP High	3		14.7767
Sulphidized Low, 160 nm	3		15.0000
Uncoated Low	3		15.1133
PVP Low	3		16.1133
Ionic Low	3		16.6667
Ionic High	3		17.3333
Sulphidized Low, 120 nm	3		17.7767
Uncoated High	3		18.7767
Weathered Low	3		19.3333
Significance		1.000	0.597

Table D.29: Multiple comparisons for richness Month 3 treatments using Tukey's test

Treatment (I)	Treatment (J)	Mean Difference (I-J)	Significance	95% Confidence Interval	
				Lower Bound	Upper Bound
Control	Ionic Maximum	11.99667*	0.008	2.2629	21.7304
	Weathered Low	-2.33333	0.998	-12.0671	7.4004
	Sulphidized Low, 120 nm	-0.66667	1.000	-10.4004	9.0671
	Sulphidized Low, 160 nm	4.55333	0.834	-5.1804	14.2871
	Ionic Low	1.33333	1.000	-8.4004	11.0671
	Ionic High	3.11000	0.983	-6.6237	12.8437
	PVP Low	3.11000	0.983	-6.6237	12.8437
	PVP High	1.00000	1.000	-8.7337	10.7337
	Uncoated Low	1.77667	1.000	-7.9571	11.5104
	Uncoated High	1.77667	1.000	-7.9571	11.5104
Ionic Maximum	Control	-11.99667*	0.008	-21.7304	-2.2629
	Weathered Low	-14.33000*	0.001	-24.0637	-4.5963
	Sulphidized Low, 120 nm	-12.66333*	0.005	-22.3971	-2.9296
	Sulphidized Low, 160 nm	-7.44333	0.247	-17.1771	2.2904
	Ionic Low	-10.66333*	0.024	-20.3971	-0.9296
	Ionic High	-8.88667	0.094	-18.6204	0.8471
	PVP Low	-8.88667	0.094	-18.6204	0.8471
	PVP High	-10.99667*	0.018	-20.7304	-1.2629
	Uncoated Low	-10.22000*	0.034	-19.9537	-0.4863
	Uncoated High	-10.22000*	0.034	-19.9537	-0.4863
Weathered Low	Control	2.33333	0.998	-7.4004	12.0671
	Ionic Maximum	14.33000*	0.001	4.5963	24.0637
	Sulphidized Low, 120 nm	1.66667	1.000	-8.0671	11.4004
	Sulphidized Low, 160 nm	6.88667	0.340	-2.8471	16.6204
	Ionic Low	3.66667	0.949	-6.0671	13.4004
	Ionic High	5.44333	0.651	-4.2904	15.1771
	PVP Low	5.44333	0.651	-4.2904	15.1771
	PVP High	3.33333	0.972	-6.4004	13.0671
	Uncoated Low	4.11000	0.901	-5.6237	13.8437
	Uncoated High	4.11000	0.901	-5.6237	13.8437
Sulphidized Low, 120 nm	Control	0.66667	1.000	-9.0671	10.4004
	Ionic Maximum	12.66333*	0.005	2.9296	22.3971
	Weathered Low	-1.66667	1.000	-11.4004	8.0671
	Sulphidized Low, 160 nm	5.22000	0.701	-4.5137	14.9537
	Ionic Low	2.00000	0.999	-7.7337	11.7337

	Ionic High	3.77667	0.939	-5.9571	13.5104
	PVP Low	3.77667	0.939	-5.9571	13.5104
	PVP High	1.66667	1.000	-8.0671	11.4004
	Uncoated Low	2.44333	0.997	-7.2904	12.1771
	Uncoated High	2.44333	0.997	-7.2904	12.1771
Sulphidized Low, 160 nm	Control	-4.55333	0.834	-14.2871	5.1804
	Ionic Maximum	7.44333	0.247	-2.2904	17.1771
	Weathered Low	-6.88667	0.340	-16.6204	2.8471
	Sulphidized Low, 120 nm	-5.22000	0.701	-14.9537	4.5137
	Ionic Low	-3.22000	0.978	-12.9537	6.5137
	Ionic High	-1.44333	1.000	-11.1771	8.2904
	PVP Low	-1.44333	1.000	-11.1771	8.2904
	PVP High	-3.55333	0.958	-13.2871	6.1804
	Uncoated Low	-2.77667	0.992	-12.5104	6.9571
	Uncoated High	-2.77667	0.992	-12.5104	6.9571
Ionic Low	Control	-1.33333	1.000	-11.0671	8.4004
	Ionic Maximum	10.66333*	0.024	0.9296	20.3971
	Weathered Low	-3.66667	0.949	-13.4004	6.0671
	Sulphidized Low, 120 nm	-2.00000	0.999	-11.7337	7.7337
	Sulphidized Low, 160 nm	3.22000	0.978	-6.5137	12.9537
	Ionic High	1.77667	1.000	-7.9571	11.5104
	PVP Low	1.77667	1.000	-7.9571	11.5104
	PVP High	-0.33333	1.000	-10.0671	9.4004
	Uncoated Low	0.44333	1.000	-9.2904	10.1771
	Uncoated High	0.44333	1.000	-9.2904	10.1771
Ionic High	Control	-3.11000	0.983	-12.8437	6.6237
	Ionic Maximum	8.88667	0.094	-0.8471	18.6204
	Weathered Low	-5.44333	0.651	-15.1771	4.2904
	Sulphidized Low, 120 nm	-3.77667	0.939	-13.5104	5.9571
	Sulphidized Low, 160 nm	1.44333	1.000	-8.2904	11.1771
	Ionic Low	-1.77667	1.000	-11.5104	7.9571
	PVP Low	0.00000	1.000	-9.7337	9.7337
	PVP High	-2.11000	0.999	-11.8437	7.6237
	Uncoated Low	-1.33333	1.000	-11.0671	8.4004
	Uncoated High	-1.33333	1.000	-11.0671	8.4004
PVP Low	Control	-3.11000	0.983	-12.8437	6.6237
	Ionic Maximum	8.88667	0.094	-0.8471	18.6204
	Weathered Low	-5.44333	0.651	-15.1771	4.2904
	Sulphidized Low, 120 nm	-3.77667	0.939	-13.5104	5.9571

	Sulphidized Low, 160 nm	1.44333	1.000	-8.2904	11.1771
	Ionic Low	-1.77667	1.000	-11.5104	7.9571
	Ionic High	0.00000	1.000	-9.7337	9.7337
	PVP High	-2.11000	0.999	-11.8437	7.6237
	Uncoated Low	-1.33333	1.000	-11.0671	8.4004
	Uncoated High	-1.33333	1.000	-11.0671	8.4004
PVP High	Control	-1.00000	1.000	-10.7337	8.7337
	Ionic Maximum	10.99667*	0.018	1.2629	20.7304
	Weathered Low	-3.33333	0.972	-13.0671	6.4004
	Sulphidized Low, 120 nm	-1.66667	1.000	-11.4004	8.0671
	Sulphidized Low, 160 nm	3.55333	0.958	-6.1804	13.2871
	Ionic Low	0.33333	1.000	-9.4004	10.0671
	Ionic High	2.11000	0.999	-7.6237	11.8437
	PVP Low	2.11000	0.999	-7.6237	11.8437
	Uncoated Low	0.77667	1.000	-8.9571	10.5104
	Uncoated High	0.77667	1.000	-8.9571	10.5104
Uncoated Low	Control	-1.77667	1.000	-11.5104	7.9571
	Ionic Maximum	10.22000*	0.034	0.4863	19.9537
	Weathered Low	-4.11000	0.901	-13.8437	5.6237
	Sulphidized Low, 120 nm	-2.44333	0.997	-12.1771	7.2904
	Sulphidized Low, 160 nm	2.77667	0.992	-6.9571	12.5104
	Ionic Low	-0.44333	1.000	-10.1771	9.2904
	Ionic High	1.33333	1.000	-8.4004	11.0671
	PVP Low	1.33333	1.000	-8.4004	11.0671
	PVP High	-0.77667	1.000	-10.5104	8.9571
	Uncoated High	0.00000	1.000	-9.7337	9.7337
Uncoated High	Control	-1.77667	1.000	-11.5104	7.9571
	Ionic Maximum	10.22000*	0.034	0.4863	19.9537
	Weathered Low	-4.11000	0.901	-13.8437	5.6237
	Sulphidized Low, 120 nm	-2.44333	0.997	-12.1771	7.2904
	Sulphidized Low, 160 nm	2.77667	0.992	-6.9571	12.5104
	Ionic Low	-0.44333	1.000	-10.1771	9.2904
	Ionic High	1.33333	1.000	-8.4004	11.0671
	PVP Low	1.33333	1.000	-8.4004	11.0671
	PVP High	-0.77667	1.000	-10.5104	8.9571
	Uncoated Low	0.00000	1.000	-9.7337	9.7337

Table D.30: Tukey test subset treatment groups for Month 3 richness

Treatment	N	Subsets	
		1	2
Ionic Maximum	3	5.7800	
Sulphidized Low, 160 nm	3	13.2233	13.2233
Ionic High	3	14.6667	14.6667
PVP Low	3	14.6667	14.6667
Uncoated Low	3		16.0000
Uncoated High	3		16.0000
Ionic Low	3		16.4433
PVP High	3		16.7767
Control	3		17.7767
Sulphidized Low, 120 nm	3		18.4433
Weathered Low	3		20.1100
Significance		0.094	0.340

Table D.31: One-way ANOVA for Month 1 Guilds

		Sum of Squares	DOF	Mean Square	F	Significance
Carbohydrates	Between Groups	0.128	10	0.013	2.137	0.066
	Within Groups	0.132	22	0.006		
	Total	0.260	32			
Polymers	Between Groups	0.009	10	0.001	5.997	0.000
	Within Groups	0.003	22	0.000		
	Total	0.013	32			
Carboxylic acids	Between Groups	0.031	10	0.003	2.263	0.053
	Within Groups	0.030	22	0.001		
	Total	0.062	32			
Amino acids	Between Groups	0.013	10	0.001	2.801	0.021
	Within Groups	0.010	22	0.000		
	Total	0.022	32			
Amides/ amides	Between Groups	0.001	10	0.000	1.286	0.297
	Within Groups	0.002	22	0.000		
	Total	0.003	32			
Root Exudates	Between Groups	0.069	10	0.007	2.488	0.036
	Within Groups	0.061	22	0.003		
	Total	0.130	32			

Table D.32: Multiple comparisons for polymers Month 1 treatments using Tukey's test

Treatment (I)	Treatment (J)	Mean Difference (I-J)	Significance	95% Confidence Interval	
				Lower Bound	Upper Bound
Control	Ionic Maximum	0.04599*	0.006	0.0099	0.0821
	Weathered Low	-0.01275	0.966	-0.0489	0.0234
	Sulphidized Low, 120 nm	-0.01066	0.990	-0.0468	0.0255
	Sulphidized Low, 160 nm	-0.00193	1.000	-0.0380	0.0342
	Ionic Low	-0.00449	1.000	-0.0406	0.0316
	Ionic High	-0.01054	0.991	-0.0467	0.0256
	PVP Low	-0.00233	1.000	-0.0385	0.0338
	PVP High	-0.02101	0.602	-0.0571	0.0151
	Uncoated Low	-0.01151	0.983	-0.0476	0.0246
	Uncoated High	-0.00962	0.996	-0.0457	0.0265
Ionic Maximum	Control	-0.04599*	0.006	-0.0821	-0.0099
	Weathered Low	-0.05874*	0.000	-0.0949	-0.0226
	Sulphidized Low, 120 nm	-0.05665*	0.001	-0.0928	-0.0205
	Sulphidized Low, 160 nm	-0.04792*	0.004	-0.0840	-0.0118
	Ionic Low	-0.05048*	0.002	-0.0866	-0.0144
	Ionic High	-0.05653*	0.001	-0.0926	-0.0204
	PVP Low	-0.04832*	0.003	-0.0844	-0.0122
	PVP High	-0.06700*	0.000	-0.1031	-0.0309
	Uncoated Low	-0.05749*	0.000	-0.0936	-0.0214
	Uncoated High	-0.05561*	0.001	-0.0917	-0.0195
Weathered Low	Control	0.01275	0.966	-0.0234	0.0489
	Ionic Maximum	0.05874*	0.000	0.0226	0.0949
	Sulphidized Low, 120 nm	0.00209	1.000	-0.0340	0.0382
	Sulphidized Low, 160 nm	0.01082	0.989	-0.0253	0.0469
	Ionic Low	0.00826	0.999	-0.0279	0.0444
	Ionic High	0.00222	1.000	-0.0339	0.0383
	PVP Low	0.01042	0.992	-0.0257	0.0465
	PVP High	-0.00825	0.999	-0.0444	0.0279
	Uncoated Low	0.00125	1.000	-0.0349	0.0374
	Uncoated High	0.00313	1.000	-0.0330	0.0392
Sulphidized Low, 120 nm	Control	0.01066	0.990	-0.0255	0.0468
	Ionic Maximum	0.05665*	0.001	0.0205	0.0928
	Weathered Low	-0.00209	1.000	-0.0382	0.0340
	Sulphidized Low, 160 nm	0.00873	0.998	-0.0274	0.0448
	Ionic Low	0.00617	1.000	-0.0299	0.0423

	Ionic High	0.00012	1.000	-0.0360	0.0362
	PVP Low	0.00833	0.999	-0.0278	0.0444
	PVP High	-0.01035	0.992	-0.0465	0.0258
	Uncoated Low	-0.00085	1.000	-0.0370	0.0353
	Uncoated High	0.00104	1.000	-0.0351	0.0372
Sulphidized Low, 160 nm	Control	0.00193	1.000	-0.0342	0.0380
	Ionic Maximum	0.04792*	0.004	0.0118	0.0840
	Weathered Low	-0.01082	0.989	-0.0469	0.0253
	Sulphidized Low, 120 nm	-0.00873	0.998	-0.0448	0.0274
	Ionic Low	-0.00256	1.000	-0.0387	0.0336
	Ionic High	-0.00861	0.998	-0.0447	0.0275
	PVP Low	-0.00041	1.000	-0.0365	0.0357
	PVP High	-0.01908	0.718	-0.0552	0.0170
	Uncoated Low	-0.00958	0.996	-0.0457	0.0265
	Uncoated High	-0.0077	0.999	-0.0438	0.0284
Ionic Low	Control	0.00449	1.000	-0.0316	0.0406
	Ionic Maximum	0.05048*	0.002	0.0144	0.0866
	Weathered Low	-0.00826	0.999	-0.0444	0.0279
	Sulphidized Low, 120 nm	-0.00617	1.000	-0.0423	0.0299
	Sulphidized Low, 160 nm	0.00256	1.000	-0.0336	0.0387
	Ionic High	-0.00605	1.000	-0.0422	0.0301
	PVP Low	0.00216	1.000	-0.0340	0.0383
	PVP High	-0.01652	0.851	-0.0526	0.0196
	Uncoated Low	-0.00701	1.000	-0.0431	0.0291
	Uncoated High	-0.00513	1.000	-0.0413	0.0310
Ionic High	Control	0.01054	0.991	-0.0256	0.0467
	Ionic Maximum	0.05653*	0.001	0.0204	0.0926
	Weathered Low	-0.00222	1.000	-0.0383	0.0339
	Sulphidized Low, 120 nm	-0.00012	1.000	-0.0362	0.0360
	Sulphidized Low, 160 nm	0.00861	0.998	-0.0275	0.0447
	Ionic Low	0.00605	1.000	-0.0301	0.0422
	PVP Low	0.0082	0.999	-0.0279	0.0443
	PVP High	-0.01047	0.991	-0.0466	0.0256
	Uncoated Low	-0.00097	1.000	-0.0371	0.0351
	Uncoated High	0.00091	1.000	-0.0352	0.0370
PVP Low	Control	0.00233	1.000	-0.0338	0.0385
	Ionic Maximum	0.04832*	0.003	0.0122	0.0844
	Weathered Low	-0.01042	0.992	-0.0465	0.0257
	Sulphidized Low, 120 nm	-0.00833	0.999	-0.0444	0.0278

	Sulphidized Low, 160 nm	0.00041	1.000	-0.0357	0.0365
	Ionic Low	-0.00216	1.000	-0.0383	0.0340
	Ionic High	-0.0082	0.999	-0.0443	0.0279
	PVP High	-0.01867	0.741	-0.0548	0.0174
	Uncoated Low	-0.00917	0.997	-0.0453	0.0269
	Uncoated High	-0.00729	1.000	-0.0434	0.0288
PVP High	Control	0.02101	0.602	-0.0151	0.0571
	Ionic Maximum	0.06700*	0.000	0.0309	0.1031
	Weathered Low	0.00825	0.999	-0.0279	0.0444
	Sulphidized Low, 120 nm	0.01035	0.992	-0.0258	0.0465
	Sulphidized Low, 160 nm	0.01908	0.718	-0.0170	0.0552
	Ionic Low	0.01652	0.851	-0.0196	0.0526
	Ionic High	0.01047	0.991	-0.0256	0.0466
	PVP Low	0.01867	0.741	-0.0174	0.0548
	Uncoated Low	0.0095	0.996	-0.0266	0.0456
	Uncoated High	0.01138	0.984	-0.0247	0.0475
Uncoated Low	Control	0.01151	0.983	-0.0246	0.0476
	Ionic Maximum	0.05749*	0.000	0.0214	0.0936
	Weathered Low	-0.00125	1.000	-0.0374	0.0349
	Sulphidized Low, 120 nm	0.00085	1.000	-0.0353	0.0370
	Sulphidized Low, 160 nm	0.00958	0.996	-0.0265	0.0457
	Ionic Low	0.00701	1.000	-0.0291	0.0431
	Ionic High	0.00097	1.000	-0.0351	0.0371
	PVP Low	0.00917	0.997	-0.0269	0.0453
	PVP High	-0.0095	0.996	-0.0456	0.0266
	Uncoated High	0.00188	1.000	-0.0342	0.0380
Uncoated High	Control	0.00962	0.996	-0.0265	0.0457
	Ionic Maximum	.05561*	0.001	0.0195	0.0917
	Weathered Low	-0.00313	1.000	-0.0392	0.0330
	Sulphidized Low, 120 nm	-0.00104	1.000	-0.0372	0.0351
	Sulphidized Low, 160 nm	0.0077	0.999	-0.0284	0.0438
	Ionic Low	0.00513	1.000	-0.0310	0.0413
	Ionic High	-0.00091	1.000	-0.0370	0.0352
	PVP Low	0.00729	1.000	-0.0288	0.0434
	PVP High	-0.01138	0.984	-0.0475	0.0247
	Uncoated Low	-0.00188	1.000	-0.0380	0.0342

Table D.33: Tukey test subset treatment groups for polymers in Month 1

Treatment	N	Subsets	
		1	2
Ionic Maximum	3	0.0312	
Control	3		0.0772
Sulphidized Low, 160 nm	3		0.0791
PVP Low	3		0.0795
Ionic Low	3		0.0817
Uncoated High	3		0.0868
Ionic High	3		0.0877
Sulphidized Low, 120 nm	3		0.0879
Uncoated Low	3		0.0887
Weathered Low	3		0.0900
PVP High	3		0.0982
Significance		1.000	0.602

Table D.34: Multiple comparisons for amino acids Month 1 treatments using Tukey's test

Treatment (I)	Treatment (J)	Mean Difference (I-J)	Significance	95% Confidence Interval	
				Lower Bound	Upper Bound
Control	Ionic Maximum	0.06780*	0.024	0.0061	0.1296
	Weathered Low	0.00669	1.000	-0.0551	0.0684
	Sulphidized Low, 120 nm	0.00852	1.000	-0.0532	0.0703
	Sulphidized Low, 160 nm	0.03034	0.792	-0.0314	0.0921
	Ionic Low	0.01971	0.983	-0.0420	0.0815
	Ionic High	0.01849	0.989	-0.0433	0.0802
	PVP Low	0.00725	1.000	-0.0545	0.0690
	PVP High	0.00433	1.000	-0.0574	0.0661
	Uncoated Low	0.01418	0.999	-0.0476	0.0759
	Uncoated High	-0.00971	1.000	-0.0715	0.0520
Ionic Maximum	Control	-0.06780*	0.024	-0.1296	-0.0061
	Weathered Low	-0.06111	0.054	-0.1229	0.0006
	Sulphidized Low, 120 nm	-0.05928	0.067	-0.1210	0.0025
	Sulphidized Low, 160 nm	-0.03746	0.546	-0.0992	0.0243
	Ionic Low	-0.04809	0.227	-0.1098	0.0137
	Ionic High	-0.04932	0.201	-0.1111	0.0124
	PVP Low	-0.06055	0.058	-0.1223	0.0012
	PVP High	-0.06348*	0.041	-0.1252	-0.0017
	Uncoated Low	-0.05362	0.128	-0.1154	0.0081
	Uncoated High	-0.07751*	0.007	-0.1393	-0.0158

Weathered Low	Control	-0.00669	1.000	-0.0684	0.0551
	Ionic Maximum	0.06111	0.054	-0.0006	0.1229
	Sulphidized Low, 120 nm	0.00183	1.000	-0.0599	0.0636
	Sulphidized Low, 160 nm	0.02366	0.944	-0.0381	0.0854
	Ionic Low	0.01302	0.999	-0.0487	0.0748
	Ionic High	0.01180	1.000	-0.0500	0.0736
	PVP Low	0.00057	1.000	-0.0612	0.0623
	PVP High	-0.00236	1.000	-0.0641	0.0594
	Uncoated Low	0.00749	1.000	-0.0543	0.0692
	Uncoated High	-0.01640	0.996	-0.0781	0.0454
Sulphidized Low, 120 nm	Control	-0.00852	1.000	-0.0703	0.0532
	Ionic Maximum	0.05928	0.067	-0.0025	0.1210
	Weathered Low	-0.00183	1.000	-0.0636	0.0599
	Sulphidized Low, 160 nm	0.02182	0.966	-0.0399	0.0836
	Ionic Low	0.01119	1.000	-0.0506	0.0729
	Ionic High	0.00997	1.000	-0.0518	0.0717
	PVP Low	-0.00127	1.000	-0.0630	0.0605
	PVP High	-0.00419	1.000	-0.0659	0.0576
	Uncoated Low	0.00566	1.000	-0.0561	0.0674
	Uncoated High	-0.01823	0.990	-0.0800	0.0435
Sulphidized Low, 160 nm	Control	-0.03034	0.792	-0.0921	0.0314
	Ionic Maximum	0.03746	0.546	-0.0243	0.0992
	Weathered Low	-0.02366	0.944	-0.0854	0.0381
	Sulphidized Low, 120 nm	-0.02182	0.966	-0.0836	0.0399
	Ionic Low	-0.01063	1.000	-0.0724	0.0511
	Ionic High	-0.01186	1.000	-0.0736	0.0499
	PVP Low	-0.02309	0.951	-0.0848	0.0387
	PVP High	-0.02602	0.903	-0.0878	0.0357
	Uncoated Low	-0.01616	0.996	-0.0779	0.0456
	Uncoated High	-0.04005	0.456	-0.1018	0.0217
Ionic Low	Control	-0.01971	0.983	-0.0815	0.0420
	Ionic Maximum	0.04809	0.227	-0.0137	0.1098
	Weathered Low	-0.01302	0.999	-0.0748	0.0487
	Sulphidized Low, 120 nm	-0.01119	1.000	-0.0729	0.0506
	Sulphidized Low, 160 nm	0.01063	1.000	-0.0511	0.0724
	Ionic High	-0.00122	1.000	-0.0630	0.0605
	PVP Low	-0.01246	1.000	-0.0742	0.0493
	PVP High	-0.01538	0.997	-0.0771	0.0464
	Uncoated Low	-0.00553	1.000	-0.0673	0.0562
	Uncoated High	-0.02942	0.819	-0.0912	0.0323

Ionic High	Control	-0.01849	0.989	-0.0802	0.0433
	Ionic Maximum	0.04932	0.201	-0.0124	0.1111
	Weathered Low	-0.01180	1.000	-0.0736	0.0500
	Sulphidized Low, 120 nm	-0.00997	1.000	-0.0717	0.0518
	Sulphidized Low, 160 nm	0.01186	1.000	-0.0499	0.0736
	Ionic Low	0.00122	1.000	-0.0605	0.0630
	PVP Low	-0.01123	1.000	-0.0730	0.0505
	PVP High	-0.01416	0.999	-0.0759	0.0476
	Uncoated Low	-0.00431	1.000	-0.0661	0.0574
	Uncoated High	-0.02820	0.852	-0.0899	0.0336
PVP Low	Control	-0.00725	1.000	-0.0690	0.0545
	Ionic Maximum	0.06055	0.058	-0.0012	0.1223
	Weathered Low	-0.00057	1.000	-0.0623	0.0612
	Sulphidized Low, 120 nm	0.00127	1.000	-0.0605	0.0630
	Sulphidized Low, 160 nm	0.02309	0.951	-0.0387	0.0848
	Ionic Low	0.01246	1.000	-0.0493	0.0742
	Ionic High	0.01123	1.000	-0.0505	0.0730
	PVP High	-0.00293	1.000	-0.0647	0.0588
	Uncoated Low	0.00692	1.000	-0.0548	0.0687
	Uncoated High	-0.01696	0.994	-0.0787	0.0448
PVP High	Control	-0.00433	1.000	-0.0661	0.0574
	Ionic Maximum	0.06348*	0.041	0.0017	0.1252
	Weathered Low	0.00236	1.000	-0.0594	0.0641
	Sulphidized Low, 120 nm	0.00419	1.000	-0.0576	0.0659
	Sulphidized Low, 160 nm	0.02602	0.903	-0.0357	0.0878
	Ionic Low	0.01538	0.997	-0.0464	0.0771
	Ionic High	0.01416	0.999	-0.0476	0.0759
	PVP Low	0.00293	1.000	-0.0588	0.0647
	Uncoated Low	0.00985	1.000	-0.0519	0.0716
	Uncoated High	-0.01404	0.999	-0.0758	0.0477
Uncoated Low	Control	-0.01418	0.999	-0.0759	0.0476
	Ionic Maximum	0.05362	0.128	-0.0081	0.1154
	Weathered Low	-0.00749	1.000	-0.0692	0.0543
	Sulphidized Low, 120 nm	-0.00566	1.000	-0.0674	0.0561
	Sulphidized Low, 160 nm	0.01616	0.996	-0.0456	0.0779
	Ionic Low	0.00553	1.000	-0.0562	0.0673
	Ionic High	0.00431	1.000	-0.0574	0.0661
	PVP Low	-0.00692	1.000	-0.0687	0.0548

	PVP High	-0.00985	1.000	-0.0716	0.0519
	Uncoated High	-0.02389	0.940	-0.0856	0.0379
Uncoated High	Control	0.00971	1.000	-0.0520	0.0715
	Ionic Maximum	0.07751*	0.007	0.0158	0.1393
	Weathered Low	0.01640	0.996	-0.0454	0.0781
	Sulphidized Low, 120 nm	0.01823	0.990	-0.0435	0.0800
	Sulphidized Low, 160 nm	0.04005	0.456	-0.0217	0.1018
	Ionic Low	0.02942	0.819	-0.0323	0.0912
	Ionic High	0.02820	0.852	-0.0336	0.0899
	PVP Low	0.01696	0.994	-0.0448	0.0787
	PVP High	0.01404	0.999	-0.0477	0.0758
	Uncoated Low	0.02389	0.940	-0.0379	0.0856

Table D.35: Post-hoc Tukey test subset treatment groups for amino acids in Month 1

Treatment	N	Subsets	
		1	2
Ionic Maximum	3	0.0548	
Sulphidized Low, 160 nm	3	0.0923	0.0923
Ionic Low	3	0.1029	0.1029
Ionic High	3	0.1042	0.1042
Uncoated Low	3	0.1085	0.1085
Sulphidized Low, 120 nm	3	0.1141	0.1141
PVP Low	3	0.1154	0.1154
Weathered Low	3	0.1159	0.1159
PVP High	3		0.1183
Control	3		0.1226
Uncoated High	3		0.1323
Significance		0.054	0.456

Table D.36: Multiple comparisons for root exudates Month 1 treatments using Tukey's test

Treatment (I)	Treatment (J)	Mean Difference (I-J)	Significance	95% Confidence Interval	
				Lower Bound	Upper Bound
Control	Ionic Maximum	0.11696	0.253	-0.0368	0.2708
	Weathered Low	-0.00131	1.000	-0.1551	0.1525
	Sulphidized Low, 120 nm	-0.04135	0.995	-0.1951	0.1124
	Sulphidized Low, 160 nm	0.04306	0.993	-0.1107	0.1969
	Ionic Low	0.02517	1.000	-0.1286	0.179

	Ionic High	0.03814	0.997	-0.1157	0.1919
	PVP Low	-0.00963	1.000	-0.1634	0.1442
	PVP High	-0.04697	0.987	-0.2008	0.1068
	Uncoated Low	0.02426	1.000	-0.1295	0.1781
	Uncoated High	-0.04263	0.994	-0.1964	0.1112
Ionic Maximum	Control	-0.11696	0.253	-0.2708	0.0368
	Weathered Low	-0.11827	0.241	-0.2721	0.0355
	Sulphidized Low, 120 nm	-0.15831*	0.040	-0.3121	-0.0045
	Sulphidized Low, 160 nm	-0.07390	0.812	-0.2277	0.0799
	Ionic Low	-0.09179	0.568	-0.2456	0.062
	Ionic High	-0.07882	0.750	-0.2326	0.075
	PVP Low	-0.12659	0.172	-0.2804	0.0272
	PVP High	-0.16393*	0.030	-0.3177	-0.0101
	Uncoated Low	-0.09270	0.555	-0.2465	0.0611
	Uncoated High	-0.15959*	0.038	-0.3134	-0.0058
Weathered Low	Control	0.00131	1.000	-0.1525	0.1551
	Ionic Maximum	0.11827	0.241	-0.0355	0.2721
	Sulphidized Low, 120 nm	-0.04004	0.996	-0.1938	0.1137
	Sulphidized Low, 160 nm	0.04437	0.992	-0.1094	0.1982
	Ionic Low	0.02648	1.000	-0.1273	0.1803
	Ionic High	0.03945	0.997	-0.1143	0.1932
	PVP Low	-0.00833	1.000	-0.1621	0.1455
	PVP High	-0.04566	0.990	-0.1995	0.1081
	Uncoated Low	0.02557	1.000	-0.1282	0.1794
	Uncoated High	-0.04133	0.995	-0.1951	0.1125
Sulphidized Low, 120 nm	Control	0.04135	0.995	-0.1124	0.1951
	Ionic Maximum	0.15831*	0.040	0.0045	0.3121
	Weathered Low	0.04004	0.996	-0.1137	0.1938
	Sulphidized Low, 160 nm	0.08441	0.674	-0.0694	0.2382
	Ionic Low	0.06652	0.888	-0.0873	0.2203
	Ionic High	0.07949	0.742	-0.0743	0.2333
	PVP Low	0.03172	0.999	-0.1221	0.1855
	PVP High	-0.00562	1.000	-0.1594	0.1482
	Uncoated Low	0.06561	0.896	-0.0882	0.2194
	Uncoated High	-0.00128	1.000	-0.1551	0.1525
Sulphidized Low, 160 nm	Control	-0.04306	0.993	-0.1969	0.1107
	Ionic Maximum	0.07390	0.812	-0.0799	0.2277
	Weathered Low	-0.04437	0.992	-0.1982	0.1094
	Sulphidized Low, 120 nm	-0.08441	0.674	-0.2382	0.0694
	Ionic Low	-0.01789	1.000	-0.1717	0.1359

	Ionic High	-0.00492	1.000	-0.1587	0.1489
	PVP Low	-0.05270	0.972	-0.2065	0.1011
	PVP High	-0.09003	0.593	-0.2438	0.0638
	Uncoated Low	-0.01880	1.000	-0.1726	0.135
	Uncoated High	-0.08570	0.656	-0.2395	0.0681
Ionic Low	Control	-0.02517	1.000	-0.179	0.1286
	Ionic Maximum	0.09179	0.568	-0.062	0.2456
	Weathered Low	-0.02648	1.000	-0.1803	0.1273
	Sulphidized Low, 120 nm	-0.06652	0.888	-0.2203	0.0873
	Sulphidized Low, 160 nm	0.01789	1.000	-0.1359	0.1717
	Ionic High	0.01297	1.000	-0.1408	0.1668
	PVP Low	-0.03481	0.999	-0.1886	0.119
	PVP High	-0.07214	0.832	-0.2259	0.0816
	Uncoated Low	-0.00091	1.000	-0.1547	0.1529
	Uncoated High	-0.06781	0.876	-0.2216	0.086
Ionic High	Control	-0.03814	0.997	-0.1919	0.1157
	Ionic Maximum	0.07882	0.750	-0.075	0.2326
	Weathered Low	-0.03945	0.997	-0.1932	0.1143
	Sulphidized Low, 120 nm	-0.07949	0.742	-0.2333	0.0743
	Sulphidized Low, 160 nm	0.00492	1.000	-0.1489	0.1587
	Ionic Low	-0.01297	1.000	-0.1668	0.1408
	PVP Low	-0.04777	0.986	-0.2016	0.106
	PVP High	-0.08511	0.664	-0.2389	0.0687
	Uncoated Low	-0.01388	1.000	-0.1677	0.1399
	Uncoated High	-0.08077	0.724	-0.2346	0.073
PVP Low	Control	0.00963	1.000	-0.1442	0.1634
	Ionic Maximum	0.12659	0.172	-0.0272	0.2804
	Weathered Low	0.00833	1.000	-0.1455	0.1621
	Sulphidized Low, 120 nm	-0.03172	0.999	-0.1855	0.1221
	Sulphidized Low, 160 nm	0.05270	0.972	-0.1011	0.2065
	Ionic Low	0.03481	0.999	-0.119	0.1886
	Ionic High	0.04777	0.986	-0.106	0.2016
	PVP High	-0.03734	0.998	-0.1911	0.1165
	Uncoated Low	0.03390	0.999	-0.1199	0.1877
	Uncoated High	-0.03300	0.999	-0.1868	0.1208
PVP High	Control	0.04697	0.987	-0.1068	0.2008
	Ionic Maximum	0.16393*	0.030	0.0101	0.3177
	Weathered Low	0.04566	0.990	-0.1081	0.1995
	Sulphidized Low, 120 nm	0.00562	1.000	-0.1482	0.1594

	Sulphidized Low, 160 nm	0.09003	0.593	-0.0638	0.2438
	Ionic Low	0.07214	0.832	-0.0816	0.2259
	Ionic High	0.08511	0.664	-0.0687	0.2389
	PVP Low	0.03734	0.998	-0.1165	0.1911
	Uncoated Low	0.07123	0.842	-0.0826	0.225
	Uncoated High	0.00434	1.000	-0.1495	0.1581
Uncoated Low	Control	-0.02426	1.000	-0.1781	0.1295
	Ionic Maximum	0.09270	0.555	-0.0611	0.2465
	Weathered Low	-0.02557	1.000	-0.1794	0.1282
	Sulphidized Low, 120 nm	-0.06561	0.896	-0.2194	0.0882
	Sulphidized Low, 160 nm	0.01880	1.000	-0.135	0.1726
	Ionic Low	0.00091	1.000	-0.1529	0.1547
	Ionic High	0.01388	1.000	-0.1399	0.1677
	PVP Low	-0.03390	0.999	-0.1877	0.1199
	PVP High	-0.07123	0.842	-0.225	0.0826
	Uncoated High	-0.06690	0.885	-0.2207	0.0869
Uncoated High	Control	0.04263	0.994	-0.1112	0.1964
	Ionic Maximum	0.15959*	0.038	0.0058	0.3134
	Weathered Low	0.04133	0.995	-0.1125	0.1951
	Sulphidized Low, 120 nm	0.00128	1.000	-0.1525	0.1551
	Sulphidized Low, 160 nm	0.08570	0.656	-0.0681	0.2395
	Ionic Low	0.06781	0.876	-0.086	0.2216
	Ionic High	0.08077	0.724	-0.073	0.2346
	PVP Low	0.03300	0.999	-0.1208	0.1868
	PVP High	-0.00434	1.000	-0.1581	0.1495
	Uncoated Low	0.06690	0.885	-0.0869	0.2207

Table D.37: Tukey test subset treatment groups for root exudates in Month 1

Treatment	N	Subsets	
		1	2
Ionic Maximum	3	0.0822	
Sulphidized Low, 160 nm	3	0.1561	0.1561
Ionic High	3	0.1610	0.1610
Ionic Low	3	0.1740	0.1740
Uncoated Low	3	0.1749	0.1749
Control	3	0.1992	0.1992
Weathered Low	3	0.2005	0.2005
PVP Low	3	0.2088	0.2088
Sulphidized Low, 120 nm	3		0.2405
Uncoated High	3		0.2418
PVP High	3		0.2461
Significance		0.1720	0.5930

Table D.38: One-way ANOVA for Month 2 Guilds

		Sum of Squares	DOF	Mean Square	F	Significance
Carbohydrates	Between Groups	0.077	10	0.008	1.496	0.206
	Within Groups	0.113	22	0.005		
	Total	0.190	32			
Polymers	Between Groups	0.011	10	0.001	5.280	0.001
	Within Groups	0.005	22	0.000		
	Total	0.015	32			
Carboxylic acids	Between Groups	0.042	10	0.004	2.884	0.018
	Within Groups	0.032	22	0.001		
	Total	0.074	32			
Amino acids	Between Groups	0.024	10	0.002	3.835	0.004
	Within Groups	0.013	22	0.001		
	Total	0.037	32			
Amides/ amides	Between Groups	0.001	10	0.000	1.381	0.252
	Within Groups	0.002	22	0.000		
	Total	0.004	32			
Root Exudates	Between Groups	0.059	10	0.006	2.021	0.081
	Within Groups	0.065	22	0.003		
	Total	0.124	32			

Table D.39: Multiple comparisons for polymers Month 2 treatments using Tukey's test

Treatment (I)	Treatment (J)	Mean Difference (I-J)	Significance	95% Confidence Interval	
				Lower Bound	Upper Bound
Control	Ionic Maximum	0.05301*	0.006	0.0112	0.0948
	Weathered Low	-0.01306	0.985	-0.0548	0.0287
	Sulphidized Low, 120 nm	-0.01277	0.987	-0.0545	0.029
	Sulphidized Low, 160 nm	0.01720	0.914	-0.0246	0.059
	Ionic Low	-0.00249	1.000	-0.0443	0.0393
	Ionic High	-0.00551	1.000	-0.0473	0.0363
	PVP Low	0.00403	1.000	-0.0377	0.0458
	PVP High	-0.00459	1.000	-0.0464	0.0372
	Uncoated Low	-0.00795	1.000	-0.0497	0.0338
	Uncoated High	-0.00792	1.000	-0.0497	0.0338
Ionic Maximum	Control	-0.05301*	0.006	-0.0948	-0.0112
	Weathered Low	-0.06608*	0.000	-0.1078	-0.0243
	Sulphidized Low, 120 nm	-0.06578*	0.000	-0.1076	-0.024
	Sulphidized Low, 160 nm	-0.03581	0.138	-0.0776	0.006
	Ionic Low	-0.05550*	0.004	-0.0973	-0.0137
	Ionic High	-0.05852*	0.002	-0.1003	-0.0168
	PVP Low	-0.04898*	0.013	-0.0907	-0.0072
	PVP High	-0.05760*	0.002	-0.0994	-0.0158
	Uncoated Low	-0.06096*	0.001	-0.1027	-0.0192
	Uncoated High	-0.06094*	0.001	-0.1027	-0.0192
Weathered Low	Control	0.01306	0.985	-0.0287	0.0548
	Ionic Maximum	0.06608*	0.000	0.0243	0.1078
	Sulphidized Low, 120 nm	0.00029	1.000	-0.0415	0.0421
	Sulphidized Low, 160 nm	0.03027	0.310	-0.0115	0.072
	Ionic Low	0.01058	0.997	-0.0312	0.0523
	Ionic High	0.00756	1.000	-0.0342	0.0493
	PVP Low	0.01710	0.917	-0.0247	0.0589
	PVP High	0.00848	1.000	-0.0333	0.0502
	Uncoated Low	0.00512	1.000	-0.0366	0.0469
	Uncoated High	0.00514	1.000	-0.0366	0.0469
Sulphidized Low, 120 nm	Control	0.01277	0.987	-0.029	0.0545
	Ionic Maximum	0.06578*	0.000	0.024	0.1076
	Weathered Low	-0.00029	1.000	-0.0421	0.0415

	Sulphidized Low, 160 nm	0.02997	0.322	-0.0118	0.0717
	Ionic Low	0.01028	0.998	-0.0315	0.052
	Ionic High	0.00727	1.000	-0.0345	0.049
	PVP Low	0.01680	0.925	-0.025	0.0586
	PVP High	0.00818	1.000	-0.0336	0.0499
	Uncoated Low	0.00482	1.000	-0.0369	0.0466
	Uncoated High	0.00485	1.000	-0.0369	0.0466
Sulphidized Low, 160 nm	Control	-0.01720	0.914	-0.059	0.0246
	Ionic Maximum	0.03581	0.138	-0.006	0.0776
	Weathered Low	-0.03027	0.310	-0.072	0.0115
	Sulphidized Low, 120 nm	-0.02997	0.322	-0.0717	0.0118
	Ionic Low	-0.01969	0.828	-0.0615	0.0221
	Ionic High	-0.02271	0.685	-0.0645	0.0191
	PVP Low	-0.01317	0.984	-0.0549	0.0286
	PVP High	-0.02179	0.732	-0.0636	0.02
	Uncoated Low	-0.02515	0.556	-0.0669	0.0166
	Uncoated High	-0.02512	0.558	-0.0669	0.0166
Ionic Low	Control	0.00249	1.000	-0.0393	0.0443
	Ionic Maximum	0.05550*	0.004	0.0137	0.0973
	Weathered Low	-0.01058	0.997	-0.0523	0.0312
	Sulphidized Low, 120 nm	-0.01028	0.998	-0.052	0.0315
	Sulphidized Low, 160 nm	0.01969	0.828	-0.0221	0.0615
	Ionic High	-0.00302	1.000	-0.0448	0.0387
	PVP Low	0.00652	1.000	-0.0352	0.0483
	PVP High	-0.00210	1.000	-0.0439	0.0397
	Uncoated Low	-0.00546	1.000	-0.0472	0.0363
	Uncoated High	-0.00543	1.000	-0.0472	0.0363
Ionic High	Control	0.00551	1.000	-0.0363	0.0473
	Ionic Maximum	0.05852*	0.002	0.0168	0.1003
	Weathered Low	-0.00756	1.000	-0.0493	0.0342
	Sulphidized Low, 120 nm	-0.00727	1.000	-0.049	0.0345
	Sulphidized Low, 160 nm	0.02271	0.685	-0.0191	0.0645
	Ionic Low	0.00302	1.000	-0.0387	0.0448
	PVP Low	0.00954	0.999	-0.0322	0.0513
	PVP High	0.00092	1.000	-0.0408	0.0427
	Uncoated Low	-0.00244	1.000	-0.0442	0.0393
	Uncoated High	-0.00242	1.000	-0.0442	0.0394
PVP Low	Control	-0.00403	1.000	-0.0458	0.0377
	Ionic Maximum	0.04898*	0.013	0.0072	0.0907
	Weathered Low	-0.01710	0.917	-0.0589	0.0247

	Sulphidized Low, 120 nm	-0.01680	0.925	-0.0586	0.025
	Sulphidized Low, 160 nm	0.01317	0.984	-0.0286	0.0549
	Ionic Low	-0.00652	1.000	-0.0483	0.0352
	Ionic High	-0.00954	0.999	-0.0513	0.0322
	PVP High	-0.00862	0.999	-0.0504	0.0331
	Uncoated Low	-0.01198	0.992	-0.0537	0.0298
	Uncoated High	-0.01195	0.992	-0.0537	0.0298
PVP High	Control	0.00459	1.000	-0.0372	0.0464
	Ionic Maximum	0.05760*	0.002	0.0158	0.0994
	Weathered Low	-0.00848	1.000	-0.0502	0.0333
	Sulphidized Low, 120 nm	-0.00818	1.000	-0.0499	0.0336
	Sulphidized Low, 160 nm	0.02179	0.732	-0.02	0.0636
	Ionic Low	0.00210	1.000	-0.0397	0.0439
	Ionic High	-0.00092	1.000	-0.0427	0.0408
	PVP Low	0.00862	0.999	-0.0331	0.0504
	Uncoated Low	-0.00336	1.000	-0.0451	0.0384
	Uncoated High	-0.00333	1.000	-0.0451	0.0384
Uncoated Low	Control	0.00795	1.000	-0.0338	0.0497
	Ionic Maximum	0.06096*	0.001	0.0192	0.1027
	Weathered Low	-0.00512	1.000	-0.0469	0.0366
	Sulphidized Low, 120 nm	-0.00482	1.000	-0.0466	0.0369
	Sulphidized Low, 160 nm	0.02515	0.556	-0.0166	0.0669
	Ionic Low	0.00546	1.000	-0.0363	0.0472
	Ionic High	0.00244	1.000	-0.0393	0.0442
	PVP Low	0.01198	0.992	-0.0298	0.0537
	PVP High	0.00336	1.000	-0.0384	0.0451
	Uncoated High	0.00003	1.000	-0.0417	0.0418
Uncoated High	Control	0.00792	1.000	-0.0338	0.0497
	Ionic Maximum	0.06094*	0.001	0.0192	0.1027
	Weathered Low	-0.00514	1.000	-0.0469	0.0366
	Sulphidized Low, 120 nm	-0.00485	1.000	-0.0466	0.0369
	Sulphidized Low, 160 nm	0.02512	0.558	-0.0166	0.0669
	Ionic Low	0.00543	1.000	-0.0363	0.0472
	Ionic High	0.00242	1.000	-0.0394	0.0442
	PVP Low	0.01195	0.992	-0.0298	0.0537
	PVP High	0.00333	1.000	-0.0384	0.0451
	Uncoated Low	-0.00003	1.000	-0.0418	0.0417

Table D.40: Tukey test subset treatment groups for polymers in Month 2

Treatment	N	Subsets	
		1	2
Ionic Maximum	3	0.0287	
Sulphidized Low, 160 nm	3	0.0645	0.0645
PVP Low	3		0.0777
Control	3		0.0817
Ionic Low	3		0.0842
PVP High	3		0.0863
Ionic High	3		0.0872
Uncoated Low	3		0.0896
Uncoated High	3		0.0896
Sulphidized Low, 120 nm	3		0.0945
Weathered Low	3		0.0948
Significance		0.138	0.310

Table D.41: Multiple comparisons for carboxylic acids Month 2 treatments using Tukey's test

Treatment (I)	Treatment (J)	Mean Difference (I-J)	Significance	95% Confidence Interval	
				Lower Bound	Upper Bound
Control	Ionic Maximum	0.09671	0.130	-0.015	0.2084
	Weathered Low	-0.04089	0.957	-0.1526	0.0708
	Sulphidized Low, 120 nm	-0.03889	0.969	-0.1506	0.0728
	Sulphidized Low, 160 nm	0.00703	1.000	-0.1047	0.1187
	Ionic Low	0.00308	1.000	-0.1086	0.1148
	Ionic High	-0.02572	0.999	-0.1374	0.086
	PVP Low	0.01125	1.000	-0.1005	0.123
	PVP High	0.01686	1.000	-0.0949	0.1286
	Uncoated Low	0.00793	1.000	-0.1038	0.1197
	Uncoated High	-0.01956	1.000	-0.1313	0.0922
Ionic Maximum	Control	-0.09671	0.130	-0.2084	0.015
	Weathered Low	-0.13760*	0.008	-0.2493	-0.0259
	Sulphidized Low, 120 nm	-0.13560*	0.009	-0.2473	-0.0239
	Sulphidized Low, 160 nm	-0.08968	0.196	-0.2014	0.022
	Ionic Low	-0.09363	0.156	-0.2054	0.0181
	Ionic High	-0.12243*	0.024	-0.2341	-0.0107
	PVP Low	-0.08546	0.247	-0.1972	0.0263
	PVP High	-0.07985	0.327	-0.1916	0.0319

	Uncoated Low	-0.08878	0.206	-0.2005	0.0229
	Uncoated High	-0.11627*	0.037	-0.228	-0.0046
Weathered Low	Control	0.04089	0.957	-0.0708	0.1526
	Ionic Maximum	0.13760*	0.008	0.0259	0.2493
	Sulphidized Low, 120 nm	0.00200	1.000	-0.1097	0.1137
	Sulphidized Low, 160 nm	0.04792	0.893	-0.0638	0.1596
	Ionic Low	0.04397	0.934	-0.0678	0.1557
	Ionic High	0.01518	1.000	-0.0965	0.1269
	PVP Low	0.05214	0.836	-0.0596	0.1639
	PVP High	0.05775	0.742	-0.054	0.1695
	Uncoated Low	0.04882	0.882	-0.0629	0.1605
	Uncoated High	0.02133	1.000	-0.0904	0.1331
Sulphidized Low, 120 nm	Control	0.03889	0.969	-0.0728	0.1506
	Ionic Maximum	0.13560*	0.009	0.0239	0.2473
	Weathered Low	-0.00200	1.000	-0.1137	0.1097
	Sulphidized Low, 160 nm	0.04591	0.915	-0.0658	0.1576
	Ionic Low	0.04196	0.950	-0.0698	0.1537
	Ionic High	0.01317	1.000	-0.0985	0.1249
	PVP Low	0.05014	0.864	-0.0616	0.1619
	PVP High	0.05575	0.777	-0.056	0.1675
	Uncoated Low	0.04682	0.905	-0.0649	0.1585
	Uncoated High	0.01933	1.000	-0.0924	0.131
Sulphidized Low, 160 nm	Control	-0.00703	1.000	-0.1187	0.1047
	Ionic Maximum	0.08968	0.196	-0.022	0.2014
	Weathered Low	-0.04792	0.893	-0.1596	0.0638
	Sulphidized Low, 120 nm	-0.04591	0.915	-0.1576	0.0658
	Ionic Low	-0.00395	1.000	-0.1157	0.1078
	Ionic High	-0.03274	0.991	-0.1445	0.079
	PVP Low	0.00422	1.000	-0.1075	0.1159
	PVP High	0.00983	1.000	-0.1019	0.1216
	Uncoated Low	0.00091	1.000	-0.1108	0.1126
	Uncoated High	-0.02659	0.998	-0.1383	0.0851
Ionic Low	Control	-0.00308	1.000	-0.1148	0.1086
	Ionic Maximum	0.09363	0.156	-0.0181	0.2054
	Weathered Low	-0.04397	0.934	-0.1557	0.0678
	Sulphidized Low, 120 nm	-0.04196	0.950	-0.1537	0.0698
	Sulphidized Low, 160 nm	0.00395	1.000	-0.1078	0.1157
	Ionic High	-0.02879	0.997	-0.1405	0.0829
	PVP Low	0.00817	1.000	-0.1035	0.1199
	PVP High	0.01378	1.000	-0.0979	0.1255

	Uncoated Low	0.00486	1.000	-0.1069	0.1166
	Uncoated High	-0.02264	1.000	-0.1344	0.0891
Ionic High	Control	0.02572	0.999	-0.086	0.1374
	Ionic Maximum	0.12243*	0.024	0.0107	0.2341
	Weathered Low	-0.01518	1.000	-0.1269	0.0965
	Sulphidized Low, 120 nm	-0.01317	1.000	-0.1249	0.0985
	Sulphidized Low, 160 nm	0.03274	0.991	-0.079	0.1445
	Ionic Low	0.02879	0.997	-0.0829	0.1405
	PVP Low	0.03696	0.978	-0.0748	0.1487
	PVP High	0.04257	0.945	-0.0691	0.1543
	Uncoated Low	0.03365	0.989	-0.0781	0.1454
	Uncoated High	0.00615	1.000	-0.1056	0.1179
PVP Low	Control	-0.01125	1.000	-0.123	0.1005
	Ionic Maximum	0.08546	0.247	-0.0263	0.1972
	Weathered Low	-0.05214	0.836	-0.1639	0.0596
	Sulphidized Low, 120 nm	-0.05014	0.864	-0.1619	0.0616
	Sulphidized Low, 160 nm	-0.00422	1.000	-0.1159	0.1075
	Ionic Low	-0.00817	1.000	-0.1199	0.1035
	Ionic High	-0.03696	0.978	-0.1487	0.0748
	PVP High	0.00561	1.000	-0.1061	0.1173
	Uncoated Low	-0.00332	1.000	-0.115	0.1084
	Uncoated High	-0.03081	0.994	-0.1425	0.0809
PVP High	Control	-0.01686	1.000	-0.1286	0.0949
	Ionic Maximum	0.07985	0.327	-0.0319	0.1916
	Weathered Low	-0.05775	0.742	-0.1695	0.054
	Sulphidized Low, 120 nm	-0.05575	0.777	-0.1675	0.056
	Sulphidized Low, 160 nm	-0.00983	1.000	-0.1216	0.1019
	Ionic Low	-0.01378	1.000	-0.1255	0.0979
	Ionic High	-0.04257	0.945	-0.1543	0.0691
	PVP Low	-0.00561	1.000	-0.1173	0.1061
	Uncoated Low	-0.00892	1.000	-0.1206	0.1028
	Uncoated High	-0.03642	0.980	-0.1481	0.0753
Uncoated Low	Control	-0.00793	1.000	-0.1197	0.1038
	Ionic Maximum	0.08878	0.206	-0.0229	0.2005
	Weathered Low	-0.04882	0.882	-0.1605	0.0629
	Sulphidized Low, 120 nm	-0.04682	0.905	-0.1585	0.0649
	Sulphidized Low, 160 nm	-0.00091	1.000	-0.1126	0.1108
	Ionic Low	-0.00486	1.000	-0.1166	0.1069

	Ionic High	-0.03365	0.989	-0.1454	0.0781
	PVP Low	0.00332	1.000	-0.1084	0.115
	PVP High	0.00892	1.000	-0.1028	0.1206
	Uncoated High	-0.02749	0.998	-0.1392	0.0842
Uncoated High	Control	0.01956	1.000	-0.0922	0.1313
	Ionic Maximum	0.11627*	0.037	0.0046	0.228
	Weathered Low	-0.02133	1.000	-0.1331	0.0904
	Sulphidized Low, 120 nm	-0.01933	1.000	-0.131	0.0924
	Sulphidized Low, 160 nm	0.02659	0.998	-0.0851	0.1383
	Ionic Low	0.02264	1.000	-0.0891	0.1344
	Ionic High	-0.00615	1.000	-0.1179	0.1056
	PVP Low	0.03081	0.994	-0.0809	0.1425
	PVP High	0.03642	0.980	-0.0753	0.1481
	Uncoated Low	0.02749	0.998	-0.0842	0.1392

Table D.42: Tukey test subset treatment groups for carboxylic acids in Month 2

Treatment	N	Subsets	
		1	2
Ionic Maximum	3	0.0162	
PVP High	3	0.0961	0.0961
PVP Low	3	0.1017	0.1017
Uncoated Low	3	0.1050	0.1050
Sulphidized Low, 160 nm	3	0.1059	0.1059
Ionic Low	3	0.1099	0.1099
Control	3	0.1129	0.1129
Uncoated High	3		0.1325
Ionic High	3		0.1387
Sulphidized Low, 120 nm	3		0.1518
Weathered Low	3		0.1538
Significance		0.130	0.742

Table D.43: Multiple comparisons for amino acids Month 2 treatments using Tukey's test

Treatment (I)	Treatment (J)	Mean Difference (I-J)	Significance	95% Confidence Interval	
				Lower Bound	Upper Bound
Control	Ionic Maximum	0.07316*	0.046	0.0009	0.1454
	Weathered Low	-0.01035	1.000	-0.0826	0.0619
	Sulphidized Low, 120 nm	-0.02893	0.927	-0.1012	0.0433
	Sulphidized Low, 160 nm	0.02034	0.993	-0.0519	0.0926

	Ionic Low	0.00972	1.000	-0.0626	0.082
	Ionic High	-0.01418	1.000	-0.0864	0.0581
	PVP Low	0.00990	1.000	-0.0624	0.0822
	PVP High	0.01596	0.999	-0.0563	0.0882
	Uncoated Low	0.00178	1.000	-0.0705	0.074
	Uncoated High	-0.02661	0.956	-0.0989	0.0457
Ionic Maximum	Control	-0.07316*	0.046	-0.1454	-0.0009
	Weathered Low	-0.08352*	0.015	-0.1558	-0.0112
	Sulphidized Low, 120 nm	-0.10209*	0.002	-0.1744	-0.0298
	Sulphidized Low, 160 nm	-0.05282	0.300	-0.1251	0.0195
	Ionic Low	-0.06344	0.120	-0.1357	0.0088
	Ionic High	-0.08734*	0.010	-0.1596	-0.0151
	PVP Low	-0.06327	0.122	-0.1355	0.009
	PVP High	-0.05720	0.210	-0.1295	0.0151
	Uncoated Low	-0.07138	0.055	-0.1437	0.0009
	Uncoated High	-0.09977*	0.002	-0.172	-0.0275
Weathered Low	Control	0.01035	1.000	-0.0619	0.0826
	Ionic Maximum	0.08352*	0.015	0.0112	0.1558
	Sulphidized Low, 120 nm	-0.01857	0.997	-0.0908	0.0537
	Sulphidized Low, 160 nm	0.03070	0.898	-0.0416	0.103
	Ionic Low	0.02007	0.994	-0.0522	0.0923
	Ionic High	-0.00382	1.000	-0.0761	0.0684
	PVP Low	0.02025	0.993	-0.052	0.0925
	PVP High	0.02631	0.959	-0.046	0.0986
	Uncoated Low	0.01213	1.000	-0.0601	0.0844
	Uncoated High	-0.01626	0.999	-0.0885	0.056
Sulphidized Low, 120 nm	Control	0.02893	0.927	-0.0433	0.1012
	Ionic Maximum	0.10209*	0.002	0.0298	0.1744
	Weathered Low	0.01857	0.997	-0.0537	0.0908
	Sulphidized Low, 160 nm	0.04927	0.389	-0.023	0.1215
	Ionic Low	0.03865	0.704	-0.0336	0.1109
	Ionic High	0.01475	0.999	-0.0575	0.087
	PVP Low	0.03882	0.699	-0.0334	0.1111
	PVP High	0.04489	0.515	-0.0274	0.1172
	Uncoated Low	0.03071	0.898	-0.0416	0.103
	Uncoated High	0.00232	1.000	-0.07	0.0746
Sulphidized Low, 160 nm	Control	-0.02034	0.993	-0.0926	0.0519
	Ionic Maximum	0.05282	0.300	-0.0195	0.1251
	Weathered Low	-0.03070	0.898	-0.103	0.0416
	Sulphidized Low, 120 nm	-0.04927	0.389	-0.1215	0.023

	Ionic Low	-0.01063	1.000	-0.0829	0.0616
	Ionic High	-0.03452	0.817	-0.1068	0.0377
	PVP Low	-0.01045	1.000	-0.0827	0.0618
	PVP High	-0.00439	1.000	-0.0767	0.0679
	Uncoated Low	-0.01857	0.997	-0.0908	0.0537
	Uncoated High	-0.04696	0.453	-0.1192	0.0253
Ionic Low	Control	-0.00972	1.000	-0.082	0.0626
	Ionic Maximum	0.06344	0.120	-0.0088	0.1357
	Weathered Low	-0.02007	0.994	-0.0923	0.0522
	Sulphidized Low, 120 nm	-0.03865	0.704	-0.1109	0.0336
	Sulphidized Low, 160 nm	0.01063	1.000	-0.0616	0.0829
	Ionic High	-0.02390	0.978	-0.0962	0.0484
	PVP Low	0.00018	1.000	-0.0721	0.0724
	PVP High	0.00624	1.000	-0.066	0.0785
	Uncoated Low	-0.00794	1.000	-0.0802	0.0643
	Uncoated High	-0.03633	0.770	-0.1086	0.0359
Ionic High	Control	0.01418	1.000	-0.0581	0.0864
	Ionic Maximum	0.08734*	0.010	0.0151	0.1596
	Weathered Low	0.00382	1.000	-0.0684	0.0761
	Sulphidized Low, 120 nm	-0.01475	0.999	-0.087	0.0575
	Sulphidized Low, 160 nm	0.03452	0.817	-0.0377	0.1068
	Ionic Low	0.02390	0.978	-0.0484	0.0962
	PVP Low	0.02408	0.977	-0.0482	0.0963
	PVP High	0.03014	0.908	-0.0421	0.1024
	Uncoated Low	0.01596	0.999	-0.0563	0.0882
	Uncoated High	-0.01243	1.000	-0.0847	0.0598
PVP Low	Control	-0.00990	1.000	-0.0822	0.0624
	Ionic Maximum	0.06327	0.122	-0.009	0.1355
	Weathered Low	-0.02025	0.993	-0.0925	0.052
	Sulphidized Low, 120 nm	-0.03882	0.699	-0.1111	0.0334
	Sulphidized Low, 160 nm	0.01045	1.000	-0.0618	0.0827
	Ionic Low	-0.00018	1.000	-0.0724	0.0721
	Ionic High	-0.02408	0.977	-0.0963	0.0482
	PVP High	0.00606	1.000	-0.0662	0.0783
	Uncoated Low	-0.00812	1.000	-0.0804	0.0641
	Uncoated High	-0.03651	0.765	-0.1088	0.0358
PVP High	Control	-0.01596	0.999	-0.0882	0.0563
	Ionic Maximum	0.05720	0.210	-0.0151	0.1295
	Weathered Low	-0.02631	0.959	-0.0986	0.046

	Sulphidized Low, 120 nm	-0.04489	0.515	-0.1172	0.0274
	Sulphidized Low, 160 nm	0.00439	1.000	-0.0679	0.0767
	Ionic Low	-0.00624	1.000	-0.0785	0.066
	Ionic High	-0.03014	0.908	-0.1024	0.0421
	PVP Low	-0.00606	1.000	-0.0783	0.0662
	Uncoated Low	-0.01418	1.000	-0.0864	0.0581
	Uncoated High	-0.04257	0.585	-0.1148	0.0297
Uncoated Low	Control	-0.00178	1.000	-0.074	0.0705
	Ionic Maximum	0.07138	0.055	-0.0009	0.1437
	Weathered Low	-0.01213	1.000	-0.0844	0.0601
	Sulphidized Low, 120 nm	-0.03071	0.898	-0.103	0.0416
	Sulphidized Low, 160 nm	0.01857	0.997	-0.0537	0.0908
	Ionic Low	0.00794	1.000	-0.0643	0.0802
	Ionic High	-0.01596	0.999	-0.0882	0.0563
	PVP Low	0.00812	1.000	-0.0641	0.0804
	PVP High	0.01418	1.000	-0.0581	0.0864
	Uncoated High	-0.02839	0.935	-0.1007	0.0439
Uncoated High	Control	0.02661	0.956	-0.0457	0.0989
	Ionic Maximum	0.09977*	0.002	0.0275	0.172
	Weathered Low	0.01626	0.999	-0.056	0.0885
	Sulphidized Low, 120 nm	-0.00232	1.000	-0.0746	0.07
	Sulphidized Low, 160 nm	0.04696	0.453	-0.0253	0.1192
	Ionic Low	0.03633	0.770	-0.0359	0.1086
	Ionic High	0.01243	1.000	-0.0598	0.0847
	PVP Low	0.03651	0.765	-0.0358	0.1088
	PVP High	0.04257	0.585	-0.0297	0.1148
	Uncoated Low	0.02839	0.935	-0.0439	0.1007

Table D.44: Tukey test subset treatment groups for amino acids in Month 2

Treatment	N	Subsets	
		1	2
Ionic Maximum	3	0.0259	
Sulphidized Low, 160 nm	3	0.0787	0.0787
PVP High	3	0.0831	0.0831
PVP Low	3	0.0892	0.0892
Ionic Low	3	0.0894	0.0894
Uncoated Low	3	0.0973	0.0973
Control	3		0.0991
Weathered Low	3		0.1094
Ionic High	3		0.1133
Uncoated High	3		0.1257
Sulphidized Low, 120 nm	3		0.1280
Significance		0.055	0.389

Table D.45: One-way ANOVA for Month 3 Guilds

		Sum of Squares	DOF	Mean Square	F	Significance
Carbohydrates	Between Groups	0.118	10	0.012	1.232	0.325
	Within Groups	0.211	22	0.010		
	Total	0.329	32			
Polymers	Between Groups	0.012	10	0.001	3.086	0.013
	Within Groups	0.008	22	0.000		
	Total	0.020	32			
Carboxylic acids	Between Groups	0.065	10	0.006	3.330	0.009
	Within Groups	0.043	22	0.002		
	Total	0.108	32			
Amino acids	Between Groups	0.034	10	0.003	3.083	0.013
	Within Groups	0.024	22	0.001		
	Total	0.058	32			
Amides/ amides	Between Groups	0.002	10	0.000	2.253	0.054
	Within Groups	0.002	22	0.000		
	Total	0.005	32			
Root Exudates	Between Groups	0.131	10	0.013	2.407	0.041
	Within Groups	0.119	22	0.005		
	Total	0.250	32			

Table D.46: Multiple comparisons for polymers Month 3 treatments using Tukey's test

Treatment (I)	Treatment (J)	Mean Difference (I-J)	Significance	95% Confidence Interval	
				Lower Bound	Upper Bound
Control	Ionic Maximum	0.05407	0.071	-0.0027	0.1108
	Weathered Low	-0.02415	0.897	-0.0809	0.0326
	Sulphidized Low, 120 nm	-0.00551	1.000	-0.0622	0.0512
	Sulphidized Low, 160 nm	0.02230	0.934	-0.0344	0.0790
	Ionic Low	-0.00530	1.000	-0.0620	0.0514
	Ionic High	0.00463	1.000	-0.0521	0.0614
	PVP Low	0.00607	1.000	-0.0507	0.0628
	PVP High	0.00439	1.000	-0.0523	0.0611
	Uncoated Low	-0.00286	1.000	-0.0596	0.0539
	Uncoated High	-0.00008	1.000	-0.0568	0.0567
Ionic Maximum	Control	-0.05407	0.071	-0.1108	0.0027
	Weathered Low	-0.07822*	0.002	-0.1350	-0.0215
	Sulphidized Low, 120 nm	-0.05958*	0.034	-0.1163	-0.0028
	Sulphidized Low, 160 nm	-0.03177	0.650	-0.0885	0.0250
	Ionic Low	-0.05937*	0.035	-0.1161	-0.0026
	Ionic High	-0.04944	0.125	-0.1062	0.0073
	PVP Low	-0.04800	0.149	-0.1047	0.0087
	PVP High	-0.04968	0.122	-0.1064	0.0071
	Uncoated Low	-0.05693*	0.049	-0.1137	-0.0002
	Uncoated High	-0.05415	0.070	-0.1109	0.0026
Weathered Low	Control	0.02415	0.897	-0.0326	0.0809
	Ionic Maximum	0.07822*	0.002	0.0215	0.1350
	Sulphidized Low, 120 nm	0.01864	0.979	-0.0381	0.0754
	Sulphidized Low, 160 nm	0.04645	0.177	-0.0103	0.1032
	Ionic Low	0.01885	0.977	-0.0379	0.0756
	Ionic High	0.02878	0.761	-0.0280	0.0855
	PVP Low	0.03022	0.709	-0.0265	0.0870
	PVP High	0.02854	0.769	-0.0282	0.0853
	Uncoated Low	0.02129	0.950	-0.0354	0.0780
	Uncoated High	0.02407	0.899	-0.0327	0.0808
Sulphidized Low, 120 nm	Control	0.00551	1.000	-0.0512	0.0622
	Ionic Maximum	0.05958*	0.034	0.0028	0.1163
	Weathered Low	-0.01864	0.979	-0.0754	0.0381
	Sulphidized Low, 160 nm	0.02781	0.794	-0.0289	0.0845
	Ionic Low	0.00021	1.000	-0.0565	0.0569

	Ionic High	0.01014	1.000	-0.0466	0.0669
	PVP Low	0.01158	0.999	-0.0452	0.0683
	PVP High	0.00990	1.000	-0.0468	0.0666
	Uncoated Low	0.00265	1.000	-0.0541	0.0594
	Uncoated High	0.00543	1.000	-0.0513	0.0622
Sulphidized Low, 160 nm	Control	-0.02230	0.934	-0.0790	0.0344
	Ionic Maximum	0.03177	0.650	-0.0250	0.0885
	Weathered Low	-0.04645	0.177	-0.1032	0.0103
	Sulphidized Low, 120 nm	-0.02781	0.794	-0.0845	0.0289
	Ionic Low	-0.02760	0.801	-0.0843	0.0291
	Ionic High	-0.01767	0.986	-0.0744	0.0391
	PVP Low	-0.01623	0.992	-0.0730	0.0405
	PVP High	-0.01791	0.984	-0.0746	0.0388
	Uncoated Low	-0.02516	0.872	-0.0819	0.0316
	Uncoated High	-0.02238	0.933	-0.0791	0.0344
Ionic Low	Control	0.00530	1.000	-0.0514	0.0620
	Ionic Maximum	0.05937*	0.035	0.0026	0.1161
	Weathered Low	-0.01885	0.977	-0.0756	0.0379
	Sulphidized Low, 120 nm	-0.00021	1.000	-0.0569	0.0565
	Sulphidized Low, 160 nm	0.02760	0.801	-0.0291	0.0843
	Ionic High	0.00993	1.000	-0.0468	0.0667
	PVP Low	0.01137	1.000	-0.0454	0.0681
	PVP High	0.00969	1.000	-0.0470	0.0664
	Uncoated Low	0.00244	1.000	-0.0543	0.0592
	Uncoated High	0.00522	1.000	-0.0515	0.0620
Ionic High	Control	-0.00463	1.000	-0.0614	0.0521
	Ionic Maximum	0.04944	0.125	-0.0073	0.1062
	Weathered Low	-0.02878	0.761	-0.0855	0.0280
	Sulphidized Low, 120 nm	-0.01014	1.000	-0.0669	0.0466
	Sulphidized Low, 160 nm	0.01767	0.986	-0.0391	0.0744
	Ionic Low	-0.00993	1.000	-0.0667	0.0468
	PVP Low	0.00144	1.000	-0.0553	0.0582
	PVP High	-0.00024	1.000	-0.0570	0.0565
	Uncoated Low	-0.00749	1.000	-0.0642	0.0492
	Uncoated High	-0.00471	1.000	-0.0615	0.0520
PVP Low	Control	-0.00607	1.000	-0.0628	0.0507
	Ionic Maximum	0.04800	0.149	-0.0087	0.1047
	Weathered Low	-0.03022	0.709	-0.0870	0.0265
	Sulphidized Low, 120 nm	-0.01158	0.999	-0.0683	0.0452

	Sulphidized Low, 160 nm	0.01623	0.992	-0.0405	0.0730
	Ionic Low	-0.01137	1.000	-0.0681	0.0454
	Ionic High	-0.00144	1.000	-0.0582	0.0553
	PVP High	-0.00168	1.000	-0.0584	0.0551
	Uncoated Low	-0.00893	1.000	-0.0657	0.0478
	Uncoated High	-0.00615	1.000	-0.0629	0.0506
PVP High	Control	-0.00439	1.000	-0.0611	0.0523
	Ionic Maximum	0.04968	0.122	-0.0071	0.1064
	Weathered Low	-0.02854	0.769	-0.0853	0.0282
	Sulphidized Low, 120 nm	-0.00990	1.000	-0.0666	0.0468
	Sulphidized Low, 160 nm	0.01791	0.984	-0.0388	0.0746
	Ionic Low	-0.00969	1.000	-0.0664	0.0470
	Ionic High	0.00024	1.000	-0.0565	0.0570
	PVP Low	0.00168	1.000	-0.0551	0.0584
	Uncoated Low	-0.00725	1.000	-0.0640	0.0495
	Uncoated High	-0.00447	1.000	-0.0612	0.0523
Uncoated Low	Control	0.00286	1.000	-0.0539	0.0596
	Ionic Maximum	0.05693*	0.049	0.0002	0.1137
	Weathered Low	-0.02129	0.950	-0.0780	0.0354
	Sulphidized Low, 120 nm	-0.00265	1.000	-0.0594	0.0541
	Sulphidized Low, 160 nm	0.02516	0.872	-0.0316	0.0819
	Ionic Low	-0.00244	1.000	-0.0592	0.0543
	Ionic High	0.00749	1.000	-0.0492	0.0642
	PVP Low	0.00893	1.000	-0.0478	0.0657
	PVP High	0.00725	1.000	-0.0495	0.0640
	Uncoated High	0.00278	1.000	-0.0540	0.0595
Uncoated High	Control	0.00008	1.000	-0.0567	0.0568
	Ionic Maximum	0.05415	0.070	-0.0026	0.1109
	Weathered Low	-0.02407	0.899	-0.0808	0.0327
	Sulphidized Low, 120 nm	-0.00543	1.000	-0.0622	0.0513
	Sulphidized Low, 160 nm	0.02238	0.933	-0.0344	0.0791
	Ionic Low	-0.00522	1.000	-0.0620	0.0515
	Ionic High	0.00471	1.000	-0.0520	0.0615
	PVP Low	0.00615	1.000	-0.0506	0.0629
	PVP High	0.00447	1.000	-0.0523	0.0612
	Uncoated Low	-0.00278	1.000	-0.0595	0.0540

Table D.47: Tukey test subset treatment groups for polymers in Month 3

Treatment	N	Subsets	
		1	2
Ionic Maximum	3	0.0251	
Sulphidized Low, 160 nm	3	0.0569	0.0569
PVP Low	3	0.0731	0.0731
Ionic High	3	0.0746	0.0746
PVP High	3	0.0748	0.0748
Control	3	0.0792	0.0792
Uncoated High	3	0.0793	0.0793
Uncoated Low	3		0.0821
Ionic Low	3		0.0845
Sulphidized Low, 120 nm	3		0.0847
Weathered Low	3		0.1034
Significance		0.070	0.177

Table D.48: Multiple comparisons for carboxylic acids Month 3 treatments using Tukey's test

Treatment (I)	Treatment (J)	Mean Difference (I-J)	Significance	95% Confidence Interval	
				Lower Bound	Upper Bound
Control	Ionic Maximum	0.12504	0.062	-0.0037	0.2537
	Weathered Low	-0.05473	0.898	-0.1834	0.0740
	Sulphidized Low, 120 nm	-0.00467	1.000	-0.1334	0.1240
	Sulphidized Low, 160 nm	0.06993	0.686	-0.0588	0.1986
	Ionic Low	0.05154	0.927	-0.0772	0.1802
	Ionic High	0.05579	0.887	-0.0729	0.1845
	PVP Low	0.03103	0.998	-0.0977	0.1597
	PVP High	0.02590	1.000	-0.1028	0.1546
	Uncoated Low	0.02592	1.000	-0.1028	0.1546
	Uncoated High	0.00741	1.000	-0.1213	0.1361
Ionic Maximum	Control	-0.12504	0.062	-0.2537	0.0037
	Weathered Low	-0.17977*	0.002	-0.3085	-0.0511
	Sulphidized Low, 120 nm	-0.12971*	0.047	-0.2584	-0.0010
	Sulphidized Low, 160 nm	-0.05511	0.894	-0.1838	0.0736
	Ionic Low	-0.07349	0.625	-0.2022	0.0552
	Ionic High	-0.06925	0.697	-0.1979	0.0595
	PVP Low	-0.09401	0.301	-0.2227	0.0347
	PVP High	-0.09914	0.239	-0.2278	0.0296

	Uncoated Low	-0.09911	0.239	-0.2278	0.0296
	Uncoated High	-0.11763	0.093	-0.2463	0.0111
Weathered Low	Control	0.05473	0.898	-0.0740	0.1834
	Ionic Maximum	0.17977*	0.002	0.0511	0.3085
	Sulphidized Low, 120 nm	0.05006	0.938	-0.0786	0.1788
	Sulphidized Low, 160 nm	0.12466	0.063	-0.0040	0.2534
	Ionic Low	0.10628	0.170	-0.0224	0.2350
	Ionic High	0.11052	0.137	-0.0182	0.2392
	PVP Low	0.08576	0.419	-0.0429	0.2145
	PVP High	0.08063	0.503	-0.0481	0.2093
	Uncoated Low	0.08066	0.502	-0.0480	0.2094
	Uncoated High	0.06214	0.807	-0.0666	0.1908
Sulphidized Low, 120 nm	Control	0.00467	1.000	-0.1240	0.1334
	Ionic Maximum	0.12971*	0.047	0.0010	0.2584
	Weathered Low	-0.05006	0.938	-0.1788	0.0786
	Sulphidized Low, 160 nm	0.07460	0.606	-0.0541	0.2033
	Ionic Low	0.05622	0.882	-0.0725	0.1849
	Ionic High	0.06046	0.830	-0.0682	0.1892
	PVP Low	0.03570	0.994	-0.0930	0.1644
	PVP High	0.03057	0.998	-0.0981	0.1593
	Uncoated Low	0.03060	0.998	-0.0981	0.1593
	Uncoated High	0.01208	1.000	-0.1166	0.1408
Sulphidized Low, 160 nm	Control	-0.06993	0.686	-0.1986	0.0588
	Ionic Maximum	0.05511	0.894	-0.0736	0.1838
	Weathered Low	-0.12466	0.063	-0.2534	0.0040
	Sulphidized Low, 120 nm	-0.07460	0.606	-0.2033	0.0541
	Ionic Low	-0.01839	1.000	-0.1471	0.1103
	Ionic High	-0.01414	1.000	-0.1428	0.1146
	PVP Low	-0.03890	0.988	-0.1676	0.0898
	PVP High	-0.04403	0.973	-0.1727	0.0847
	Uncoated Low	-0.04400	0.973	-0.1727	0.0847
	Uncoated High	-0.06252	0.802	-0.1912	0.0662
Ionic Low	Control	-0.05154	0.927	-0.1802	0.0772
	Ionic Maximum	0.07349	0.625	-0.0552	0.2022
	Weathered Low	-0.10628	0.170	-0.2350	0.0224
	Sulphidized Low, 120 nm	-0.05622	0.882	-0.1849	0.0725
	Sulphidized Low, 160 nm	0.01839	1.000	-0.1103	0.1471
	Ionic High	0.00425	1.000	-0.1245	0.1329
	PVP Low	-0.02051	1.000	-0.1492	0.1082
	PVP High	-0.02564	1.000	-0.1543	0.1031

	Uncoated Low	-0.02562	1.000	-0.1543	0.1031
	Uncoated High	-0.04413	0.972	-0.1728	0.0846
Ionic High	Control	-0.05579	0.887	-0.1845	0.0729
	Ionic Maximum	0.06925	0.697	-0.0595	0.1979
	Weathered Low	-0.11052	0.137	-0.2392	0.0182
	Sulphidized Low, 120 nm	-0.06046	0.830	-0.1892	0.0682
	Sulphidized Low, 160 nm	0.01414	1.000	-0.1146	0.1428
	Ionic Low	-0.00425	1.000	-0.1329	0.1245
	PVP Low	-0.02476	1.000	-0.1535	0.1039
	PVP High	-0.02989	0.999	-0.1586	0.0988
	Uncoated Low	-0.02986	0.999	-0.1586	0.0988
	Uncoated High	-0.04838	0.950	-0.1771	0.0803
PVP Low	Control	-0.03103	0.998	-0.1597	0.0977
	Ionic Maximum	0.09401	0.301	-0.0347	0.2227
	Weathered Low	-0.08576	0.419	-0.2145	0.0429
	Sulphidized Low, 120 nm	-0.03570	0.994	-0.1644	0.0930
	Sulphidized Low, 160 nm	0.03890	0.988	-0.0898	0.1676
	Ionic Low	0.02051	1.000	-0.1082	0.1492
	Ionic High	0.02476	1.000	-0.1039	0.1535
	PVP High	-0.00513	1.000	-0.1338	0.1236
	Uncoated Low	-0.00510	1.000	-0.1338	0.1236
	Uncoated High	-0.02362	1.000	-0.1523	0.1051
PVP High	Control	-0.02590	1.000	-0.1546	0.1028
	Ionic Maximum	0.09914	0.239	-0.0296	0.2278
	Weathered Low	-0.08063	0.503	-0.2093	0.0481
	Sulphidized Low, 120 nm	-0.03057	0.998	-0.1593	0.0981
	Sulphidized Low, 160 nm	0.04403	0.973	-0.0847	0.1727
	Ionic Low	0.02564	1.000	-0.1031	0.1543
	Ionic High	0.02989	0.999	-0.0988	0.1586
	PVP Low	0.00513	1.000	-0.1236	0.1338
	Uncoated Low	0.00003	1.000	-0.1287	0.1287
	Uncoated High	-0.01849	1.000	-0.1472	0.1102
Uncoated Low	Control	-0.02592	1.000	-0.1546	0.1028
	Ionic Maximum	0.09911	0.239	-0.0296	0.2278
	Weathered Low	-0.08066	0.502	-0.2094	0.0480
	Sulphidized Low, 120 nm	-0.03060	0.998	-0.1593	0.0981
	Sulphidized Low, 160 nm	0.04400	0.973	-0.0847	0.1727
	Ionic Low	0.02562	1.000	-0.1031	0.1543

	Ionic High	0.02986	0.999	-0.0988	0.1586
	PVP Low	0.00510	1.000	-0.1236	0.1338
	PVP High	-0.00003	1.000	-0.1287	0.1287
	Uncoated High	-0.01852	1.000	-0.1472	0.1102
Uncoated High	Control	-0.00741	1.000	-0.1361	0.1213
	Ionic Maximum	0.11763	0.093	-0.0111	0.2463
	Weathered Low	-0.06214	0.807	-0.1908	0.0666
	Sulphidized Low, 120 nm	-0.01208	1.000	-0.1408	0.1166
	Sulphidized Low, 160 nm	0.06252	0.802	-0.0662	0.1912
	Ionic Low	0.04413	0.972	-0.0846	0.1728
	Ionic High	0.04838	0.950	-0.0803	0.1771
	PVP Low	0.02362	1.000	-0.1051	0.1523
	PVP High	0.01849	1.000	-0.1102	0.1472
	Uncoated Low	0.01852	1.000	-0.1102	0.1472

Table D.49: Tukey test subset treatment groups for carboxylic acids in Month 3

Treatment	N	Subsets	
		1	2
Ionic Maximum	3	0.0309	
Sulphidized Low, 160 nm	3	0.0860	0.0860
Ionic High	3	0.1002	0.1002
Ionic Low	3	0.1044	0.1044
PVP Low	3	0.1249	0.1249
Uncoated Low	3	0.1300	0.1300
PVP High	3	0.1301	0.1301
Uncoated High	3	0.1486	0.1486
Control	3	0.1560	0.1560
Sulphidized Low, 120 nm	3		0.1606
Weathered Low	3		0.2107
Significance		0.062	0.063

Table D.50: Multiple comparisons for amino acids Month 3 treatments using Tukey's test

Treatment (I)	Treatment (J)	Mean Difference (I-J)	Significance	95% Confidence Interval	
				Lower Bound	Upper Bound
Control	Ionic Maximum	0.08406	0.128	-0.0127	0.1808
	Weathered Low	-0.02235	0.999	-0.1191	0.0744
	Sulphidized Low, 120 nm	-0.02987	0.987	-0.1267	0.0669

	Sulphidized Low, 160 nm	0.05990	0.519	-0.0369	0.1567
	Ionic Low	0.01517	1.000	-0.0816	0.1120
	Ionic High	0.00710	1.000	-0.0897	0.1039
	PVP Low	0.03021	0.985	-0.0666	0.1270
	PVP High	0.00494	1.000	-0.0918	0.1017
	Uncoated Low	0.01172	1.000	-0.0851	0.1085
	Uncoated High	-0.00701	1.000	-0.1038	0.0898
Ionic Maximum	Control	-0.08406	0.128	-0.1808	0.0127
	Weathered Low	-0.10641*	0.023	-0.2032	-0.0096
	Sulphidized Low, 120 nm	-0.11393*	0.013	-0.2107	-0.0171
	Sulphidized Low, 160 nm	-0.02416	0.997	-0.1209	0.0726
	Ionic Low	-0.06889	0.333	-0.1657	0.0279
	Ionic High	-0.07696	0.206	-0.1737	0.0198
	PVP Low	-0.05385	0.658	-0.1506	0.0429
	PVP High	-0.07913	0.179	-0.1759	0.0177
	Uncoated Low	-0.07234	0.273	-0.1691	0.0244
	Uncoated High	-0.09108	0.077	-0.1879	0.0057
Weathered Low	Control	0.02235	0.999	-0.0744	0.1191
	Ionic Maximum	0.10641*	0.023	0.0096	0.2032
	Sulphidized Low, 120 nm	-0.00752	1.000	-0.1043	0.0893
	Sulphidized Low, 160 nm	0.08225	0.145	-0.0145	0.1790
	Ionic Low	0.03752	0.939	-0.0593	0.1343
	Ionic High	0.02946	0.988	-0.0673	0.1262
	PVP Low	0.05256	0.687	-0.0442	0.1493
	PVP High	0.02729	0.993	-0.0695	0.1241
	Uncoated Low	0.03407	0.967	-0.0627	0.1309
	Uncoated High	0.01534	1.000	-0.0814	0.1121
Sulphidized Low, 120 nm	Control	0.02987	0.987	-0.0669	0.1267
	Ionic Maximum	0.11393*	0.013	0.0171	0.2107
	Weathered Low	0.00752	1.000	-0.0893	0.1043
	Sulphidized Low, 160 nm	0.08977	0.085	-0.0070	0.1866
	Ionic Low	0.04504	0.838	-0.0517	0.1418
	Ionic High	0.03697	0.945	-0.0598	0.1338
	PVP Low	0.06008	0.515	-0.0367	0.1569
	PVP High	0.03481	0.962	-0.0620	0.1316
	Uncoated Low	0.04159	0.892	-0.0552	0.1384
	Uncoated High	0.02286	0.998	-0.0739	0.1196
Sulphidized Low, 160 nm	Control	-0.05990	0.519	-0.1567	0.0369
	Ionic Maximum	0.02416	0.997	-0.0726	0.1209
	Weathered Low	-0.08225	0.145	-0.1790	0.0145

	Sulphidized Low, 120 nm	-0.08977	0.085	-0.1866	0.0070
	Ionic Low	-0.04473	0.843	-0.1415	0.0521
	Ionic High	-0.05280	0.681	-0.1496	0.0440
	PVP Low	-0.02969	0.987	-0.1265	0.0671
	PVP High	-0.05496	0.632	-0.1517	0.0418
	Uncoated Low	-0.04818	0.779	-0.1450	0.0486
	Uncoated High	-0.06691	0.370	-0.1637	0.0299
Ionic Low	Control	-0.01517	1.000	-0.1120	0.0816
	Ionic Maximum	0.06889	0.333	-0.0279	0.1657
	Weathered Low	-0.03752	0.939	-0.1343	0.0593
	Sulphidized Low, 120 nm	-0.04504	0.838	-0.1418	0.0517
	Sulphidized Low, 160 nm	0.04473	0.843	-0.0521	0.1415
	Ionic High	-0.00807	1.000	-0.1049	0.0887
	PVP Low	0.01504	1.000	-0.0817	0.1118
	PVP High	-0.01024	1.000	-0.1070	0.0865
	Uncoated Low	-0.00346	1.000	-0.1002	0.0933
	Uncoated High	-0.02219	0.999	-0.1190	0.0746
Ionic High	Control	-0.00710	1.000	-0.1039	0.0897
	Ionic Maximum	0.07696	0.206	-0.0198	0.1737
	Weathered Low	-0.02946	0.988	-0.1262	0.0673
	Sulphidized Low, 120 nm	-0.03697	0.945	-0.1338	0.0598
	Sulphidized Low, 160 nm	0.05280	0.681	-0.0440	0.1496
	Ionic Low	0.00807	1.000	-0.0887	0.1049
	PVP Low	0.02311	0.998	-0.0737	0.1199
	PVP High	-0.00217	1.000	-0.0990	0.0946
	Uncoated Low	0.00461	1.000	-0.0922	0.1014
	Uncoated High	-0.01412	1.000	-0.1109	0.0827
PVP Low	Control	-0.03021	0.985	-0.1270	0.0666
	Ionic Maximum	0.05385	0.658	-0.0429	0.1506
	Weathered Low	-0.05256	0.687	-0.1493	0.0442
	Sulphidized Low, 120 nm	-0.06008	0.515	-0.1569	0.0367
	Sulphidized Low, 160 nm	0.02969	0.987	-0.0671	0.1265
	Ionic Low	-0.01504	1.000	-0.1118	0.0817
	Ionic High	-0.02311	0.998	-0.1199	0.0737
	PVP High	-0.02528	0.996	-0.1221	0.0715
	Uncoated Low	-0.01849	1.000	-0.1153	0.0783
	Uncoated High	-0.03723	0.942	-0.1340	0.0596
PVP High	Control	-0.00494	1.000	-0.1017	0.0918
	Ionic Maximum	0.07913	0.179	-0.0177	0.1759

	Weathered Low	-0.02729	0.993	-0.1241	0.0695
	Sulphidized Low, 120 nm	-0.03481	0.962	-0.1316	0.0620
	Sulphidized Low, 160 nm	0.05496	0.632	-0.0418	0.1517
	Ionic Low	0.01024	1.000	-0.0865	0.1070
	Ionic High	0.00217	1.000	-0.0946	0.0990
	PVP Low	0.02528	0.996	-0.0715	0.1221
	Uncoated Low	0.00678	1.000	-0.0900	0.1036
	Uncoated High	-0.01195	1.000	-0.1087	0.0848
Uncoated Low	Control	-0.01172	1.000	-0.1085	0.0851
	Ionic Maximum	0.07234	0.273	-0.0244	0.1691
	Weathered Low	-0.03407	0.967	-0.1309	0.0627
	Sulphidized Low, 120 nm	-0.04159	0.892	-0.1384	0.0552
	Sulphidized Low, 160 nm	0.04818	0.779	-0.0486	0.1450
	Ionic Low	0.00346	1.000	-0.0933	0.1002
	Ionic High	-0.00461	1.000	-0.1014	0.0922
	PVP Low	0.01849	1.000	-0.0783	0.1153
	PVP High	-0.00678	1.000	-0.1036	0.0900
	Uncoated High	-0.01873	1.000	-0.1155	0.0781
Uncoated High	Control	0.00701	1.000	-0.0898	0.1038
	Ionic Maximum	0.09108	0.077	-0.0057	0.1879
	Weathered Low	-0.01534	1.000	-0.1121	0.0814
	Sulphidized Low, 120 nm	-0.02286	0.998	-0.1196	0.0739
	Sulphidized Low, 160 nm	0.06691	0.370	-0.0299	0.1637
	Ionic Low	0.02219	0.999	-0.0746	0.1190
	Ionic High	0.01412	1.000	-0.0827	0.1109
	PVP Low	0.03723	0.942	-0.0596	0.1340
	PVP High	0.01195	1.000	-0.0848	0.1087
	Uncoated Low	0.01873	1.000	-0.0781	0.1155

Table D.51: Tukey test subset treatment groups for amino acids in Month 3

Treatment	N	Subsets	
		1	2
Ionic Maximum	3	0.0322	
Sulphidized Low, 160 nm	3	0.0563	0.0563
PVP Low	3	0.0860	0.0860
Ionic Low	3	0.1011	0.1011
Uncoated Low	3	0.1045	0.1045
Ionic High	3	0.1091	0.1091
PVP High	3	0.1113	0.1113
Control	3	0.1162	0.1162
Uncoated High	3	0.1232	0.1232
Weathered Low	3		0.1386
Sulphidized Low, 120 nm	3		0.1461
Significance		0.077	0.085

Table D.52: Multiple comparisons for root exudates Month 3 treatments using Tukey's test

Treatment (I)	Treatment (J)	Mean Difference (I-J)	Significance	95% Confidence Interval	
				Lower Bound	Upper Bound
Control	Ionic Maximum	0.17724	0.171	-0.0378	0.3922
	Weathered Low	-0.04726	0.999	-0.2623	0.1677
	Sulphidized Low, 120 nm	-0.03609	1.000	-0.2511	0.1789
	Sulphidized Low, 160 nm	0.11941	0.660	-0.0956	0.3344
	Ionic Low	0.05407	0.997	-0.1609	0.2691
	Ionic High	0.05276	0.998	-0.1622	0.2678
	PVP Low	0.06748	0.985	-0.1475	0.2825
	PVP High	0.01131	1.000	-0.2037	0.2263
	Uncoated Low	0.00910	1.000	-0.2059	0.2241
	Uncoated High	0.01751	1.000	-0.1975	0.2325
Ionic Maximum	Control	-0.17724	0.171	-0.3922	0.0378
	Weathered Low	-0.22450*	0.036	-0.4395	-0.0095
	Sulphidized Low, 120 nm	-0.21333	0.053	-0.4283	0.0017
	Sulphidized Low, 160 nm	-0.05783	0.995	-0.2728	0.1572
	Ionic Low	-0.12317	0.621	-0.3382	0.0918
	Ionic High	-0.12448	0.608	-0.3395	0.0905
	PVP Low	-0.10976	0.754	-0.3248	0.1052
	PVP High	-0.16593	0.237	-0.3809	0.0491
	Uncoated Low	-0.16814	0.223	-0.3831	0.0469

	Uncoated High	-0.15973	0.280	-0.3747	0.0553
Weathered Low	Control	0.04726	0.999	-0.1677	0.2623
	Ionic Maximum	0.22450*	0.036	0.0095	0.4395
	Sulphidized Low, 120 nm	0.01116	1.000	-0.2038	0.2262
	Sulphidized Low, 160 nm	0.16667	0.232	-0.0483	0.3817
	Ionic Low	0.10133	0.828	-0.1137	0.3163
	Ionic High	0.10002	0.838	-0.1150	0.3150
	PVP Low	0.11474	0.707	-0.1003	0.3297
	PVP High	0.05857	0.995	-0.1564	0.2736
	Uncoated Low	0.05636	0.996	-0.1586	0.2714
	Uncoated High	0.06476	0.989	-0.1502	0.2798
Sulphidized Low, 120 nm	Control	0.03609	1.000	-0.1789	0.2511
	Ionic Maximum	0.21333	0.053	-0.0017	0.4283
	Weathered Low	-0.01116	1.000	-0.2262	0.2038
	Sulphidized Low, 160 nm	0.15551	0.313	-0.0595	0.3705
	Ionic Low	0.09016	0.905	-0.1248	0.3052
	Ionic High	0.08885	0.912	-0.1261	0.3038
	PVP Low	0.10358	0.809	-0.1114	0.3186
	PVP High	0.04741	0.999	-0.1676	0.2624
	Uncoated Low	0.04520	0.999	-0.1698	0.2602
	Uncoated High	0.05360	0.997	-0.1614	0.2686
Sulphidized Low, 160 nm	Control	-0.11941	0.660	-0.3344	0.0956
	Ionic Maximum	0.05783	0.995	-0.1572	0.2728
	Weathered Low	-0.16667	0.232	-0.3817	0.0483
	Sulphidized Low, 120 nm	-0.15551	0.313	-0.3705	0.0595
	Ionic Low	-0.06534	0.988	-0.2803	0.1497
	Ionic High	-0.06665	0.986	-0.2816	0.1483
	PVP Low	-0.05193	0.998	-0.2669	0.1631
	PVP High	-0.10810	0.770	-0.3231	0.1069
	Uncoated Low	-0.11031	0.749	-0.3253	0.1047
	Uncoated High	-0.10191	0.823	-0.3169	0.1131
Ionic Low	Control	-0.05407	0.997	-0.2691	0.1609
	Ionic Maximum	0.12317	0.621	-0.0918	0.3382
	Weathered Low	-0.10133	0.828	-0.3163	0.1137
	Sulphidized Low, 120 nm	-0.09016	0.905	-0.3052	0.1248
	Sulphidized Low, 160 nm	0.06534	0.988	-0.1497	0.2803
	Ionic High	-0.00131	1.000	-0.2163	0.2137
	PVP Low	0.01341	1.000	-0.2016	0.2284
	PVP High	-0.04276	1.000	-0.2578	0.1722
	Uncoated Low	-0.04497	0.999	-0.2600	0.1700

	Uncoated High	-0.03657	1.000	-0.2516	0.1784
Ionic High	Control	-0.05276	0.998	-0.2678	0.1622
	Ionic Maximum	0.12448	0.608	-0.0905	0.3395
	Weathered Low	-0.10002	0.838	-0.3150	0.1150
	Sulphidized Low, 120 nm	-0.08885	0.912	-0.3038	0.1261
	Sulphidized Low, 160 nm	0.06665	0.986	-0.1483	0.2816
	Ionic Low	0.00131	1.000	-0.2137	0.2163
	PVP Low	0.01472	1.000	-0.2003	0.2297
	PVP High	-0.04145	1.000	-0.2564	0.1735
	Uncoated Low	-0.04366	1.000	-0.2587	0.1713
	Uncoated High	-0.03525	1.000	-0.2502	0.1797
PVP Low	Control	-0.06748	0.985	-0.2825	0.1475
	Ionic Maximum	0.10976	0.754	-0.1052	0.3248
	Weathered Low	-0.11474	0.707	-0.3297	0.1003
	Sulphidized Low, 120 nm	-0.10358	0.809	-0.3186	0.1114
	Sulphidized Low, 160 nm	0.05193	0.998	-0.1631	0.2669
	Ionic Low	-0.01341	1.000	-0.2284	0.2016
	Ionic High	-0.01472	1.000	-0.2297	0.2003
	PVP High	-0.05617	0.996	-0.2712	0.1588
	Uncoated Low	-0.05838	0.995	-0.2734	0.1566
	Uncoated High	-0.04998	0.998	-0.2650	0.1650
PVP High	Control	-0.01131	1.000	-0.2263	0.2037
	Ionic Maximum	0.16593	0.237	-0.0491	0.3809
	Weathered Low	-0.05857	0.995	-0.2736	0.1564
	Sulphidized Low, 120 nm	-0.04741	0.999	-0.2624	0.1676
	Sulphidized Low, 160 nm	0.10810	0.770	-0.1069	0.3231
	Ionic Low	0.04276	1.000	-0.1722	0.2578
	Ionic High	0.04145	1.000	-0.1735	0.2564
	PVP Low	0.05617	0.996	-0.1588	0.2712
	Uncoated Low	-0.00221	1.000	-0.2172	0.2128
	Uncoated High	0.00619	1.000	-0.2088	0.2212
Uncoated Low	Control	-0.00910	1.000	-0.2241	0.2059
	Ionic Maximum	0.16814	0.223	-0.0469	0.3831
	Weathered Low	-0.05636	0.996	-0.2714	0.1586
	Sulphidized Low, 120 nm	-0.04520	0.999	-0.2602	0.1698
	Sulphidized Low, 160 nm	0.11031	0.749	-0.1047	0.3253
	Ionic Low	0.04497	0.999	-0.1700	0.2600
	Ionic High	0.04366	1.000	-0.1713	0.2587

	PVP Low	0.05838	0.995	-0.1566	0.2734
	PVP High	0.00221	1.000	-0.2128	0.2172
	Uncoated High	0.00840	1.000	-0.2066	0.2234
Uncoated High	Control	-0.01751	1.000	-0.2325	0.1975
	Ionic Maximum	0.15973	0.280	-0.0553	0.3747
	Weathered Low	-0.06476	0.989	-0.2798	0.1502
	Sulphidized Low, 120 nm	-0.05360	0.997	-0.2686	0.1614
	Sulphidized Low, 160 nm	0.10191	0.823	-0.1131	0.3169
	Ionic Low	0.03657	1.000	-0.1784	0.2516
	Ionic High	0.03525	1.000	-0.1797	0.2502
	PVP Low	0.04998	0.998	-0.1650	0.2650
	PVP High	-0.00619	1.000	-0.2212	0.2088
	Uncoated Low	-0.00840	1.000	-0.2234	0.2066

Table D.53: Tukey test subset treatment groups for root exudates in Month 3

Treatment	N	Subsets	
		1	2
Ionic Maximum	3	0.0482	
Sulphidized Low, 160 nm	3	0.1060	0.1060
PVP Low	3	0.1580	0.1580
Ionic Low	3	0.1714	0.1714
Ionic High	3	0.1727	0.1727
Uncoated High	3	0.2080	0.2080
PVP High	3	0.2141	0.2141
Uncoated Low	3	0.2164	0.2164
Control	3	0.2255	0.2255
Sulphidized Low, 120 nm	3	0.2616	0.2616
Weathered Low	3		0.2727
Significance		0.053	0.232

Table D.54: One-way ANOVA for Month 1 enzyme assays

		Sum of Squares	DOF	Mean Square	F	Significance
Leucine aminopeptidase	Between Groups	1455.014	10	145.501	1.581	0.178
	Within Groups	2024.127	22	92.006		
	Total	3479.141	32			

Table D.55: Kruskal Wallis test for Month 1 β-glucosidase enzymatic activity

	β-glucosidase
X^2	17.783
DOF	10
Significance	0.059

Table D.56: One-way ANOVA for Month 2 enzyme assays

		Sum of Squares	DOF	Mean Square	F	Significance
β- glucosidase	Between Groups	401.349	10	40.135	0.895	0.553
	Within Groups	986.784	22	44.854		
	Total	1388.133	32			
α- glucosidase	Between Groups	118.058	10	11.806	0.368	0.948
	Within Groups	705.081	22	32.049		
	Total	823.140	32			
Xylosidase	Between Groups	116.310	10	11.631	0.361	0.951
	Within Groups	708.564	22	32.207		
	Total	824.874	32			
Cellobiosidase	Between Groups	113.946	10	11.395	0.365	0.949
	Within Groups	687.672	22	31.258		
	Total	801.618	32			
n-acetylglucosaminase	Between Groups	283.335	10	28.334	0.611	0.788
	Within Groups	1020.518	22	46.387		
	Total	1303.854	32			
Phosphatase	Between Groups	328.416	10	32.842	0.699	0.716
	Within Groups	1034.012	22	47.001		
	Total	1362.428	32			
Leucine aminopeptidase	Between Groups	81.375	10	8.138	0.361	0.951
	Total	496.519	22	22.569		

Table D.57: One-way ANOVA for Month 3 enzyme assays

		Sum of Squares	DOF	Mean Square	F	Significance
β- glucosidase	Between Groups	1345.976	10	134.598	1.041	0.444
	Within Groups	2845.656	22	129.348		
	Total	4191.632	32			
Phosphatase	Between Groups	5052.753	10	505.275	1.039	0.445
	Within Groups	10697.901	22	486.268		
	Total	15750.654	32			
Leucine aminopeptidase	Between Groups	4930.129	10	493.013	.892	0.555
	Total	12158.577	22	552.663		

Table D.58: Enzymatic activity (nmol/ g d.w. soil h) of various enzymes in soil treatments over three months of exposure

	Enzyme Activity (nmol/ g d.w. soil h)											
	β-glucosidase			α-glucosidase			Xylosidase			Cellobiosidase		
Treatment	Month 1	Month 2	Month 3	Month 1	Month 2	Month 3	Month 1	Month 2	Month 3	Month 1	Month 2	Month 3
Control	0.7±0.6	17.8±8.7	2.7±3.8	0.0±0.0	8.7±6.4	0.0±0.0	0.0±0.0	8.8±6.5	0.0±0.0	0.0±0.0	9.6±6.7	0.0±0.0
Sulphidized Low, 120 nm	3.2±2.3	20.9±7.3	0.0±0.0	0.0±0.0	11.0±8.4	0.0±0.0	0.0±0.0	11.1±8.4	0.0±0.0	0.0±0.0	12.0±8.4	0.0±0.0
Sulphidized Low, 160 nm	0.0±0.0	18.5±3.8	0.0±0.0	0.0±0.0	11.4±0.9	0.0±0.0	0.0±0.0	11.4±1.5	0.0±0.0	0.0±0.0	12.0±1.3	0.0±0.0
Weathered Low	8.1±7.3	16.6±2.3	22.5±30.0	0.0±0.0	12.1±0.9	0.0±0.0	0.0±0.0	12.1±0.9	0.0±0.0	0.0±0.0	12.7±0.9	0.0±0.0
Uncoated Low	0.4±0.6	18.9±6.3	0.0±0.0	0.0±0.0	10.3±3.2	0.0±0.0	0.0±0.0	10.3±3.3	0.0±0.0	0.0±0.0	11.2±3.5	0.0±0.0
PVP Low	0.0±0.0	18.9±7.1	0.0±0.0	0.0±0.0	12.4±0.0	0.0±0.0	0.0±0.0	12.4±0.0	0.0±0.0	0.0±0.0	13.6±0.8	0.0±0.0
Ionic Low	0.0±0.0	12.9±3.6	0.0±0.0	0.0±0.0	6.8±4.2	0.0±0.0	0.0±0.0	6.8±4.1	0.0±0.0	0.0±0.0	7.6±3.7	0.0±0.0
Uncoated High	0.7±1.0	23.4±3.9	4.1±5.8	0.0±0.0	12.1±2.6	0.0±0.0	0.0±0.0	12.2±2.6	0.0±0.0	0.0±0.0	13.1±2.7	0.0±0.0
PVP High	0.9±1.9	18.5±0.1	0.0±0.0	0.0±0.0	12.9±0.0	0.0±0.0	0.0±0.0	12.9±0.0	0.0±0.0	0.0±0.0	13.7±0.0	0.0±0.0
Ionic High	8.0±2.4	24.6±2.5	0.3±0.4	0.0±0.0	11.8±3.3	0.0±0.0	0.0±0.0	11.8±3.3	0.0±0.0	0.0±0.0	12.9±3.1	0.0±0.0
Ionic Maximum	3.1±2.2	25.3±5.6	0.5±0.7	0.0±0.0	8.1±2.4	0.0±0.0	0.0±0.0	8.2±2.4	0.0±0.0	0.0±0.0	9.5±2.2	0.0±0.0

	Enzyme Activity (nmol/ g d.w. soil h)								
	N-acetylglucosaminadase			Phosphatase			Leucine aminopeptidase		
Treatment	Month 1	Month 2	Month 3	Month 1	Month 2	Month 3	Month 1	Month 2	Month 3
Control	0.0±0.0	12.9±7.6	0.0±0.0	0.0±0.0	22.6±8.7	12.9±18.2	34.3±8.4	3.2±4.5	0.0±0.0
Sulphidized Low, 120 nm	0.0±0.0	13.6±0.2	0.0±0.0	0.0±0.0	28.1±8.6	20.9±3.5	24.0±1.8	1.2±1.7	23.3±18.0
Sulphidized Low, 160 nm	0.0±0.0	16.1±9.4	0.0±0.0	0.0±0.0	22.8±3.9	0.0±0.0	31.7±5.6	5.5±7.7	30.8±43.6
Weathered Low	0.0±0.0	17.0±5.5	0.0±0.0	0.0±0.0	21.6±1.8	44.1±48.1	14.2±10.1	1.4±2.0	33.4±23.2
Uncoated Low	0.0±0.0	13.3±4.3	0.0±0.0	0.0±0.0	30.3±8.4	5.7±4.1	23.0±4.5	0.0±0.0	12.1±17.1
PVP Low	0.0±0.0	14.8±0.5	0.0±0.0	0.0±0.0	24.0±0.1	6.1±0.8	17.3±1.7	3.5±0.2	1.6±0.2
Ionic Low	0.0±0.0	8.9±6.4	0.0±0.0	0.0±0.0	19.0±3.4	11.2±15.8	27.4±3.2	3.5±0.0	0.0±0.0
Uncoated High	0.0±0.0	16.0±3.2	0.0±0.0	0.0±0.0	25.0±1.9	31.9±17.0	15.2±10.8	2.6±2.4	0.9±1.2
PVP High	0.0±0.0	17.6±2.9	0.0±0.0	0.0±0.0	23.4±0.1	11.1±1.9	14.7±0.8	1.2±0.2	18.3±0.9
Ionic High	0.0±0.0	17.1±3.2	0.0±0.0	0.0±0.0	26.8±6.5	16.3±7.7	27.6±5.5	4.8±5.0	0.0±0.0
Ionic Maximum	0.0±0.0	20.6±6.1	0.0±0.0	0.0±0.0	19.7±3.3	7.5±10.6	23.5±3.6	2.9±4.1	12.5±17.7

Table D.59: One-way ANOVA for heterotrophic plate count

	Sum of Squares	DOF	Mean Square	F	Significance
Between Groups	1.5E+15	10	1.5E+14	0.981	0.487
Within Groups	3.4E+15	22	1.5E+14		
Total	4.9E+15	32			

Table D.60: One-way ANOVA for substrate-induced respiration

	Sum of Squares	DOF	Mean Square	F	Significance
Between Groups	364.617	10	36.462	6.201	0.000
Within Groups	129.363	22	5.880		
Total	493.980	32			

Table D.61: Multiple comparisons of substrate-induced respiration using Tukey's test

Treatment (I)	Treatment (J)	Mean Difference (I-J)	Significance	95% Confidence Interval	
				Lower Bound	Upper Bound
Control	Ionic Low	-0.5719	1.000	-7.6498	6.5059
	Ionic High	-0.7475	1.000	-7.8253	6.3303
	Ionic Maximum	7.0053	0.054	-0.0725	14.0832
	PVP Low	-2.3133	0.980	-9.3912	4.7645
	PVP High	-4.9355	0.359	-12.0133	2.1424
	Sulphidized Low, 120 nm	-2.7812	0.934	-9.8590	4.2966
	Sulphidized Low, 160 nm	-3.7585	0.712	-10.8363	3.3193
	Uncoated Low	-3.2625	0.845	-10.3403	3.8154
	Uncoated High	-0.3637	1.000	-7.4415	6.7142
	Weathered Low	3.8556	0.683	-3.2222	10.9334
Ionic Low	Control	0.5719	1.000	-6.5059	7.6498
	Ionic High	-0.1756	1.000	-7.2534	6.9023
	Ionic Maximum	7.5773*	0.029	0.4994	14.6551
	PVP Low	-1.7414	0.998	-8.8192	5.3364
	PVP High	-4.3636	0.525	-11.4414	2.7143
	Sulphidized Low, 120 nm	-2.2093	0.985	-9.2871	4.8686
	Sulphidized Low, 160 nm	-3.1866	0.862	-10.2644	3.8913
	Uncoated Low	-2.6905	0.946	-9.7684	4.3873
	Uncoated High	0.2083	1.000	-6.8696	7.2861
	Weathered Low	4.4275	0.505	-2.6503	11.5054

Ionic High	Control	0.7475	1.000	-6.3303	7.8253
	Ionic Low	0.1756	1.000	-6.9023	7.2534
	Ionic Maximum	7.7528*	0.024	0.6750	14.8307
	PVP Low	-1.5658	0.999	-8.6437	5.5120
	PVP High	-4.1880	0.579	-11.2658	2.8899
	Sulphidized Low, 120 nm	-2.0337	0.992	-9.1116	5.0441
	Sulphidized Low, 160 nm	-3.0110	0.897	-10.0888	4.0668
	Uncoated Low	-2.5150	0.965	-9.5928	4.5629
	Uncoated High	0.3838	1.000	-6.6940	7.4617
	Weathered Low	4.6031	0.452	-2.4748	11.6809
Ionic Maximum	Control	-7.0053	0.054	-14.0832	0.0725
	Ionic Low	-7.5773*	0.029	-14.6551	-0.4994
	Ionic High	-7.7528*	0.024	-14.8307	-0.6750
	PVP Low	-9.3187*	0.004	-16.3965	-2.2408
	PVP High	-11.9408*	0.000	-19.0187	-4.8630
	Sulphidized Low, 120 nm	-9.7866*	0.002	-16.8644	-2.7087
	Sulphidized Low, 160 nm	-10.7638*	0.001	-17.8417	-3.6860
	Uncoated Low	-10.2678*	0.001	-17.3457	-3.1900
	Uncoated High	-7.3690*	0.037	-14.4469	-0.2912
	Weathered Low	-3.1498	0.870	-10.2276	3.9281
PVP Low	Control	2.3133	0.980	-4.7645	9.3912
	Ionic Low	1.7414	0.998	-5.3364	8.8192
	Ionic High	1.5658	0.999	-5.5120	8.6437
	Ionic Maximum	9.3187*	0.004	2.2408	16.3965
	PVP High	-2.6222	0.954	-9.7000	4.4557
	Sulphidized Low, 120 nm	-0.4679	1.000	-7.5457	6.6100
	Sulphidized Low, 160 nm	-1.4452	0.999	-8.5230	5.6327
	Uncoated Low	-0.9492	1.000	-8.0270	6.1287
	Uncoated High	1.9496	0.994	-5.1282	9.0275
	Weathered Low	6.1689	0.125	-0.9089	13.2468
PVP High	Control	4.9355	0.359	-2.1424	12.0133
	Ionic Low	4.3636	0.525	-2.7143	11.4414
	Ionic High	4.1880	0.579	-2.8899	11.2658
	Ionic Maximum	11.9408*	0.000	4.8630	19.0187
	PVP Low	2.6222	0.954	-4.4557	9.7000
	Sulphidized Low, 120 nm	2.1543	0.988	-4.9236	9.2321
	Sulphidized Low, 160 nm	1.1770	1.000	-5.9009	8.2548
	Uncoated Low	1.6730	0.998	-5.4048	8.7509

	Uncoated High	4.5718	0.461	-2.5060	11.6497
	Weathered Low	8.7911*	0.007	1.7132	15.8689
Sulphidized Low, 120 nm	Control	2.7812	0.934	-4.2966	9.8590
	Ionic Low	2.2093	0.985	-4.8686	9.2871
	Ionic High	2.0337	0.992	-5.0441	9.1116
	Ionic Maximum	9.7866*	0.002	2.7087	16.8644
	PVP Low	0.4679	1.000	-6.6100	7.5457
	PVP High	-2.1543	0.988	-9.2321	4.9236
	Sulphidized Low, 160 nm	-0.9773	1.000	-8.0551	6.1006
	Uncoated Low	-0.4813	1.000	-7.5591	6.5966
	Uncoated High	2.4175	0.973	-4.6603	9.4954
	Weathered Low	6.6368	0.079	-0.4410	13.7146
Sulphidized Low, 160 nm	Control	3.7585	0.712	-3.3193	10.8363
	Ionic Low	3.1866	0.862	-3.8913	10.2644
	Ionic High	3.0110	0.897	-4.0668	10.0888
	Ionic Maximum	10.7638*	0.001	3.6860	17.8417
	PVP Low	1.4452	0.999	-5.6327	8.5230
	PVP High	-1.1770	1.000	-8.2548	5.9009
	Sulphidized Low, 120 nm	0.9773	1.000	-6.1006	8.0551
	Uncoated Low	0.4960	1.000	-6.5818	7.5739
	Uncoated High	3.3948	0.813	-3.6830	10.4727
	Weathered Low	7.6141*	0.028	0.5362	14.6919
Uncoated Low	Control	3.2625	0.845	-3.8154	10.3403
	Ionic Low	2.6905	0.946	-4.3873	9.7684
	Ionic High	2.5150	0.965	-4.5629	9.5928
	Ionic Maximum	10.2678*	0.001	3.1900	17.3457
	PVP Low	0.9492	1.000	-6.1287	8.0270
	PVP High	-1.6730	0.998	-8.7509	5.4048
	Sulphidized Low, 120 nm	0.4813	1.000	-6.5966	7.5591
	Sulphidized Low, 160 nm	-0.4960	1.000	-7.5739	6.5818
	Uncoated High	2.8988	0.917	-4.1790	9.9766
	Weathered Low	7.1181*	0.048	0.0402	14.1959
Uncoated High	Control	0.3637	1.000	-6.7142	7.4415
	Ionic Low	-0.2083	1.000	-7.2861	6.8696
	Ionic High	-0.3838	1.000	-7.4617	6.6940
	Ionic Maximum	7.3690*	0.037	0.2912	14.4469
	PVP Low	-1.9496	0.994	-9.0275	5.1282
	PVP High	-4.5718	0.461	-11.6497	2.5060
	Sulphidized Low, 120 nm	-2.4175	0.973	-9.4954	4.6603
	Sulphidized Low, 160 nm	-3.3948	0.813	-10.4727	3.6830

	Uncoated Low	-2.8988	0.917	-9.9766	4.1790
	Weathered Low	4.2193	0.570	-2.8586	11.2971
Weathered Low	Control	-3.8556	0.683	-10.9334	3.2222
	Ionic Low	-4.4275	0.505	-11.5054	2.6503
	Ionic High	-4.6031	0.452	-11.6809	2.4748
	Ionic Maximum	3.1498	0.870	-3.9281	10.2276
	PVP Low	-6.1689	0.125	-13.2468	0.9089
	PVP High	-8.7911*	0.007	-15.8689	-1.7132
	Sulphidized Low, 120 nm	-6.6368	0.079	-13.7146	0.4410
	Sulphidized Low, 160 nm	-7.6141*	0.028	-14.6919	-0.5362
	Uncoated Low	-7.1181*	0.048	-14.1959	-0.0402
	Uncoated High	-4.2193	0.570	-11.2971	2.8586

Table D.62: Tukey test subset treatment groups for substrate-induced respiration

Treatment	N	Subsets		
		1	2	3
Ionic Maximum	3	8.0985		
Weathered Low	3	11.2483	11.2483	
Control	3	15.1039	15.1039	15.1039
Uncoated High	3		15.4675	15.4675
Ionic Low	3		15.6758	15.6758
Ionic High	3		15.8514	15.8514
PVP Low	3		17.4172	17.4172
Sulphidized Low, 120 nm	3		17.8851	17.8851
Uncoated Low	3			18.3663
Sulphidized Low, 160 nm	3			18.8624
PVP High	3			20.0394
Significance		0.054	0.079	0.359

Table D.63: One-way ANOVA of DNA extracted from treatments after three months of exposure

	Sum of Squares	DOF	Mean Square	F	Significance
Between Groups	457680.323	11	41607.302	1.711	0.14
Within Groups	510806.669	21	24324.127		
Total	968486.992	32			

Table D.64: One-way ANOVA of Shannon diversity index, species richness and evenness from metagenomic sequencing after three months exposure

		Sum of Squares	DOF	Mean Square	F	Significance
Shannon Diversity Index (H)	Between Groups	0.031	10	0.003	3.090	0.013
	Within Groups	0.022	22	0.001		
	Total	0.053	32			
Species Richness (S)	Between Groups	45094.727	10	4509.473	0.248	0.987
	Within Groups	400221.333	22	18191.879		
	Total	445316.061	32			
Evenness (E)	Between Groups	0.001	10	6.250E-05	0.992	0.479
	Within Groups	0.001	22	6.299E-05		
	Total	0.002	32			

Table D.65: Multiple comparisons for Shannon diversity index using Tukey's test

Treatment (I)	Treatment (J)	Mean Difference (I-J)	Significance	95% Confidence Interval	
				Lower Bound	Upper Bound
Control	Ionic Low	-0.0470	0.754	-0.1390	0.0450
	Ionic High	-0.0593	0.464	-0.1513	0.0327
	Ionic Maximum	-0.1040*	0.018	-0.1960	-0.0120
	PVP Low	-0.0410	0.869	-0.1330	0.0510
	PVP High	-0.0407	0.875	-0.1327	0.0513
	Sulphidized Low, 120 nm	-0.0110	1.000	-0.1030	0.0810
	Sulphidized Low, 160 nm	-0.0357	0.940	-0.1277	0.0563
	Uncoated Low	-0.0547	0.574	-0.1467	0.0373
	Uncoated High	-0.0500	0.686	-0.1420	0.0420
	Weathered Low	-0.1030*	0.020	-0.1950	-0.0110
Ionic Low	Control	0.0470	0.754	-0.0450	0.1390
	Ionic High	-0.0123	1.000	-0.1043	0.0797
	Ionic Maximum	-0.0570	0.518	-0.1490	0.0350
	PVP Low	0.0060	1.000	-0.0860	0.0980
	PVP High	0.0063	1.000	-0.0857	0.0983
	Sulphidized Low, 120 nm	0.0360	0.936	-0.0560	0.1280
	Sulphidized Low, 160 nm	0.0113	1.000	-0.0807	0.1033
	Uncoated Low	-0.0077	1.000	-0.0997	0.0843
	Uncoated High	-0.0030	1.000	-0.0950	0.0890
	Weathered Low	-0.0560	0.542	-0.1480	0.0360
Ionic High	Control	0.0593	0.464	-0.0327	0.1513
	Ionic Low	0.0123	1.000	-0.0797	0.1043

	Ionic Maximum	-0.0447	0.803	-0.1367	0.0473
	PVP Low	0.0183	1.000	-0.0737	0.1103
	PVP High	0.0187	1.000	-0.0733	0.1107
	Sulphidized Low, 120 nm	0.0483	0.724	-0.0437	0.1403
	Sulphidized Low, 160 nm	0.0237	0.997	-0.0683	0.1157
	Uncoated Low	0.0047	1.000	-0.0873	0.0967
	Uncoated High	0.0093	1.000	-0.0827	0.1013
	Weathered Low	-0.0437	0.822	-0.1357	0.0483
Ionic Maximum	Control	0.1040*	0.018	0.0120	0.1960
	Ionic Low	0.0570	0.518	-0.0350	0.1490
	Ionic High	0.0447	0.803	-0.0473	0.1367
	PVP Low	0.0630	0.383	-0.0290	0.1550
	PVP High	0.0633	0.376	-0.0287	0.1553
	Sulphidized Low, 120 nm	0.0930*	0.046	0.0010	0.1850
	Sulphidized Low, 160 nm	0.0683	0.281	-0.0237	0.1603
	Uncoated Low	0.0493	0.701	-0.0427	0.1413
	Uncoated High	0.0540	0.590	-0.0380	0.1460
	Weathered Low	0.0010	1.000	-0.0910	0.0930
PVP Low	Control	0.0410	0.869	-0.0510	0.1330
	Ionic Low	-0.0060	1.000	-0.0980	0.0860
	Ionic High	-0.0183	1.000	-0.1103	0.0737
	Ionic Maximum	-0.0630	0.383	-0.1550	0.0290
	PVP High	0.0003	1.000	-0.0917	0.0923
	Sulphidized Low, 120 nm	0.0300	0.980	-0.0620	0.1220
	Sulphidized Low, 160 nm	0.0053	1.000	-0.0867	0.0973
	Uncoated Low	-0.0137	1.000	-0.1057	0.0783
	Uncoated High	-0.0090	1.000	-0.1010	0.0830
	Weathered Low	-0.0620	0.404	-0.1540	0.0300
PVP High	Control	0.0407	0.875	-0.0513	0.1327
	Ionic Low	-0.0063	1.000	-0.0983	0.0857
	Ionic High	-0.0187	1.000	-0.1107	0.0733
	Ionic Maximum	-0.0633	0.376	-0.1553	0.0287
	PVP Low	-0.0003	1.000	-0.0923	0.0917
	Sulphidized Low, 120 nm	0.0297	0.982	-0.0623	0.1217
	Sulphidized Low, 160 nm	0.0050	1.000	-0.0870	0.0970
	Uncoated Low	-0.0140	1.000	-0.1060	0.0780
	Uncoated High	-0.0093	1.000	-0.1013	0.0827
	Weathered Low	-0.0623	0.397	-0.1543	0.0297

Sulphidized Low, 120 nm	Control	0.0110	1.000	-0.0810	0.1030
	Ionic Low	-0.0360	0.936	-0.1280	0.0560
	Ionic High	-0.0483	0.724	-0.1403	0.0437
	Ionic Maximum	-0.0930*	0.046	-0.1850	-0.0010
	PVP Low	-0.0300	0.980	-0.1220	0.0620
	PVP High	-0.0297	0.982	-0.1217	0.0623
	Sulphidized Low, 160 nm	-0.0247	0.995	-0.1167	0.0673
	Uncoated Low	-0.0437	0.822	-0.1357	0.0483
	Uncoated High	-0.0390	0.899	-0.1310	0.0530
	Weathered Low	-0.0920	0.050	-0.1840	0.0000
Sulphidized Low, 160 nm	Control	0.0357	0.940	-0.0563	0.1277
	Ionic Low	-0.0113	1.000	-0.1033	0.0807
	Ionic High	-0.0237	0.997	-0.1157	0.0683
	Ionic Maximum	-0.0683	0.281	-0.1603	0.0237
	PVP Low	-0.0053	1.000	-0.0973	0.0867
	PVP High	-0.0050	1.000	-0.0970	0.0870
	Sulphidized Low, 120 nm	0.0247	0.995	-0.0673	0.1167
	Uncoated Low	-0.0190	0.999	-0.1110	0.0730
	Uncoated High	-0.0143	1.000	-0.1063	0.0777
	Weathered Low	-0.0673	0.298	-0.1593	0.0247
Uncoated Low	Control	0.0547	0.574	-0.0373	0.1467
	Ionic Low	0.0077	1.000	-0.0843	0.0997
	Ionic High	-0.0047	1.000	-0.0967	0.0873
	Ionic Maximum	-0.0493	0.701	-0.1413	0.0427
	PVP Low	0.0137	1.000	-0.0783	0.1057
	PVP High	0.0140	1.000	-0.0780	0.1060
	Sulphidized Low, 120 nm	0.0437	0.822	-0.0483	0.1357
	Sulphidized Low, 160 nm	0.0190	0.999	-0.0730	0.1110
	Uncoated High	0.0047	1.000	-0.0873	0.0967
	Weathered Low	-0.0483	0.724	-0.1403	0.0437
Uncoated High	Control	0.0500	0.686	-0.0420	0.1420
	Ionic Low	0.0030	1.000	-0.0890	0.0950
	Ionic High	-0.0093	1.000	-0.1013	0.0827
	Ionic Maximum	-0.0540	0.590	-0.1460	0.0380
	PVP Low	0.0090	1.000	-0.0830	0.1010
	PVP High	0.0093	1.000	-0.0827	0.1013
	Sulphidized Low, 120 nm	0.0390	0.899	-0.0530	0.1310
	Sulphidized Low, 160 nm	0.0143	1.000	-0.0777	0.1063
	Uncoated Low	-0.0047	1.000	-0.0967	0.0873
	Weathered Low	-0.0530	0.614	-0.1450	0.0390

Weathered Low	Control	0.1030*	0.020	0.0110	0.1950
	Ionic Low	0.0560	0.542	-0.0360	0.1480
	Ionic High	0.0437	0.822	-0.0483	0.1357
	Ionic Maximum	-0.0010	1.000	-0.0930	0.0910
	PVP Low	0.0620	0.404	-0.0300	0.1540
	PVP High	0.0623	0.397	-0.0297	0.1543
	Sulphidized Low, 120 nm	0.0920	0.050	0.0000	0.1840
	Sulphidized Low, 160 nm	0.0673	0.298	-0.0247	0.1593
	Uncoated Low	0.0483	0.724	-0.0437	0.1403
	Uncoated High	0.0530	0.614	-0.0390	0.1450

Table D.66: Tukey test subset treatment groups for Shannon diversity index

Treatment	N	Subsets		
		1	2	3
Control	3	2.3983		
Sulphidized Low, 120 nm	3	2.4093	2.4093	
Sulphidized Low, 160 nm	3	2.4340	2.4340	2.4340
PVP High	3	2.4390	2.4390	2.4390
PVP Low	3	2.4393	2.4393	2.4393
Ionic Low	3	2.4453	2.4453	2.4453
Uncoated High	3	2.4483	2.4483	2.4483
Uncoated Low	3	2.4530	2.4530	2.4530
Ionic High	3	2.4577	2.4577	2.4577
Weathered Low	3		2.5013	2.5013
Ionic Maximum	3			2.5023
Significance		0.464	0.050	0.281

Table D.67: One-way ANOVA of relative abundance of *R. limosa*, *F. alni*, *A. malthae* and *X. oryzae* from DNA sequencing after three month's exposure

		Sum of Squares	DOF	Mean Square	F	Significance
R. limosa	Between Groups	13.643	10	1.364	8.668	0.000
	Within Groups	3.463	22	0.157		
	Total	17.106	32			
B. pachyrhizi	Between Groups	0.149	10	0.015	15.410	0.000
	Within Groups	0.021	22	0.001		
	Total	0.171	32			
F. alni	Between Groups	4.183	10	0.418	6.592	0.000
	Within Groups	1.396	22	0.063		
	Total	5.580	32			
A. malthae	Between Groups	13.521	10	1.352	7.628	0.000
	Within Groups	3.900	22	0.177		
	Total	17.420	32			
X. oryzae	Between Groups	0.021	10	0.002	10.564	0.000
	Within Groups	0.004	22	0.000		
	Total	0.025	32			

Table D.68: Tukey test subset treatment groups for *R. limosa* abundance

Treatment	N	Subsets	
		1	2
Control	3	0.2380	
Sulphidized Low, 120 nm	3	0.2433	
PVP Low	3	0.2606	
Uncoated Low	3	0.2625	
Sulphidized Low, 160 nm	3	0.2635	
Ionic Low	3	0.2721	
Weathered Low	3	0.3054	
Uncoated High	3	0.3229	
PVP High	3	0.3768	
Ionic High	3	0.4852	
Ionic Maximum	3		2.5267
Significance		0.999	1.000

Table D.69: Tukey test subset treatment groups for *B. pachyrhizi* abundance

Treatment	N	Subsets	
		1	2
Control	3	0.2601	
Weathered Low	3	0.2638	
Sulphidized Low, 120 nm	3	0.2666	
Uncoated Low	3	0.2726	
Sulphidized Low, 160 nm	3	0.2784	
Uncoated High	3	0.2816	
PVP Low	3	0.2909	
Ionic Low	3	0.2931	
PVP High	3	0.3000	
Ionic High	3	0.3353	
Ionic Maximum	3		0.5074
Significance		0.167	1.000

Table D.70: Tukey test subset treatment groups for *F. alni* abundance

Treatment	N	Subsets	
		1	2
Weathered Low	3	0.1583	
Sulphidized Low, 120 nm	3	0.1612	
Ionic Low	3	0.1619	
Control	3	0.1646	
Sulphidized Low, 160 nm	3	0.1697	
PVP Low	3	0.1796	
Uncoated High	3	0.1887	
PVP High	3	0.1966	
Uncoated Low	3	0.2001	
Ionic High	3	0.2922	
Ionic Maximum	3		1.4194
Significance		1.000	1.000

Table D.71: Tukey test subset treatment groups for *A. malthae* abundance

Treatment	N	Subsets	
		1	2
Uncoated High	3	0.0518	
Sulphidized Low, 120 nm	3	0.0519	
Control	3	0.0553	
Ionic Low	3	0.0604	
Weathered Low	3	0.0656	
PVP Low	3	0.0689	
Sulphidized Low, 160 nm	3	0.0706	
Uncoated Low	3	0.0742	
PVP High	3	0.1319	
Ionic High	3	0.2038	
Ionic Maximum	3		2.3048
Significance		1.000	1.000

Table D.72: Tukey test subset treatment groups for *X. oryzae* abundance

Treatment	N	Subsets	
		1	2
Control	3	0.0024	
Ionic Low	3	0.0028	
Weathered Low	3	0.0029	
Uncoated Low	3	0.0030	
Sulphidized Low, 120 nm	3	0.0032	
Uncoated High	3	0.0038	
PVP Low	3	0.0043	
Sulphidized Low, 160 nm	3	0.0050	
Ionic High	3	0.0089	
PVP High	3	0.0098	
Ionic Maximum	3		0.0919
Significance		1.000	1.000

Table D.73: One-way ANOVA of plant germination rate

	Sum of squares	DOF	Mean square	F	Significance
Between Groups	156.7273	10	15.67273	0.849261	0.583117
Within Groups	1624	88	18.45455		
Total	1780.727	98			

Table D. 74: One-way ANOVA of shoot biomass

		Sum of Squares	DOF	Mean Square	F	Significance
Month 1	Between Groups	0.025	10	0.002	0.555	0.832
	Within Groups	0.098	22	0.004		
	Total	0.123	32			
Month 2	Between Groups	0.327	10	0.033	2.168	0.063
	Within Groups	0.332	22	0.015		
	Total	0.659	32			
Month 3	Between Groups	0.210	10	0.021	0.664	0.745
	Within Groups	0.695	22	0.032		
	Total	0.905	32			

Table D. 75: One-way ANOVA of shoot silver concentrations

		Sum of Squares	DOF	Mean Square	F	Significance
Month 1	Between Groups	4.629	10	0.463	4.322	0.002
	Within Groups	2.356	22	0.107		
	Total	6.986	32			
Month 2	Between Groups	20.542	10	2.054	3.631	0.006
	Within Groups	12.448	22	0.566		
	Total	32.990	32			
Month 3	Between Groups	1.544	10	0.154	1.094	0.408
	Within Groups	3.105	22	0.141		
	Total	4.650	32			

Table D.76: Multiple comparisons for shoot silver concentrations in Month 1 treatments using Tukey's test

Treatment (I)	Treatment (J)	Mean Difference (I-J)	Significance	95% Confidence Interval	
				Lower Bound	Upper Bound
Control	Ionic Low	-0.9617*	0.048	-1.9169	-0.0064
	Ionic High	-0.5926	0.516	-1.5478	0.3627
	Ionic Maximum	-1.2154*	0.006	-2.1707	-0.2601
	PVP Low	-0.3371	0.966	-1.2923	0.6182
	PVP High	-0.3488	0.958	-1.3041	0.6065
	Sulphidized Low, 120 nm	-0.2731	0.992	-1.2284	0.6821
	Sulphidized Low, 160 nm	-0.9089	0.072	-1.8642	0.0464
	Uncoated Low	-0.1544	1.000	-1.1097	0.8009
	Uncoated High	-0.2651	0.994	-1.2204	0.6902
	Weathered Low	-0.1467	1.000	-1.1019	0.8086
	Control	0.9617*	0.048	0.0064	1.9169

Ionic Low	Ionic High	0.3691	0.941	-0.5862	1.3244
	Ionic Maximum	-0.2537	0.996	-1.2090	0.7015
	PVP Low	0.6246	0.445	-0.3307	1.5799
	PVP High	0.6129	0.471	-0.3424	1.5681
	Sulphidized Low, 120 nm	0.6885	0.317	-0.2668	1.6438
	Sulphidized Low, 160 nm	0.0528	1.000	-0.9025	1.0081
	Uncoated Low	0.8073	0.150	-0.1480	1.7625
	Uncoated High	0.6966	0.303	-0.2587	1.6518
	Weathered Low	0.8150	0.142	-0.1403	1.7703
Ionic High	Control	0.5926	0.516	-0.3627	1.5478
	Ionic Low	-0.3691	0.941	-1.3244	0.5862
	Ionic Maximum	-0.6228	0.449	-1.5781	0.3324
	PVP Low	0.2555	0.995	-0.6998	1.2108
	PVP High	0.2438	0.997	-0.7115	1.1990
	Sulphidized Low, 120 nm	0.3194	0.976	-0.6359	1.2747
	Sulphidized Low, 160 nm	-0.3163	0.978	-1.2716	0.6390
	Uncoated Low	0.4382	0.849	-0.5171	1.3934
	Uncoated High	0.3275	0.972	-0.6278	1.2827
	Weathered Low	0.4459	0.836	-0.5094	1.4012
Ionic Maximum	Control	1.2154*	0.006	0.2601	2.1707
	Ionic Low	0.2537	0.996	-0.7015	1.2090
	Ionic High	0.6228	0.449	-0.3324	1.5781
	PVP Low	0.8783	0.090	-0.0769	1.8336
	PVP High	0.8666	0.098	-0.0887	1.8219
	Sulphidized Low, 120 nm	0.9423	0.055	-0.0130	1.8975
	Sulphidized Low, 160 nm	0.3065	0.982	-0.6488	1.2618
	Uncoated Low	1.0610*	0.021	0.1057	2.0163
	Uncoated High	0.9503	0.052	-0.0050	1.9056
	Weathered Low	1.0688*	0.020	0.1135	2.0240
PVP Low	Control	0.3371	0.966	-0.6182	1.2923
	Ionic Low	-0.6246	0.445	-1.5799	0.3307
	Ionic High	-0.2555	0.995	-1.2108	0.6998
	Ionic Maximum	-0.8783	0.090	-1.8336	0.0769
	PVP High	-0.0117	1.000	-0.9670	0.9435
	Sulphidized Low, 120 nm	0.0639	1.000	-0.8914	1.0192
	Sulphidized Low, 160 nm	-0.5718	0.564	-1.5271	0.3835
	Uncoated Low	0.1827	1.000	-0.7726	1.1379
	Uncoated High	0.0720	1.000	-0.8833	1.0272

	Weathered Low	0.1904	1.000	-0.7649	1.1457
PVP High	Control	0.3488	0.958	-0.6065	1.3041
	Ionic Low	-0.6129	0.471	-1.5681	0.3424
	Ionic High	-0.2438	0.997	-1.1990	0.7115
	Ionic Maximum	-0.8666	0.098	-1.8219	0.0887
	PVP Low	0.0117	1.000	-0.9435	0.9670
	Sulphidized Low, 120 nm	0.0757	1.000	-0.8796	1.0309
	Sulphidized Low, 160 nm	-0.5601	0.591	-1.5154	0.3952
	Uncoated Low	0.1944	1.000	-0.7609	1.1497
	Uncoated High	0.0837	1.000	-0.8716	1.0390
	Weathered Low	0.2022	0.999	-0.7531	1.1574
Sulphidized Low, 120 nm	Control	0.2731	0.992	-0.6821	1.2284
	Ionic Low	-0.6885	0.317	-1.6438	0.2668
	Ionic High	-0.3194	0.976	-1.2747	0.6359
	Ionic Maximum	-0.9423	0.055	-1.8975	0.0130
	PVP Low	-0.0639	1.000	-1.0192	0.8914
	PVP High	-0.0757	1.000	-1.0309	0.8796
	Sulphidized Low, 160 nm	-0.6357	0.421	-1.5910	0.3195
	Uncoated Low	0.1188	1.000	-0.8365	1.0740
	Uncoated High	0.0081	1.000	-0.9472	0.9633
	Weathered Low	0.1265	1.000	-0.8288	1.0818
Sulphidized Low, 160 nm	Control	0.9089	0.072	-0.0464	1.8642
	Ionic Low	-0.0528	1.000	-1.0081	0.9025
	Ionic High	0.3163	0.978	-0.6390	1.2716
	Ionic Maximum	-0.3065	0.982	-1.2618	0.6488
	PVP Low	0.5718	0.564	-0.3835	1.5271
	PVP High	0.5601	0.591	-0.3952	1.5154
	Sulphidized Low, 120 nm	0.6357	0.421	-0.3195	1.5910
	Uncoated Low	0.7545	0.212	-0.2008	1.7098
	Uncoated High	0.6438	0.404	-0.3115	1.5991
	Weathered Low	0.7622	0.202	-0.1930	1.7175
Uncoated Low	Control	0.1544	1.000	-0.8009	1.1097
	Ionic Low	-0.8073	0.150	-1.7625	0.1480
	Ionic High	-0.4382	0.849	-1.3934	0.5171
	Ionic Maximum	-1.0610*	0.021	-2.0163	-0.1057
	PVP Low	-0.1827	1.000	-1.1379	0.7726
	PVP High	-0.1944	1.000	-1.1497	0.7609
	Sulphidized Low, 120 nm	-0.1188	1.000	-1.0740	0.8365
	Sulphidized Low, 160 nm	-0.7545	0.212	-1.7098	0.2008
	Uncoated High	-0.1107	1.000	-1.0660	0.8446

	Weathered Low	0.0077	1.000	-0.9475	0.9630
Uncoated High	Control	0.2651	0.994	-0.6902	1.2204
	Ionic Low	-0.6966	0.303	-1.6518	0.2587
	Ionic High	-0.3275	0.972	-1.2827	0.6278
	Ionic Maximum	-0.9503	0.052	-1.9056	0.0050
	PVP Low	-0.0720	1.000	-1.0272	0.8833
	PVP High	-0.0837	1.000	-1.0390	0.8716
	Sulphidized Low, 120 nm	-0.0081	1.000	-0.9633	0.9472
	Sulphidized Low, 160 nm	-0.6438	0.404	-1.5991	0.3115
	Uncoated Low	0.1107	1.000	-0.8446	1.0660
	Weathered Low	0.1184	1.000	-0.8368	1.0737
Weathered Low	Control	0.1467	1.000	-0.8086	1.1019
	Ionic Low	-0.8150	0.142	-1.7703	0.1403
	Ionic High	-0.4459	0.836	-1.4012	0.5094
	Ionic Maximum	-1.0688*	0.020	-2.0240	-0.1135
	PVP Low	-0.1904	1.000	-1.1457	0.7649
	PVP High	-0.2022	0.999	-1.1574	0.7531
	Sulphidized Low, 120 nm	-0.1265	1.000	-1.0818	0.8288
	Sulphidized Low, 160 nm	-0.7622	0.202	-1.7175	0.1930
	Uncoated Low	-0.0077	1.000	-0.9630	0.9475
	Uncoated High	-0.1184	1.000	-1.0737	0.8368

Table D.77: Tukey test subset treatment groups for shoot silver concentrations in Month 1

Treatment	N	Subset		
		1	2	3
Control	3	0.4135		
Weathered Low	3	0.5601	0.5601	
Uncoated Low	3	0.5679	0.5679	
Uncoated High	3	0.6786	0.6786	0.6786
Sulphidized Low, 122nm	3	0.6866	0.6866	0.6866
PVP Low	3	0.7505	0.7505	0.7505
PVP High	3	0.7623	0.7623	0.7623
Ionic High	3	1.0060	1.0060	1.0060
Sulphidized Low, 156nm	3	1.3224	1.3224	1.3224
Ionic Low	3		1.3751	1.3751
Ionic Maximum	3			1.6289
Significance		0.072	0.142	0.052

Table D.78: Multiple comparisons for shoot silver concentrations in Month 2 treatments using Tukey's test

Treatment (I)	Treatment (J)	Mean Difference (I-J)	Significance	95% Confidence Interval	
				Lower Bound	Upper Bound
Control	Ionic Low	0.1904	1.000	-2.0051	2.3860
	Ionic High	0.6777	0.987	-1.5178	2.8732
	Ionic Maximum	-1.7217	0.220	-3.9173	0.4738
	PVP Low	0.8498	0.940	-1.3458	3.0453
	PVP High	-0.1046	1.000	-2.3001	2.0910
	Sulphidized Low, 120 nm	0.3746	1.000	-1.8209	2.5702
	Sulphidized Low, 160 nm	0.9960	0.857	-1.1995	3.1915
	Uncoated Low	0.8160	0.953	-1.3795	3.0115
	Uncoated High	-0.9866	0.864	-3.1821	1.2089
	Weathered Low	0.1566	1.000	-2.0389	2.3522
Ionic Low	Control	-0.1904	1.000	-2.3860	2.0051
	Ionic High	0.4873	0.999	-1.7083	2.6828
	Ionic Maximum	-1.9122	0.126	-4.1077	0.2833
	PVP Low	0.6593	0.989	-1.5362	2.8548
	PVP High	-0.2950	1.000	-2.4906	1.9005
	Sulphidized Low, 120 nm	0.1842	1.000	-2.0113	2.3797
	Sulphidized Low, 160 nm	0.8055	0.957	-1.3900	3.0011
	Uncoated Low	0.6255	0.993	-1.5700	2.8211
	Uncoated High	-1.1770	0.701	-3.3726	1.0185
	Weathered Low	-0.0338	1.000	-2.2293	2.1617
Ionic High	Control	-0.6777	0.987	-2.8732	1.5178
	Ionic Low	-0.4873	0.999	-2.6828	1.7083
	Ionic Maximum	-2.3994*	0.025	-4.5950	-0.2039
	PVP Low	0.1720	1.000	-2.0235	2.3676
	PVP High	-0.7823	0.964	-2.9778	1.4132
	Sulphidized Low, 120 nm	-0.3031	1.000	-2.4986	1.8925
	Sulphidized Low, 160 nm	0.3183	1.000	-1.8772	2.5138
	Uncoated Low	0.1383	1.000	-2.0573	2.3338
	Uncoated High	-1.6643	0.257	-3.8598	0.5312
	Weathered Low	-0.5211	0.998	-2.7166	1.6745
Ionic Maximum	Control	1.7217	0.220	-0.4738	3.9173
	Ionic Low	1.9122	0.126	-0.2833	4.1077
	Ionic High	2.3994*	0.025	0.2039	4.5950
	PVP Low	2.5715*	0.013	0.3760	4.7670
	PVP High	1.6172	0.290	-0.5784	3.8127

	Sulphidized Low, 120 nm	2.0964	0.070	-0.0991	4.2919
	Sulphidized Low, 160 nm	2.7177*	0.008	0.5222	4.9133
	Uncoated Low	2.5377*	0.015	0.3422	4.7332
	Uncoated High	0.7352	0.976	-1.4604	2.9307
	Weathered Low	1.8784	0.140	-0.3172	4.0739
PVP Low	Control	-0.8498	0.940	-3.0453	1.3458
	Ionic Low	-0.6593	0.989	-2.8548	1.5362
	Ionic High	-0.1720	1.000	-2.3676	2.0235
	Ionic Maximum	-2.5715*	0.013	-4.7670	-0.3760
	PVP High	-0.9543	0.885	-3.1499	1.2412
	Sulphidized Low, 120 nm	-0.4751	0.999	-2.6706	1.7204
	Sulphidized Low, 160 nm	0.1462	1.000	-2.0493	2.3418
	Uncoated Low	-0.0338	1.000	-2.2293	2.1618
	Uncoated High	-1.8363	0.158	-4.0319	0.3592
	Weathered Low	-0.6931	0.984	-2.8886	1.5024
PVP High	Control	0.1046	1.000	-2.0910	2.3001
	Ionic Low	0.2950	1.000	-1.9005	2.4906
	Ionic High	0.7823	0.964	-1.4132	2.9778
	Ionic Maximum	-1.6172	0.290	-3.8127	0.5784
	PVP Low	0.9543	0.885	-1.2412	3.1499
	Sulphidized Low, 120 nm	0.4792	0.999	-1.7163	2.6748
	Sulphidized Low, 160 nm	1.1006	0.773	-1.0950	3.2961
	Uncoated Low	0.9206	0.905	-1.2750	3.1161
	Uncoated High	-0.8820	0.925	-3.0775	1.3135
	Weathered Low	0.2612	1.000	-1.9343	2.4567
Sulphidized Low, 120 nm	Control	-0.3746	1.000	-2.5702	1.8209
	Ionic Low	-0.1842	1.000	-2.3797	2.0113
	Ionic High	0.3031	1.000	-1.8925	2.4986
	Ionic Maximum	-2.0964	0.070	-4.2919	0.0991
	PVP Low	0.4751	0.999	-1.7204	2.6706
	PVP High	-0.4792	0.999	-2.6748	1.7163
	Sulphidized Low, 160 nm	0.6213	0.993	-1.5742	2.8169
	Uncoated Low	0.4413	1.000	-1.7542	2.6369
	Uncoated High	-1.3612	0.517	-3.5568	0.8343
	Weathered Low	-0.2180	1.000	-2.4135	1.9775
Sulphidized Low, 160 nm	Control	-0.9960	0.857	-3.1915	1.1995
	Ionic Low	-0.8055	0.957	-3.0011	1.3900
	Ionic High	-0.3183	1.000	-2.5138	1.8772
	Ionic Maximum	-2.7177*	0.008	-4.9133	-0.5222

	PVP Low	-0.1462	1.000	-2.3418	2.0493
	PVP High	-1.1006	0.773	-3.2961	1.0950
	Sulphidized Low, 120 nm	-0.6213	0.993	-2.8169	1.5742
	Uncoated Low	-0.1800	1.000	-2.3755	2.0155
	Uncoated High	-1.9826	0.101	-4.1781	0.2130
	Weathered Low	-0.8393	0.944	-3.0349	1.3562
Uncoated Low	Control	-0.8160	0.953	-3.0115	1.3795
	Ionic Low	-0.6255	0.993	-2.8211	1.5700
	Ionic High	-0.1383	1.000	-2.3338	2.0573
	Ionic Maximum	-2.5377*	0.015	-4.7332	-0.3422
	PVP Low	0.0338	1.000	-2.1618	2.2293
	PVP High	-0.9206	0.905	-3.1161	1.2750
	Sulphidized Low, 120 nm	-0.4413	1.000	-2.6369	1.7542
	Sulphidized Low, 160 nm	0.1800	1.000	-2.0155	2.3755
	Uncoated High	-1.8026	0.175	-3.9981	0.3930
	Weathered Low	-0.6593	0.989	-2.8549	1.5362
Uncoated High	Control	0.9866	0.864	-1.2089	3.1821
	Ionic Low	1.1770	0.701	-1.0185	3.3726
	Ionic High	1.6643	0.257	-0.5312	3.8598
	Ionic Maximum	-0.7352	0.976	-2.9307	1.4604
	PVP Low	1.8363	0.158	-0.3592	4.0319
	PVP High	0.8820	0.925	-1.3135	3.0775
	Sulphidized Low, 120 nm	1.3612	0.517	-0.8343	3.5568
	Sulphidized Low, 160 nm	1.9826	0.101	-0.2130	4.1781
	Uncoated Low	1.8026	0.175	-0.3930	3.9981
	Weathered Low	1.1432	0.734	-1.0523	3.3388
Weathered Low	Control	-0.1566	1.000	-2.3522	2.0389
	Ionic Low	0.0338	1.000	-2.1617	2.2293
	Ionic High	0.5211	0.998	-1.6745	2.7166
	Ionic Maximum	-1.8784	0.140	-4.0739	0.3172
	PVP Low	0.6931	0.984	-1.5024	2.8886
	PVP High	-0.2612	1.000	-2.4567	1.9343
	Sulphidized Low, 120 nm	0.2180	1.000	-1.9775	2.4135
	Sulphidized Low, 160 nm	0.8393	0.944	-1.3562	3.0349
	Uncoated Low	0.6593	0.989	-1.5362	2.8549
	Uncoated High	-1.1432	0.734	-3.3388	1.0523

Table D.79: Tukey test subset treatment groups for shoot silver concentrations in Month 2

Treatment	N	Subset	
		1	2
Sulphidized Low, 156nm	3	1.2089	
PVP Low	3	1.3551	
Uncoated Low	3	1.3889	
Ionic High	3	1.5272	
Sulphidized Low, 122nm	3	1.8302	1.8302
Ionic Low	3	2.0144	2.0144
Weathered Low	3	2.0482	2.0482
Control	3	2.2049	2.2049
PVP High	3	2.3094	2.3094
Uncoated High	3	3.1914	3.1914
Ionic Maximum	3		3.9266
Significance		0.101	0.070

Table D.80: One-way ANOVA of Month 3 root biomass

	Sum of squares	DOF	Mean square	F	Significance
Between Groups	1.268	10	0.127	2.316	0.048
Within Groups	1.204	22	0.055		
Total	2.471	32			

Table D.81: Multiple comparisons of Month 3 root biomass using Tukey's test

Treatment (I)	Treatment (J)	Mean Difference (I-J)	Significance	95% Confidence Interval	
				Lower Bound	Upper Bound
Control	Ionic Low	-0.1455	0.9993	-0.8283	0.5373
	Ionic High	-0.2905	0.8973	-0.9734	0.3923
	Ionic Maximum	0.0532	1.0000	-0.6297	0.7360
	PVP Low	-0.2685	0.9342	-0.9513	0.4143
	PVP High	-0.3624	0.7129	-1.0452	0.3205
	Sulphidized Low, 120 nm	-0.0099	1.0000	-0.6927	0.6730
	Sulphidized Low, 160 nm	-0.2244	0.9791	-0.9072	0.4584
	Uncoated Low	0.0203	1.0000	-0.6625	0.7032
	Uncoated High	0.3386	0.7830	-0.3442	1.0215
	Weathered Low	0.0742	1.0000	-0.6086	0.7571

Ionic Low	Control	0.1455	0.9993	-0.5373	0.8283
	Ionic High	-0.1450	0.9993	-0.8279	0.5378
	Ionic Maximum	0.1987	0.9912	-0.4842	0.8815
	PVP Low	-0.1230	0.9998	-0.8058	0.5598
	PVP High	-0.2169	0.9835	-0.8997	0.4660
	Sulphidized Low, 120 nm	0.1356	0.9996	-0.5472	0.8185
	Sulphidized Low, 160 nm	-0.0789	1.0000	-0.7617	0.6039
	Uncoated Low	0.1658	0.9979	-0.5170	0.8487
	Uncoated High	0.4841	0.3376	-0.1987	1.1670
	Weathered Low	0.2197	0.9819	-0.4631	0.9026
Ionic High	Control	0.2905	0.8973	-0.3923	0.9734
	Ionic Low	0.1450	0.9993	-0.5378	0.8279
	Ionic Maximum	0.3437	0.7686	-0.3391	1.0265
	PVP Low	0.0220	1.0000	-0.6608	0.7049
	PVP High	-0.0718	1.0000	-0.7547	0.6110
	Sulphidized Low, 120 nm	0.2807	0.9151	-0.4022	0.9635
	Sulphidized Low, 160 nm	0.0661	1.0000	-0.6167	0.7490
	Uncoated Low	0.3109	0.8543	-0.3720	0.9937
	Uncoated High	0.6292	0.0887	-0.0537	1.3120
	Weathered Low	0.3648	0.7055	-0.3181	1.0476
Ionic Maximum	Control	-0.0532	1.0000	-0.7360	0.6297
	Ionic Low	-0.1987	0.9912	-0.8815	0.4842
	Ionic High	-0.3437	0.7686	-1.0265	0.3391
	PVP Low	-0.3217	0.8282	-1.0045	0.3612
	PVP High	-0.4155	0.5421	-1.0984	0.2673
	Sulphidized Low, 120 nm	-0.0630	1.0000	-0.7459	0.6198
	Sulphidized Low, 160 nm	-0.2776	0.9202	-0.9604	0.4053
	Uncoated Low	-0.0328	1.0000	-0.7157	0.6500
	Uncoated High	0.2855	0.9067	-0.3974	0.9683
	Weathered Low	0.0211	1.0000	-0.6618	0.7039
PVP Low	Control	0.2685	0.9342	-0.4143	0.9513
	Ionic Low	0.1230	0.9998	-0.5598	0.8058
	Ionic High	-0.0220	1.0000	-0.7049	0.6608
	Ionic Maximum	0.3217	0.8282	-0.3612	1.0045
	PVP High	-0.0939	1.0000	-0.7767	0.5890
	Sulphidized Low, 120 nm	0.2586	0.9474	-0.4242	0.9415
	Sulphidized Low, 160 nm	0.0441	1.0000	-0.6387	0.7269
	Uncoated Low	0.2888	0.9005	-0.3940	0.9717

	Uncoated High	0.6071	0.1111	-0.0757	1.2900
	Weathered Low	0.3427	0.7714	-0.3401	1.0256
PVP High	Control	0.3624	0.7129	-0.3205	1.0452
	Ionic Low	0.2169	0.9835	-0.4660	0.8997
	Ionic High	0.0718	1.0000	-0.6110	0.7547
	Ionic Maximum	0.4155	0.5421	-0.2673	1.0984
	PVP Low	0.0939	1.0000	-0.5890	0.7767
	Sulphidized Low, 120 nm	0.3525	0.7429	-0.3303	1.0353
	Sulphidized Low, 160 nm	0.1380	0.9995	-0.5449	0.8208
	Uncoated Low	0.3827	0.6487	-0.3001	1.0655
	Uncoated High	0.7010*	0.0409	0.0182	1.3838
	Weathered Low	0.4366	0.4751	-0.2462	1.1194
Sulphidized Low, 120 nm	Control	0.0099	1.0000	-0.6730	0.6927
	Ionic Low	-0.1356	0.9996	-0.8185	0.5472
	Ionic High	-0.2807	0.9151	-0.9635	0.4022
	Ionic Maximum	0.0630	1.0000	-0.6198	0.7459
	PVP Low	-0.2586	0.9474	-0.9415	0.4242
	PVP High	-0.3525	0.7429	-1.0353	0.3303
	Sulphidized Low, 160 nm	-0.2145	0.9847	-0.8974	0.4683
	Uncoated Low	0.0302	1.0000	-0.6526	0.7130
	Uncoated High	0.3485	0.7547	-0.3343	1.0313
	Weathered Low	0.0841	1.0000	-0.5987	0.7669
Sulphidized Low, 160 nm	Control	0.2244	0.9791	-0.4584	0.9072
	Ionic Low	0.0789	1.0000	-0.6039	0.7617
	Ionic High	-0.0661	1.0000	-0.7490	0.6167
	Ionic Maximum	0.2776	0.9202	-0.4053	0.9604
	PVP Low	-0.0441	1.0000	-0.7269	0.6387
	PVP High	-0.1380	0.9995	-0.8208	0.5449
	Sulphidized Low, 120 nm	0.2145	0.9847	-0.4683	0.8974
	Uncoated Low	0.2447	0.9628	-0.4381	0.9276
	Uncoated High	0.5630	0.1708	-0.1198	1.2459
	Weathered Low	0.2986	0.8812	-0.3842	0.9815
Uncoated Low	Control	-0.0203	1.0000	-0.7032	0.6625
	Ionic Low	-0.1658	0.9979	-0.8487	0.5170
	Ionic High	-0.3109	0.8543	-0.9937	0.3720
	Ionic Maximum	0.0328	1.0000	-0.6500	0.7157
	PVP Low	-0.2888	0.9005	-0.9717	0.3940
	PVP High	-0.3827	0.6487	-1.0655	0.3001
	Sulphidized Low, 120 nm	-0.0302	1.0000	-0.7130	0.6526
	Sulphidized Low, 160 nm	-0.2447	0.9628	-0.9276	0.4381

	Uncoated High	0.3183	0.8366	-0.3645	1.0011
	Weathered Low	0.0539	1.0000	-0.6289	0.7367
Uncoated High	Control	-0.3386	0.7830	-1.0215	0.3442
	Ionic Low	-0.4841	0.3376	-1.1670	0.1987
	Ionic High	-0.6292	0.0887	-1.3120	0.0537
	Ionic Maximum	-0.2855	0.9067	-0.9683	0.3974
	PVP Low	-0.6071	0.1111	-1.2900	0.0757
	PVP High	-0.7010*	0.0409	-1.3838	-0.0182
	Sulphidized Low, 120 nm	-0.3485	0.7547	-1.0313	0.3343
	Sulphidized Low, 160 nm	-0.5630	0.1708	-1.2459	0.1198
	Uncoated Low	-0.3183	0.8366	-1.0011	0.3645
	Weathered Low	-0.2644	0.9399	-0.9472	0.4184
Weathered Low	Control	-0.0742	1.0000	-0.7571	0.6086
	Ionic Low	-0.2197	0.9819	-0.9026	0.4631
	Ionic High	-0.3648	0.7055	-1.0476	0.3181
	Ionic Maximum	-0.0211	1.0000	-0.7039	0.6618
	PVP Low	-0.3427	0.7714	-1.0256	0.3401
	PVP High	-0.4366	0.4751	-1.1194	0.2462
	Sulphidized Low, 120 nm	-0.0841	1.0000	-0.7669	0.5987
	Sulphidized Low, 160 nm	-0.2986	0.8812	-0.9815	0.3842
	Uncoated Low	-0.0539	1.0000	-0.7367	0.6289
	Uncoated High	0.2644	0.9399	-0.4184	0.9472

Table D.82: Tukey test subset treatment groups for root biomass in Month 3

Treatment	N	Subsets	
		1	2
Uncoated High	3	0.5322	
Weathered Low	3	0.7966	0.7966
Ionic Maximum	3	0.8177	0.8177
Uncoated Low	3	0.8505	0.8505
Control	3	0.8708	0.8708
Sulphidized Low, 120 nm	3	0.8807	0.8807
Ionic Low	3	1.0163	1.0163
Sulphidized Low, 160 nm	3	1.0952	1.0952
PVP Low	3	1.1393	1.1393
Ionic High	3	1.1614	1.1614
PVP High	3		1.2332
Significance		0.089	0.475

Table D.83: One-way ANOVA of root silver concentrations

		Sum of Squares	DOF	Mean Square	F	Significance
Month 1	Between Groups	25941.145	10	2594.114	263.152	0.000
	Within Groups	216.873	22	9.858		
	Total	26158.018	32			
Month 2	Between Groups	6730.127	10	673.013	6.634	0.000
	Within Groups	2231.998	22	101.454		
	Total	8962.125	32			
Month 3	Between Groups	188205.717	10	18820.572	185.790	0.000
	Within Groups	2228.604	22	101.300		
	Total	190434.321	32			

Table D.84: Multiple comparisons for root silver concentrations in Month 1 treatments using Tukey's test

Treatment (I)	Treatment (J)	Mean Difference (I-J)	Significance	95% Confidence Interval	
				Lower Bound	Upper Bound
Control	Ionic Low	-3.1218	0.973	-12.2861	6.0425
	Ionic High	-24.5772*	0.000	-33.7415	-15.4129
	Ionic Maximum	-100.6097*	0.000	-109.7740	-91.4454
	PVP Low	-1.0214	1.000	-10.1857	8.1429
	PVP High	-17.2750*	0.000	-26.4393	-8.1107
	Sulphidized Low, 120 nm	-1.7267	1.000	-10.8910	7.4376
	Sulphidized Low, 160 nm	-2.3468	0.997	-11.5111	6.8175
	Uncoated Low	-0.6322	1.000	-9.7965	8.5321
	Uncoated High	-10.8704*	0.012	-20.0347	-1.7061
	Weathered Low	-4.9858	0.684	-14.1501	4.1785
Ionic Low	Control	3.1218	0.973	-6.0425	12.2861
	Ionic High	-21.4554*	0.000	-30.6197	-12.2911
	Ionic Maximum	-97.4880*	0.000	-106.6522	-88.3237
	PVP Low	2.1004	0.999	-7.0639	11.2647
	PVP High	-14.1533*	0.001	-23.3175	-4.9890
	Sulphidized Low, 120 nm	1.3951	1.000	-7.7692	10.5594
	Sulphidized Low, 160 nm	0.7750	1.000	-8.3893	9.9393
	Uncoated Low	2.4896	0.995	-6.6747	11.6539
	Uncoated High	-7.7486	0.149	-16.9129	1.4157
	Weathered Low	-1.8640	1.000	-11.0283	7.3003
Ionic High	Control	24.5772*	0.000	15.4129	33.7415
	Ionic Low	21.4554*	0.000	12.2911	30.6197
	Ionic Maximum	-76.0325*	0.000	-85.1968	-66.8682

	PVP Low	23.5558*	0.000	14.3915	32.7201
	PVP High	7.3022	0.203	-1.8621	16.4665
	Sulphidized Low, 120 nm	22.8505*	0.000	13.6862	32.0148
	Sulphidized Low, 160 nm	22.2304*	0.000	13.0661	31.3947
	Uncoated Low	23.9450*	0.000	14.7807	33.1093
	Uncoated High	13.7069*	0.001	4.5426	22.8712
	Weathered Low	19.5914*	0.000	10.4271	28.7557
Ionic Maximum	Control	100.6097*	0.000	91.4454	109.7740
	Ionic Low	97.4880*	0.000	88.3237	106.6522
	Ionic High	76.0325*	0.000	66.8682	85.1968
	PVP Low	99.5884*	0.000	90.4241	108.7527
	PVP High	83.3347*	0.000	74.1704	92.4990
	Sulphidized Low, 120 nm	98.8830*	0.000	89.7187	108.0473
	Sulphidized Low, 160 nm	98.2629*	0.000	89.0986	107.4272
	Uncoated Low	99.9775*	0.000	90.8132	109.1418
	Uncoated High	89.7394*	0.000	80.5751	98.9037
	Weathered Low	95.6239*	0.000	86.4597	104.7882
PVP Low	Control	1.0214	1.000	-8.1429	10.1857
	Ionic Low	-2.1004	0.999	-11.2647	7.0639
	Ionic High	-23.5558*	0.000	-32.7201	-14.3915
	Ionic Maximum	-99.5884*	0.000	-108.7527	-90.4241
	PVP High	-16.2537*	0.000	-25.4180	-7.0894
	Sulphidized Low, 120 nm	-0.7053	1.000	-9.8696	8.4590
	Sulphidized Low, 160 nm	-1.3254	1.000	-10.4897	7.8389
	Uncoated Low	0.3892	1.000	-8.7751	9.5535
	Uncoated High	-9.8490*	0.028	-19.0133	-0.6847
	Weathered Low	-3.9644	0.888	-13.1287	5.1999
PVP High	Control	17.2750*	0.000	8.1107	26.4393
	Ionic Low	14.1533*	0.001	4.9890	23.3175
	Ionic High	-7.3022	0.203	-16.4665	1.8621
	Ionic Maximum	-83.3347*	0.000	-92.4990	-74.1704
	PVP Low	16.2537*	0.000	7.0894	25.4180
	Sulphidized Low, 120 nm	15.5483*	0.000	6.3840	24.7126
	Sulphidized Low, 160 nm	14.9282*	0.000	5.7639	24.0925
	Uncoated Low	16.6428*	0.000	7.4785	25.8071
	Uncoated High	6.4047	0.356	-2.7596	15.5690
	Weathered Low	12.2892*	0.003	3.1249	21.4535
	Control	1.7267	1.000	-7.4376	10.8910

Sulphidized Low, 120 nm	Ionic Low	-1.3951	1.000	-10.5594	7.7692
	Ionic High	-22.8505*	0.000	-32.0148	-13.6862
	Ionic Maximum	-98.8830*	0.000	-108.0473	-89.7187
	PVP Low	0.7053	1.000	-8.4590	9.8696
	PVP High	-15.5483*	0.000	-24.7126	-6.3840
	Sulphidized Low, 160 nm	-0.6201	1.000	-9.7844	8.5442
	Uncoated Low	1.0945	1.000	-8.0698	10.2588
	Uncoated High	-9.1436	0.051	-18.3079	0.0206
	Weathered Low	-3.2591	0.965	-12.4234	5.9052
Sulphidized Low, 160 nm	Control	2.3468	0.997	-6.8175	11.5111
	Ionic Low	-0.7750	1.000	-9.9393	8.3893
	Ionic High	-22.2304*	0.000	-31.3947	-13.0661
	Ionic Maximum	-98.2629*	0.000	-107.4272	-89.0986
	PVP Low	1.3254	1.000	-7.8389	10.4897
	PVP High	-14.9282*	0.000	-24.0925	-5.7639
	Sulphidized Low, 120 nm	0.6201	1.000	-8.5442	9.7844
	Uncoated Low	1.7146	1.000	-7.4497	10.8789
	Uncoated High	-8.5236	0.083	-17.6879	0.6407
	Weathered Low	-2.6390	0.992	-11.8033	6.5253
Uncoated Low	Control	0.6322	1.000	-8.5321	9.7965
	Ionic Low	-2.4896	0.995	-11.6539	6.6747
	Ionic High	-23.9450*	0.000	-33.1093	-14.7807
	Ionic Maximum	-99.9775*	0.000	-109.1418	-90.8132
	PVP Low	-0.3892	1.000	-9.5535	8.7751
	PVP High	-16.6428*	0.000	-25.8071	-7.4785
	Sulphidized Low, 120 nm	-1.0945	1.000	-10.2588	8.0698
	Sulphidized Low, 160 nm	-1.7146	1.000	-10.8789	7.4497
	Uncoated High	-10.2381*	0.020	-19.4024	-1.0738
	Weathered Low	-4.3536	0.821	-13.5179	4.8107
Uncoated High	Control	10.8704*	0.012	1.7061	20.0347
	Ionic Low	7.7486	0.149	-1.4157	16.9129
	Ionic High	-13.7069*	0.001	-22.8712	-4.5426
	Ionic Maximum	-89.7394*	0.000	-98.9037	-80.5751
	PVP Low	9.8490*	0.028	0.6847	19.0133
	PVP High	-6.4047	0.356	-15.5690	2.7596
	Sulphidized Low, 120 nm	9.1436	0.051	-0.0206	18.3079
	Sulphidized Low, 160 nm	8.5236	0.083	-0.6407	17.6879
	Uncoated Low	10.2381*	0.020	1.0738	19.4024
	Weathered Low	5.8846	0.469	-3.2797	15.0489
	Control	4.9858	0.684	-4.1785	14.1501

Weathered Low	Ionic Low	1.8640	1.000	-7.3003	11.0283
	Ionic High	-19.5914*	0.000	-28.7557	-10.4271
	Ionic Maximum	-95.6239*	0.000	-104.7882	-86.4597
	PVP Low	3.9644	0.888	-5.1999	13.1287
	PVP High	-12.2892*	0.003	-21.4535	-3.1249
	Sulphidized Low, 120 nm	3.2591	0.965	-5.9052	12.4234
	Sulphidized Low, 160 nm	2.6390	0.992	-6.5253	11.8033
	Uncoated Low	4.3536	0.821	-4.8107	13.5179
	Uncoated High	-5.8846	0.469	-15.0489	3.2797

Table D.85: Tukey test subset treatment groups for Month 1 root silver concentrations

Treatment	N	Subset				
		1	2	3	4	5
Control	3	0.6586				
Uncoated Low	3	1.2908				
PVP Low	3	1.6800				
Sulphidized Low, 122nm	3	2.3853	2.3853			
Sulphidized Low, 156nm	3	3.0054	3.0054			
Ionic Low	3	3.7804	3.7804			
Weathered Low	3	5.6444	5.6444			
Uncoated High	3		11.5290	11.5290		
PVP High	3			17.9336	17.9336	
Ionic High	3				25.2358	
Ionic Maximum	3					101.2683
Significance		0.684	0.051	0.356	0.203	1.000

Table D.86: Multiple comparisons for root silver concentrations in Month 2 treatments using Tukey's test

Treatment (I)	Treatment (J)	Mean Difference (I-J)	Significance	95% Confidence Interval	
				Lower Bound	Upper Bound
Control	Ionic Low	-3.3574	1.000	-32.7571	26.0423
	Ionic High	-30.2685*	0.040	-59.6682	-0.8688
	Ionic Maximum	-40.8746*	0.002	-70.2743	-11.4749
	PVP Low	-1.2770	1.000	-30.6767	28.1227
	PVP High	-30.3258*	0.039	-59.7255	-0.9261
	Sulphidized Low, 120 nm	-1.3200	1.000	-30.7197	28.0798
	Sulphidized Low, 160 nm	-2.0508	1.000	-31.4505	27.3489

	Uncoated Low	-0.9622	1.000	-30.3620	28.4375
	Uncoated High	-18.2197	0.518	-47.6194	11.1800
	Weathered Low	-6.2068	0.999	-35.6065	23.1929
Ionic Low	Control	3.3574	1.000	-26.0423	32.7571
	Ionic High	-26.9111	0.093	-56.3109	2.4886
	Ionic Maximum	-37.5172*	0.006	-66.9169	-8.1175
	PVP Low	2.0804	1.000	-27.3193	31.4801
	PVP High	-26.9684	0.091	-56.3681	2.4313
	Sulphidized Low, 120 nm	2.0374	1.000	-27.3623	31.4371
	Sulphidized Low, 160 nm	1.3066	1.000	-28.0932	30.7063
	Uncoated Low	2.3951	1.000	-27.0046	31.7949
	Uncoated High	-14.8623	0.764	-44.2621	14.5374
	Weathered Low	-2.8494	1.000	-32.2491	26.5503
Ionic High	Control	30.2685*	0.040	0.8688	59.6682
	Ionic Low	26.9111	0.093	-2.4886	56.3109
	Ionic Maximum	-10.6061	0.961	-40.0058	18.7936
	PVP Low	28.9915	0.055	-0.4082	58.3913
	PVP High	-0.0573	1.000	-29.4570	29.3425
	Sulphidized Low, 120 nm	28.9486	0.056	-0.4512	58.3483
	Sulphidized Low, 160 nm	28.2177	0.067	-1.1820	57.6174
	Uncoated Low	29.3063	0.051	-0.0934	58.7060
	Uncoated High	12.0488	0.916	-17.3509	41.4485
	Weathered Low	24.0617	0.178	-5.3380	53.4614
Ionic Maximum	Control	40.8746*	0.002	11.4749	70.2743
	Ionic Low	37.5172*	0.006	8.1175	66.9169
	Ionic High	10.6061	0.961	-18.7936	40.0058
	PVP Low	39.5976*	0.003	10.1979	68.9973
	PVP High	10.5488	0.962	-18.8509	39.9485
	Sulphidized Low, 120 nm	39.5546*	0.003	10.1549	68.9544
	Sulphidized Low, 160 nm	38.8238*	0.004	9.4241	68.2235
	Uncoated Low	39.9124*	0.003	10.5126	69.3121
	Uncoated High	22.6549	0.239	-6.7448	52.0546
	Weathered Low	34.6678*	0.012	5.2681	64.0675
PVP Low	Control	1.2770	1.000	-28.1227	30.6767
	Ionic Low	-2.0804	1.000	-31.4801	27.3193
	Ionic High	-28.9915	0.055	-58.3913	0.4082
	Ionic Maximum	-39.5976*	0.003	-68.9973	-10.1979
	PVP High	-29.0488	0.055	-58.4485	0.3509
	Sulphidized Low, 120 nm	-0.0430	1.000	-29.4427	29.3568

	Sulphidized Low, 160 nm	-0.7738	1.000	-30.1736	28.6259
	Uncoated Low	0.3147	1.000	-29.0850	29.7145
	Uncoated High	-16.9427	0.614	-46.3424	12.4570
	Weathered Low	-4.9298	1.000	-34.3295	24.4699
PVP High	Control	30.3258*	0.039	0.9261	59.7255
	Ionic Low	26.9684	0.091	-2.4313	56.3681
	Ionic High	0.0573	1.000	-29.3425	29.4570
	Ionic Maximum	-10.5488	0.962	-39.9485	18.8509
	PVP Low	29.0488	0.055	-0.3509	58.4485
	Sulphidized Low, 120 nm	29.0058	0.055	-0.3939	58.4055
	Sulphidized Low, 160 nm	28.2750	0.066	-1.1248	57.6747
	Uncoated Low	29.3635	0.050	-0.0362	58.7633
	Uncoated High	12.1061	0.914	-17.2937	41.5058
	Weathered Low	24.1190	0.175	-5.2807	53.5187
Sulphidized Low, 120 nm	Control	1.3200	1.000	-28.0798	30.7197
	Ionic Low	-2.0374	1.000	-31.4371	27.3623
	Ionic High	-28.9486	0.056	-58.3483	0.4512
	Ionic Maximum	-39.5546*	0.003	-68.9544	-10.1549
	PVP Low	0.0430	1.000	-29.3568	29.4427
	PVP High	-29.0058	0.055	-58.4055	0.3939
	Sulphidized Low, 160 nm	-0.7309	1.000	-30.1306	28.6689
	Uncoated Low	0.3577	1.000	-29.0420	29.7574
	Uncoated High	-16.8998	0.617	-46.2995	12.5000
	Weathered Low	-4.8868	1.000	-34.2866	24.5129
Sulphidized Low, 160 nm	Control	2.0508	1.000	-27.3489	31.4505
	Ionic Low	-1.3066	1.000	-30.7063	28.0932
	Ionic High	-28.2177	0.067	-57.6174	1.1820
	Ionic Maximum	-38.8238*	0.004	-68.2235	-9.4241
	PVP Low	0.7738	1.000	-28.6259	30.1736
	PVP High	-28.2750	0.066	-57.6747	1.1248
	Sulphidized Low, 120 nm	0.7309	1.000	-28.6689	30.1306
	Uncoated Low	1.0886	1.000	-28.3111	30.4883
	Uncoated High	-16.1689	0.672	-45.5686	13.2308
	Weathered Low	-4.1560	1.000	-33.5557	25.2437
Uncoated Low	Control	0.9622	1.000	-28.4375	30.3620
	Ionic Low	-2.3951	1.000	-31.7949	27.0046
	Ionic High	-29.3063	0.051	-58.7060	0.0934
	Ionic Maximum	-39.9124*	0.003	-69.3121	-10.5126
	PVP Low	-0.3147	1.000	-29.7145	29.0850
	PVP High	-29.3635	0.050	-58.7633	0.0362

	Sulphidized Low, 120 nm	-0.3577	1.000	-29.7574	29.0420
	Sulphidized Low, 160 nm	-1.0886	1.000	-30.4883	28.3111
	Uncoated High	-17.2575	0.590	-46.6572	12.1423
	Weathered Low	-5.2446	1.000	-34.6443	24.1552
Uncoated High	Control	18.2197	0.518	-11.1800	47.6194
	Ionic Low	14.8623	0.764	-14.5374	44.2621
	Ionic High	-12.0488	0.916	-41.4485	17.3509
	Ionic Maximum	-22.6549	0.239	-52.0546	6.7448
	PVP Low	16.9427	0.614	-12.4570	46.3424
	PVP High	-12.1061	0.914	-41.5058	17.2937
	Sulphidized Low, 120 nm	16.8998	0.617	-12.5000	46.2995
	Sulphidized Low, 160 nm	16.1689	0.672	-13.2308	45.5686
	Uncoated Low	17.2575	0.590	-12.1423	46.6572
	Weathered Low	12.0129	0.918	-17.3868	41.4126
Weathered Low	Control	6.2068	0.999	-23.1929	35.6065
	Ionic Low	2.8494	1.000	-26.5503	32.2491
	Ionic High	-24.0617	0.178	-53.4614	5.3380
	Ionic Maximum	-34.6678*	0.012	-64.0675	-5.2681
	PVP Low	4.9298	1.000	-24.4699	34.3295
	PVP High	-24.1190	0.175	-53.5187	5.2807
	Sulphidized Low, 120 nm	4.8868	1.000	-24.5129	34.2866
	Sulphidized Low, 160 nm	4.1560	1.000	-25.2437	33.5557
	Uncoated Low	5.2446	1.000	-24.1552	34.6443
	Uncoated High	-12.0129	0.918	-41.4126	17.3868

Table D.87: Tukey test subset treatment groups for Month 2 root silver concentrations

Treatment	N	Subset		
		1	2	3
Control	3	1.0426		
Uncoated Low	3	2.0048	2.0048	
PVP Low	3	2.3196	2.3196	
Sulphidized Low, 122nm	3	2.3625	2.3625	
Sulphidized Low, 156nm	3	3.0934	3.0934	
Ionic Low	3	4.4000	4.4000	
Weathered Low	3	7.2494	7.2494	
Uncoated High	3	19.2623	19.2623	19.2623
Ionic High	3		31.3111	31.3111
PVP High	3		31.3684	31.3684
Ionic Maximum	3			41.9172
Significance		0.518	0.050	0.239

Table D.88: Multiple comparisons for root silver concentrations in Month 3 treatments using Tukey's test

Treatment (I)	Treatment (J)	Mean Difference (I-J)	Significance	95% Confidence Interval	
				Lower Bound	Upper Bound
Control	Ionic Low	-2.9427	1.000	-32.3200	26.4347
	Ionic High	-26.4908	0.102	-55.8682	2.8866
	Ionic Maximum	-267.7780*	0.000	-297.1554	-238.4007
	PVP Low	-1.0272	1.000	-30.4045	28.3502
	PVP High	-13.4670	0.849	-42.8443	15.9104
	Sulphidized Low, 120 nm	-1.0558	1.000	-30.4332	28.3215
	Sulphidized Low, 160 nm	-1.6752	1.000	-31.0525	27.7022
	Uncoated Low	-0.8198	1.000	-30.1972	28.5576
	Uncoated High	-12.8622	0.881	-42.2395	16.5152
	Weathered Low	-4.4071	1.000	-33.7845	24.9702
Ionic Low	Control	2.9427	1.000	-26.4347	32.3200
	Ionic High	-23.5481	0.198	-52.9255	5.8292
	Ionic Maximum	-264.8354*	0.000	-294.2127	-235.4580
	PVP Low	1.9155	1.000	-27.4619	31.2929
	PVP High	-10.5243	0.963	-39.9017	18.8531
	Sulphidized Low, 120 nm	1.8868	1.000	-27.4905	31.2642
	Sulphidized Low, 160 nm	1.2675	1.000	-28.1099	30.6449
	Uncoated Low	2.1229	1.000	-27.2545	31.5002

	Uncoated High	-9.9195	0.975	-39.2969	19.4579
	Weathered Low	-1.4645	1.000	-30.8418	27.9129
Ionic High	Control	26.4908	0.102	-2.8866	55.8682
	Ionic Low	23.5481	0.198	-5.8292	52.9255
	Ionic Maximum	-241.2872*	0.000	-270.6646	-211.9099
	PVP Low	25.4636	0.129	-3.9138	54.8410
	PVP High	13.0238	0.873	-16.3536	42.4012
	Sulphidized Low, 120 nm	25.4349	0.130	-3.9424	54.8123
	Sulphidized Low, 160 nm	24.8156	0.150	-4.5617	54.1930
	Uncoated Low	25.6710	0.123	-3.7064	55.0484
	Uncoated High	13.6286	0.840	-15.7487	43.0060
	Weathered Low	22.0837	0.266	-7.2937	51.4610
Ionic Maximum	Control	267.7780*	0.000	238.4007	297.1554
	Ionic Low	264.8354*	0.000	235.4580	294.2127
	Ionic High	241.2872*	0.000	211.9099	270.6646
	PVP Low	266.7509*	0.000	237.3735	296.1282
	PVP High	254.3111*	0.000	224.9337	283.6884
	Sulphidized Low, 120 nm	266.7222*	0.000	237.3448	296.0996
	Sulphidized Low, 160 nm	266.1029*	0.000	236.7255	295.4802
	Uncoated Low	266.9582*	0.000	237.5809	296.3356
	Uncoated High	254.9159*	0.000	225.5385	284.2932
	Weathered Low	263.3709*	0.000	233.9935	292.7483
PVP Low	Control	1.0272	1.000	-28.3502	30.4045
	Ionic Low	-1.9155	1.000	-31.2929	27.4619
	Ionic High	-25.4636	0.129	-54.8410	3.9138
	Ionic Maximum	-266.7509*	0.000	-296.1282	-237.3735
	PVP High	-12.4398	0.900	-41.8172	16.9376
	Sulphidized Low, 120 nm	-0.0287	1.000	-29.4060	29.3487
	Sulphidized Low, 160 nm	-0.6480	1.000	-30.0254	28.7294
	Uncoated Low	0.2074	1.000	-29.1700	29.5848
	Uncoated High	-11.8350	0.924	-41.2123	17.5424
	Weathered Low	-3.3800	1.000	-32.7573	25.9974
PVP High	Control	13.4670	0.849	-15.9104	42.8443
	Ionic Low	10.5243	0.963	-18.8531	39.9017
	Ionic High	-13.0238	0.873	-42.4012	16.3536
	Ionic Maximum	-254.3111*	0.000	-283.6884	-224.9337
	PVP Low	12.4398	0.900	-16.9376	41.8172
	Sulphidized Low, 120 nm	12.4111	0.901	-16.9662	41.7885

	Sulphidized Low, 160 nm	11.7918	0.926	-17.5855	41.1692
	Uncoated Low	12.6472	0.891	-16.7302	42.0246
	Uncoated High	0.6048	1.000	-28.7725	29.9822
	Weathered Low	9.0598	0.987	-20.3175	38.4372
Sulphidized Low, 120 nm	Control	1.0558	1.000	-28.3215	30.4332
	Ionic Low	-1.8868	1.000	-31.2642	27.4905
	Ionic High	-25.4349	0.130	-54.8123	3.9424
	Ionic Maximum	-266.7222*	0.000	-296.0996	-237.3448
	PVP Low	0.0287	1.000	-29.3487	29.4060
	PVP High	-12.4111	0.901	-41.7885	16.9662
	Sulphidized Low, 160 nm	-0.6193	1.000	-29.9967	28.7580
	Uncoated Low	0.2361	1.000	-29.1413	29.6134
	Uncoated High	-11.8063	0.925	-41.1837	17.5710
	Weathered Low	-3.3513	1.000	-32.7287	26.0261
Sulphidized Low, 160 nm	Control	1.6752	1.000	-27.7022	31.0525
	Ionic Low	-1.2675	1.000	-30.6449	28.1099
	Ionic High	-24.8156	0.150	-54.1930	4.5617
	Ionic Maximum	-266.1029*	0.000	-295.4802	-236.7255
	PVP Low	0.6480	1.000	-28.7294	30.0254
	PVP High	-11.7918	0.926	-41.1692	17.5855
	Sulphidized Low, 120 nm	0.6193	1.000	-28.7580	29.9967
	Uncoated Low	0.8554	1.000	-28.5220	30.2327
	Uncoated High	-11.1870	0.946	-40.5644	18.1904
	Weathered Low	-2.7320	1.000	-32.1093	26.6454
Uncoated Low	Control	0.8198	1.000	-28.5576	30.1972
	Ionic Low	-2.1229	1.000	-31.5002	27.2545
	Ionic High	-25.6710	0.123	-55.0484	3.7064
	Ionic Maximum	-266.9582*	0.000	-296.3356	-237.5809
	PVP Low	-0.2074	1.000	-29.5848	29.1700
	PVP High	-12.6472	0.891	-42.0246	16.7302
	Sulphidized Low, 120 nm	-0.2361	1.000	-29.6134	29.1413
	Sulphidized Low, 160 nm	-0.8554	1.000	-30.2327	28.5220
	Uncoated High	-12.0424	0.916	-41.4197	17.3350
	Weathered Low	-3.5873	1.000	-32.9647	25.7900
Uncoated High	Control	12.8622	0.881	-16.5152	42.2395
	Ionic Low	9.9195	0.975	-19.4579	39.2969
	Ionic High	-13.6286	0.840	-43.0060	15.7487
	Ionic Maximum	-254.9159*	0.000	-284.2932	-225.5385
	PVP Low	11.8350	0.924	-17.5424	41.2123
	PVP High	-0.6048	1.000	-29.9822	28.7725

	Sulphidized Low, 120 nm	11.8063	0.925	-17.5710	41.1837
	Sulphidized Low, 160 nm	11.1870	0.946	-18.1904	40.5644
	Uncoated Low	12.0424	0.916	-17.3350	41.4197
	Weathered Low	8.4550	0.992	-20.9223	37.8324
Weathered Low	Control	4.4071	1.000	-24.9702	33.7845
	Ionic Low	1.4645	1.000	-27.9129	30.8418
	Ionic High	-22.0837	0.266	-51.4610	7.2937
	Ionic Maximum	-263.3709*	0.000	-292.7483	-233.9935
	PVP Low	3.3800	1.000	-25.9974	32.7573
	PVP High	-9.0598	0.987	-38.4372	20.3175
	Sulphidized Low, 120 nm	3.3513	1.000	-26.0261	32.7287
	Sulphidized Low, 160 nm	2.7320	1.000	-26.6454	32.1093
	Uncoated Low	3.5873	1.000	-25.7900	32.9647
	Uncoated High	-8.4550	0.992	-37.8324	20.9223

Table D.89: Tukey test subset treatment groups for Month 3 root silver concentrations

Treatment	N	Subset	
		1	2
Control	3	0.2228	
Uncoated Low	3	1.0426	
PVP Low	3	1.2500	
Sulphidized Low, 122nm	3	1.2787	
Sulphidized Low, 156nm	3	1.8980	
Ionic Low	3	3.1655	
Weathered Low	3	4.6299	
Uncoated High	3	13.0850	
PVP High	3	13.6898	
Ionic High	3	26.7136	
Ionic Maximum	3		268.0008
Significance		0.102	1.000

Table D.90: One-way ANOVA of flowering plants per treatment

	Sum of Squares	DOF	Mean Square	F	Significance
Between Groups	32.24	10	3.22	1.36	0.26
Within Groups	52	22	2.36		
Total	84.24	32			

Table D.91: Multiple comparisons to the control for specified variables from a 2-sided Dunnett's test

Dependent Variable		Mean Difference (I-J)	Significance	95% Confidence Interval	
				Lower Bound	Upper Bound
pH	Weathered Low	-0.41333	0.343	-1.0479	0.2213
	Uncoated High	0.12333	0.998	-0.5113	0.7579
	Ionic High	-0.16333	0.982	-0.7979	0.4713
	Sulphidized Low, 160 nm	0.11333	0.999	-0.5213	0.7479
	Uncoated Low	0.13000	0.996	-0.5046	0.7646
	Sulphidized Low, 120 nm	0.23333	0.876	-0.4013	0.8679
	PVP High	-0.13000	0.996	-0.7646	0.5046
	Ionic Low	0.07000	1.000	-0.5646	0.7046
	PVP Low	0.16333	0.982	-0.4713	0.7979
	Ionic Maximum	-0.37333	0.451	-1.0079	0.2613
Conductivity (uS/cm)	Weathered Low	-7.83333	0.951	-33.5118	17.8452
	Uncoated High	-5.73333	0.993	-31.4118	19.9452
	Ionic High	6.33333	0.987	-19.3452	32.0118
	Sulphidized Low, 160 nm	4.96667	0.998	-20.7118	30.6452
	Uncoated Low	-6.46667	0.985	-32.1452	19.2118
	Sulphidized Low, 120 nm	1.23333	1.000	-24.4452	26.9118
	PVP High	-1.63333	1.000	-27.3118	24.0452
	Ionic Low	-1.60000	1.000	-27.2785	24.0785
	PVP Low	-10.70000	0.794	-36.3785	14.9785
	Ionic Maximum	1.76667	1.000	-23.9118	27.4452
Moisture Content	Weathered Low	13.15588*	0.008	2.8956	23.4161
	Uncoated High	10.21321	0.051	-0.0470	20.4735
	Ionic High	4.91858	0.669	-5.3417	15.1788
	Sulphidized Low, 160 nm	5.43824	0.565	-4.8220	15.6985
	Uncoated Low	7.27405	0.260	-2.9862	17.5343
	Sulphidized Low, 120 nm	8.26997	0.157	-1.9903	18.5302
	PVP High	7.75648	0.205	-2.5038	18.0167
	Ionic Low	8.01942	0.179	-2.2408	18.2797
	PVP Low	10.23446	0.051	-0.0258	20.4947
	Ionic Maximum	3.14225	0.950	-7.1180	13.4025

Organic Matter	Weathered Low	-0.35392	1.000	-5.7925	5.0847
	Uncoated High	-1.27598	0.991	-6.7146	4.1626
	Ionic High	2.61689	0.665	-2.8217	8.0555
	Sulphidized Low, 160 nm	-0.28402	1.000	-5.7226	5.1546
	Uncoated Low	0.89492	0.999	-4.5437	6.3335
	Sulphidized Low, 120 nm	-2.42059	0.739	-7.8592	3.0180
	PVP High	0.94357	0.999	-4.4950	6.3822
	Ionic Low	-0.03784	1.000	-5.4764	5.4007
	PVP Low	-0.24374	1.000	-5.6823	5.1949
	Ionic Maximum	-0.65611	1.000	-6.0947	4.7825
AWCD Month 1	Weathered Low	0.02667	1.000	-0.2785	0.3318
	Uncoated High	0.07000	0.992	-0.2351	0.3751
	Ionic High	-0.11667	0.854	-0.4218	0.1885
	Sulphidized Low, 160 nm	-0.13333	0.755	-0.4385	0.1718
	Uncoated Low	-0.08667	0.968	-0.3918	0.2185
	Sulphidized Low, 120 nm	0.07333	0.989	-0.2318	0.3785
	PVP High	0.07333	0.989	-0.2318	0.3785
	Ionic Low	-0.09333	0.951	-0.3985	0.2118
	PVP Low	-0.03000	1.000	-0.3351	0.2751
	Ionic Maximum	-0.36667*	0.013	-0.6718	-0.0615
AWCD Month 2	Weathered Low	0.15667	0.680	-0.1738	0.4872
	Uncoated High	0.09000	0.975	-0.2405	0.4205
	Ionic High	0.17333	0.576	-0.1572	0.5038
	Sulphidized Low, 160 nm	-0.05000	1.000	-0.3805	0.2805
	Uncoated Low	0.04000	1.000	-0.2905	0.3705
	Sulphidized Low, 120 nm	0.10333	0.944	-0.2272	0.4338
	PVP High	0.01333	1.000	-0.3172	0.3438
	Ionic Low	0.00333	1.000	-0.3272	0.3338
	PVP Low	-0.02000	1.000	-0.3505	0.3105
	Ionic Maximum	-0.30667	0.078	-0.6372	0.0238
AWCD Month 3	Weathered Low	0.16000	0.858	-0.2617	0.5817
	Uncoated High	-0.04667	1.000	-0.4683	0.3750
	Ionic High	-0.11667	0.972	-0.5383	0.3050
	Sulphidized Low, 160 nm	-0.23333	0.518	-0.6550	0.1883

	Uncoated Low	-0.04000	1.000	-0.4617	0.3817
	Sulphidized Low, 120 nm	0.11333	0.977	-0.3083	0.5350
	PVP High	-0.03667	1.000	-0.4583	0.3850
	Ionic Low	-0.11667	0.972	-0.5383	0.3050
	PVP Low	-0.15667	0.871	-0.5783	0.2650
	Ionic Maximum	-0.44333*	0.036	-0.8650	-0.0217
Richness Month 1	Weathered Low	0.44667	1.000	-5.5729	6.4663
	Uncoated High	0.66667	1.000	-5.3529	6.6863
	Ionic High	-2.77667	0.706	-8.7963	3.2429
	Sulphidized Low, 160 nm	-2.44333	0.813	-8.4629	3.5763
	Uncoated Low	-0.77667	1.000	-6.7963	5.2429
	Sulphidized Low, 120 nm	0.33667	1.000	-5.6829	6.3563
	PVP High	1.00000	0.999	-5.0196	7.0196
	Ionic Low	0.00000	1.000	-6.0196	6.0196
	PVP Low	-1.66667	0.972	-7.6863	4.3529
	Ionic Maximum	-10.22000*	0.000	-16.2396	-4.2004
Richness Month 2	Weathered Low	4.66667	0.265	-1.9474	11.2808
	Uncoated High	4.11000	0.392	-2.5041	10.7241
	Ionic High	2.66667	0.818	-3.9474	9.2808
	Sulphidized Low, 160 nm	0.33333	1.000	-6.2808	6.9474
	Uncoated Low	0.44667	1.000	-6.1674	7.0608
	Sulphidized Low, 120 nm	3.11000	0.688	-3.5041	9.7241
	PVP High	0.11000	1.000	-6.5041	6.7241
	Ionic Low	2.00000	0.954	-4.6141	8.6141
	PVP Low	1.44667	0.994	-5.1674	8.0608
	Ionic Maximum	-9.77667*	0.002	-16.3908	-3.1626
Richness Month 3	Weathered Low	2.33333	0.964	-5.7186	10.3853
	Uncoated High	-1.77667	0.994	-9.8286	6.2753
	Ionic High	-3.11000	0.847	-11.1620	4.9420
	Sulphidized Low, 160 nm	-4.55333	0.494	-12.6053	3.4986
	Uncoated Low	-1.77667	0.994	-9.8286	6.2753
	Sulphidized Low, 120 nm	0.66667	1.000	-7.3853	8.7186
	PVP High	-1.00000	1.000	-9.0520	7.0520

	Ionic Low	-1.33333	0.999	-9.3853	6.7186
	PVP Low	-3.11000	0.847	-11.1620	4.9420
	Ionic Maximum	-11.99667*	0.002	-20.0486	-3.9447
Carbohydrates Month 1	Weathered Low	0.02732	1.000	-0.1595	0.2141
	Uncoated High	0.03352	0.999	-0.1533	0.2203
	Ionic High	-0.08864	0.679	-0.2754	0.0981
	Sulphidized Low, 160 nm	-0.08154	0.756	-0.2683	0.1052
	Uncoated Low	-0.06034	0.934	-0.2471	0.1264
	Sulphidized Low, 120 nm	0.07010	0.865	-0.1167	0.2569
	PVP High	0.03521	0.998	-0.1516	0.2220
	Ionic Low	-0.06420	0.910	-0.2510	0.1226
	PVP Low	-0.00155	1.000	-0.1883	0.1852
	Ionic Maximum	-0.13725	0.229	-0.3240	0.0495
Polymers Month 1	Weathered Low	0.01275	0.774	-0.0171	0.0426
	Uncoated High	0.00962	0.935	-0.0203	0.0395
	Ionic High	0.01054	0.898	-0.0193	0.0404
	Sulphidized Low, 160 nm	0.00193	1.000	-0.0279	0.0318
	Uncoated Low	0.01151	0.849	-0.0184	0.0414
	Sulphidized Low, 120 nm	0.01066	0.892	-0.0192	0.0405
	PVP High	0.02101	0.268	-0.0089	0.0509
	Ionic Low	0.00449	1.000	-0.0254	0.0344
	PVP Low	0.00233	1.000	-0.0275	0.0322
	Ionic Maximum	-0.04599*	0.001	-0.0759	-0.0161
Carboxylic and acetic acids Month 1	Weathered Low	-0.00605	1.000	-0.0957	0.0836
	Uncoated High	0.00525	1.000	-0.0844	0.0949
	Ionic High	-0.03068	0.912	-0.1204	0.0590
	Sulphidized Low, 160 nm	-0.02121	0.990	-0.1109	0.0685
	Uncoated Low	-0.02116	0.990	-0.1108	0.0685
	Sulphidized Low, 120 nm	-0.00925	1.000	-0.0989	0.0804
	PVP High	0.01356	1.000	-0.0761	0.1032
	Ionic Low	-0.01392	1.000	-0.1036	0.0758
	PVP Low	-0.02438	0.975	-0.1141	0.0653
	Ionic Maximum	-0.10809*	0.013	-0.1978	-0.0184
	Weathered Low	-0.00669	1.000	-0.0578	0.0444

Amino acids Month 1	Uncoated High	0.00971	0.998	-0.0414	0.0608
	Ionic High	-0.01849	0.885	-0.0696	0.0326
	Sulphidized Low, 160 nm	-0.03034	0.441	-0.0814	0.0207
	Uncoated Low	-0.01418	0.972	-0.0653	0.0369
	Sulphidized Low, 120 nm	-0.00852	0.999	-0.0596	0.0426
	PVP High	-0.00433	1.000	-0.0554	0.0468
	Ionic Low	-0.01971	0.848	-0.0708	0.0314
	PVP Low	-0.00725	1.000	-0.0583	0.0438
	Ionic Maximum	-0.06780*	0.006	-0.1189	-0.0167
Amines/amides Month 1	Weathered Low	0.00153	1.000	-0.0219	0.0250
	Uncoated High	0.01114	0.677	-0.0123	0.0346
	Ionic High	0.01059	0.724	-0.0128	0.0340
	Sulphidized Low, 160 nm	-0.00093	1.000	-0.0244	0.0225
	Uncoated Low	0.00098	1.000	-0.0224	0.0244
	Sulphidized Low, 120 nm	0.01048	0.734	-0.0129	0.0339
	PVP High	0.00756	0.934	-0.0159	0.0310
	Ionic Low	-0.00176	1.000	-0.0252	0.0217
	PVP Low	0.00366	1.000	-0.0198	0.0271
	Ionic Maximum	-0.00921	0.835	-0.0326	0.0142
Root exudates Month 1	Weathered Low	0.00131	1.000	-0.1259	0.1285
	Uncoated High	0.04263	0.920	-0.0846	0.1699
	Ionic High	-0.03814	0.956	-0.1654	0.0891
	Sulphidized Low, 160 nm	-0.04306	0.916	-0.1703	0.0842
	Uncoated Low	-0.02426	0.998	-0.1515	0.1030
	Sulphidized Low, 120 nm	0.04135	0.932	-0.0859	0.1686
	PVP High	0.04697	0.874	-0.0802	0.1742
	Ionic Low	-0.02517	0.997	-0.1524	0.1020
	PVP Low	0.00963	1.000	-0.1176	0.1369
	Ionic Maximum	-0.11696	0.082	-0.2442	0.0103
Carbohydrates Month 2	Weathered Low	0.09594	0.516	-0.0772	0.2691
	Uncoated High	0.02336	1.000	-0.1498	0.1965
	Ionic High	0.12106	0.273	-0.0521	0.2942
	Sulphidized Low, 160 nm	-0.00182	1.000	-0.1749	0.1713
	Uncoated Low	0.04346	0.985	-0.1297	0.2166

	Sulphidized Low, 120 nm	0.01625	1.000	-0.1569	0.1894
	PVP High	0.04294	0.986	-0.1302	0.2161
	Ionic Low	0.01312	1.000	-0.1600	0.1862
	PVP Low	0.00318	1.000	-0.1699	0.1763
	Ionic Maximum	-0.06820	0.834	-0.2413	0.1049
Polymers Month 2	Weathered Low	0.01306	0.860	-0.0215	0.0476
	Uncoated High	0.00792	0.992	-0.0266	0.0425
	Ionic High	0.00551	1.000	-0.0290	0.0401
	Sulphidized Low, 160 nm	-0.01720	0.631	-0.0518	0.0173
	Uncoated Low	0.00795	0.992	-0.0266	0.0425
	Sulphidized Low, 120 nm	0.01277	0.874	-0.0218	0.0473
	PVP High	0.00459	1.000	-0.0300	0.0391
	Ionic Low	0.00249	1.000	-0.0321	0.0370
	PVP Low	-0.00403	1.000	-0.0386	0.0305
	Ionic Maximum	-0.05301*	0.001	-0.0876	-0.0185
Carboxylic and acetic acids Month 2	Weathered Low	0.04089	0.744	-0.0515	0.1333
	Uncoated High	0.01956	0.995	-0.0729	0.1120
	Ionic High	0.02572	0.971	-0.0667	0.1181
	Sulphidized Low, 160 nm	-0.00703	1.000	-0.0994	0.0854
	Uncoated Low	-0.00793	1.000	-0.1004	0.0845
	Sulphidized Low, 120 nm	0.03889	0.786	-0.0535	0.1313
	PVP High	-0.01686	0.999	-0.1093	0.0756
	Ionic Low	-0.00308	1.000	-0.0955	0.0893
	PVP Low	-0.01125	1.000	-0.1037	0.0812
	Ionic Maximum	-0.09671*	0.037	-0.1891	-0.0043
Amino acids Month 2	Weathered Low	0.01035	0.999	-0.0494	0.0701
	Uncoated High	0.02661	0.739	-0.0332	0.0864
	Ionic High	0.01418	0.990	-0.0456	0.0740
	Sulphidized Low, 160 nm	-0.02034	0.914	-0.0801	0.0394
	Uncoated Low	-0.00178	1.000	-0.0616	0.0580
	Sulphidized Low, 120 nm	0.02893	0.660	-0.0309	0.0887
	PVP High	-0.01596	0.978	-0.0757	0.0438
	Ionic Low	-0.00972	0.999	-0.0695	0.0501

	PVP Low	-0.00990	0.999	-0.0697	0.0499
	Ionic Maximum	-0.07316*	0.012	-0.1329	-0.0134
Amines/amides Month 2	Weathered Low	-0.00439	0.999	-0.0295	0.0208
	Uncoated High	0.01021	0.813	-0.0149	0.0354
	Ionic High	0.00349	1.000	-0.0217	0.0286
	Sulphidized Low, 160 nm	-0.00482	0.998	-0.0300	0.0203
	Uncoated Low	-0.00479	0.998	-0.0299	0.0204
	Sulphidized Low, 120 nm	0.00610	0.988	-0.0191	0.0312
	PVP High	-0.00414	0.999	-0.0293	0.0210
	Ionic Low	-0.00367	1.000	-0.0288	0.0215
	PVP Low	0.00168	1.000	-0.0235	0.0268
	Ionic Maximum	-0.01651	0.335	-0.0417	0.0086
Root exudates Month 2	Weathered Low	0.03523	0.977	-0.0956	0.1661
	Uncoated High	0.02063	1.000	-0.1102	0.1515
	Ionic High	0.04944	0.861	-0.0814	0.1803
	Sulphidized Low, 160 nm	-0.02660	0.997	-0.1574	0.1042
	Uncoated Low	0.01579	1.000	-0.1150	0.1466
	Sulphidized Low, 120 nm	0.04076	0.946	-0.0901	0.1716
	PVP High	0.00229	1.000	-0.1285	0.1331
	Ionic Low	-0.01378	1.000	-0.1446	0.1171
	PVP Low	-0.01576	1.000	-0.1466	0.1151
	Ionic Maximum	-0.11228	0.117	-0.2431	0.0186
Carbohydrates Month 3	Weathered Low	0.05252	0.993	-0.1837	0.2887
	Uncoated High	-0.04303	0.999	-0.2793	0.1932
	Ionic High	-0.03921	0.999	-0.2754	0.1970
	Sulphidized Low, 160 nm	-0.06558	0.972	-0.3018	0.1706
	Uncoated Low	0.00162	1.000	-0.2346	0.2378
	Sulphidized Low, 120 nm	0.07705	0.931	-0.1592	0.3133
	PVP High	0.00589	1.000	-0.2303	0.2421
	Ionic Low	-0.04158	0.999	-0.2778	0.1946
	PVP Low	-0.07738	0.929	-0.3136	0.1588
	Ionic Maximum	-0.14911	0.375	-0.3853	0.0871
Polymers Month 3	Weathered Low	0.02415	0.597	-0.0228	0.0711
	Uncoated High	0.00008	1.000	-0.0469	0.0470

	Ionic High	-0.00463	1.000	-0.0516	0.0423
	Sulphidized Low, 160 nm	-0.02230	0.678	-0.0692	0.0246
	Uncoated Low	0.00286	1.000	-0.0441	0.0498
	Sulphidized Low, 120 nm	0.00551	1.000	-0.0414	0.0524
	PVP High	-0.00439	1.000	-0.0513	0.0425
	Ionic Low	0.00530	1.000	-0.0416	0.0522
	PVP Low	-0.00607	1.000	-0.0530	0.0409
	Ionic Maximum	-0.05407*	0.019	-0.1010	-0.0071
Carboxylic and acetic acids Month 3	Weathered Low	0.05473	0.597	-0.0517	0.1612
	Uncoated High	-0.00741	1.000	-0.1139	0.0991
	Ionic High	-0.05579	0.577	-0.1623	0.0507
	Sulphidized Low, 160 nm	-0.06993	0.334	-0.1764	0.0365
	Uncoated Low	-0.02592	0.988	-0.1324	0.0805
	Sulphidized Low, 120 nm	0.00467	1.000	-0.1018	0.1111
	PVP High	-0.02590	0.988	-0.1324	0.0806
	Ionic Low	-0.05154	0.659	-0.1580	0.0549
	PVP Low	-0.03103	0.962	-0.1375	0.0754
	Ionic Maximum	-0.12504*	0.016	-0.2315	-0.0186
Amino acids Month 3	Weathered Low	0.02235	0.971	-0.0577	0.1024
	Uncoated High	0.00701	1.000	-0.0730	0.0871
	Ionic High	-0.00710	1.000	-0.0872	0.0730
	Sulphidized Low, 160 nm	-0.05990	0.214	-0.1400	0.0202
	Uncoated Low	-0.01172	1.000	-0.0918	0.0683
	Sulphidized Low, 120 nm	0.02987	0.868	-0.0502	0.1099
	PVP High	-0.00494	1.000	-0.0850	0.0751
	Ionic Low	-0.01517	0.998	-0.0952	0.0649
	PVP Low	-0.03021	0.862	-0.1103	0.0498
	Ionic Maximum	-0.08406*	0.036	-0.1641	-0.0040
Amines/amides Month 3	Weathered Low	0.00524	0.995	-0.0193	0.0298
	Uncoated High	-0.00377	1.000	-0.0283	0.0208
	Ionic High	-0.00917	0.867	-0.0337	0.0154
	Sulphidized Low, 160 nm	-0.01487	0.418	-0.0394	0.0097
	Uncoated Low	-0.00565	0.992	-0.0302	0.0189

	Sulphidized Low, 120 nm	-0.00394	0.999	-0.0285	0.0206
	PVP High	-0.00654	0.978	-0.0311	0.0180
	Ionic Low	-0.01222	0.630	-0.0367	0.0123
	PVP Low	-0.00960	0.838	-0.0341	0.0149
	Ionic Maximum	-0.02861*	0.017	-0.0531	-0.0041
Root exudates Month 3	Weathered Low	0.04726	0.979	-0.1306	0.2251
	Uncoated High	-0.01751	1.000	-0.1954	0.1603
	Ionic High	-0.05276	0.958	-0.2306	0.1251
	Sulphidized Low, 160 nm	-0.11941	0.312	-0.2973	0.0584
	Uncoated Low	-0.00910	1.000	-0.1870	0.1687
	Sulphidized Low, 120 nm	0.03609	0.997	-0.1418	0.2139
	PVP High	-0.01131	1.000	-0.1892	0.1665
	Ionic Low	-0.05407	0.952	-0.2319	0.1238
	PVP Low	-0.06748	0.858	-0.2453	0.1104
	Ionic Maximum	-0.17724	0.051	-0.3551	0.0006
CFU	Weathered Low	6.68E+06	0.9931	-2.32E+07	3.65E+07
	Uncoated High	9.48E+06	0.9395	-2.04E+07	3.93E+07
	Ionic High	8.52E+05	1.0000	-2.90E+07	3.07E+07
	Sulphidized Low, 160 nm	-7.96E+06	0.9779	-3.78E+07	2.19E+07
	Uncoated Low	1.00E+05	1.0000	-2.97E+07	2.99E+07
	Sulphidized Low, 120 nm	5.44E+06	0.9985	-2.44E+07	3.53E+07
	PVP High	-2.79E+06	1.0000	-3.26E+07	2.70E+07
	Ionic Low	-5.19E+06	0.9990	-3.50E+07	2.47E+07
	PVP Low	-4.89E+06	0.9994	-3.47E+07	2.49E+07
	Ionic Maximum	-1.51E+07	0.6122	-4.50E+07	1.47E+07
Substrate-Induced Respiration	Weathered Low	-3.85560	0.332	-9.7105	1.9993
	Uncoated High	0.36367	1.000	-5.4913	6.2186
	Ionic High	0.74749	1.000	-5.1075	6.6024
	Sulphidized Low, 160 nm	3.75849	0.358	-2.0964	9.6134
	Uncoated Low	3.26248	0.510	-2.5925	9.1174
	Sulphidized Low, 120 nm	2.78121	0.678	-3.0737	8.6362
	PVP High	4.93548	0.128	-0.9195	10.7904
	Ionic Low	0.57193	1.000	-5.2830	6.4269
	PVP Low	2.31332	0.832	-3.5416	8.1683

	Ionic Maximum	-7.00535*	0.014	-12.8603	-1.1504
β-Glucosidase	Weathered Low	19.83557	0.244	-7.6250	47.2961
	Uncoated High	1.46498	1.000	-25.9956	28.9255
	Ionic High	-2.37384	1.000	-29.8344	25.0867
	Sulphidized Low, 160 nm	-2.66554	1.000	-30.1261	24.7950
	Uncoated Low	-2.66554	1.000	-30.1261	24.7950
	Sulphidized Low, 120 nm	-2.66554	1.000	-30.1261	24.7950
	PVP High	-2.66554	1.000	-30.1261	24.7950
	Ionic Low	-2.66554	1.000	-30.1261	24.7950
	PVP Low	-2.66554	1.000	-30.1261	24.7950
	Ionic Maximum	-2.20122	1.000	-29.6618	25.2593
Acid phosphatase	Weathered Low	31.28693	0.452	-21.9567	84.5306
	Uncoated High	19.08422	0.890	-34.1594	72.3278
	Ionic High	3.44831	1.000	-49.7953	56.6919
	Sulphidized Low, 160 nm	-12.85198	0.988	-66.0956	40.3916
	Uncoated Low	-7.15349	1.000	-60.3971	46.0901
	Sulphidized Low, 120 nm	10.32004	0.998	-42.9236	63.5637
	PVP High	-1.76556	1.000	-55.0092	51.4781
	Ionic Low	-1.68831	1.000	-54.9319	51.5553
	PVP Low	-6.79239	1.000	-60.0360	46.4512
	Ionic Maximum	-5.33372	1.000	-58.5773	47.9099
Leucine aminopeptidase	Weathered Low	33.35728	0.452	-23.4050	90.1195
	Uncoated High	0.85196	1.000	-55.9103	57.6142
	Ionic High	0.00000	1.000	-56.7623	56.7623
	Sulphidized Low, 160 nm	30.83504	0.538	-25.9272	87.5973
	Uncoated Low	12.11323	0.995	-44.6490	68.8755
	Sulphidized Low, 120 nm	23.28483	0.805	-33.4774	80.0471
	PVP High	18.34988	0.934	-38.4124	75.1121
	Ionic Low	0.00000	1.000	-56.7623	56.7623
	PVP Low	1.59200	1.000	-55.1703	58.3542
	Ionic Maximum	12.52198	0.994	-44.2403	69.2842
DNA Extracted	Weathered Low	161.34129	0.792	-224.9443	547.6269
	Uncoated High	77.85411	0.997	-308.4315	464.1397
	Ionic High	-68.24074	0.999	-454.5264	318.0449

	Sulphidized Low, 160 nm	17.78006	1.000	-368.5056	404.0657
	Uncoated Low	50.00870	1.000	-336.2769	436.2943
	Sulphidized Low, 120 nm	50.28141	1.000	-336.0042	436.5670
	PVP High	17.31277	1.000	-368.9729	403.5984
	Ionic Low	84.70862	0.994	-301.5770	470.9943
	PVP Low	153.26620	0.829	-233.0194	539.5518
	Ionic Maximum	-263.16676	0.298	-649.4524	123.1189
Shannon Diversity Index	Weathered Low	0.10300*	0.005	0.0269	0.1791
	Uncoated High	0.05000	0.334	-0.0261	0.1261
	Ionic High	0.05933	0.181	-0.0168	0.1354
	Sulphidized Low, 160 nm	0.03567	0.691	-0.0404	0.1118
	Uncoated Low	0.05467	0.249	-0.0214	0.1308
	Sulphidized Low, 120 nm	0.01100	1.000	-0.0651	0.0871
	PVP High	0.04067	0.556	-0.0354	0.1168
	Ionic Low	0.04700	0.399	-0.0291	0.1231
	PVP Low	0.04100	0.547	-0.0351	0.1171
	Ionic Maximum	0.10400*	0.004	0.0279	0.1801
Species Richness	Weathered Low	52.33333	0.999	-273.3296	377.9963
	Uncoated High	-39.00000	1.000	-364.6630	286.6630
	Ionic High	66.66667	0.996	-258.9963	392.3296
	Sulphidized Low, 160 nm	66.33333	0.997	-259.3296	391.9963
	Uncoated Low	51.33333	1.000	-274.3296	376.9963
	Sulphidized Low, 120 nm	92.33333	0.968	-233.3296	417.9963
	PVP High	36.66667	1.000	-288.9963	362.3296
	Ionic Low	26.66667	1.000	-298.9963	352.3296
	PVP Low	37.33333	1.000	-288.3296	362.9963
	Ionic Maximum	92.33333	0.968	-233.3296	417.9963
Evenness	Weathered Low	0.01241	0.349	-0.0067	0.0316
	Uncoated High	0.01032	0.547	-0.0088	0.0295
	Ionic High	0.00534	0.971	-0.0138	0.0245
	Sulphidized Low, 160 nm	0.00199	1.000	-0.0172	0.0212
	Uncoated Low	0.00549	0.966	-0.0137	0.0247
	Sulphidized Low, 120 nm	-0.00250	1.000	-0.0217	0.0167

	PVP High	0.00412	0.995	-0.0150	0.0233
	Ionic Low	0.00552	0.965	-0.0136	0.0247
	PVP Low	0.00678	0.897	-0.0124	0.0259
	Ionic Maximum	0.01071	0.508	-0.0085	0.0299
Root Biomass Month 3	Weathered Low	-0.07423	1.000	-0.6391	0.4906
	Uncoated High	-0.33863	0.431	-0.9035	0.2262
	Ionic High	0.29053	0.597	-0.2743	0.8554
	Sulphidized Low, 160 nm	0.22440	0.828	-0.3404	0.7892
	Uncoated Low	-0.02033	1.000	-0.5852	0.5445
	Sulphidized Low, 120 nm	0.00987	1.000	-0.5550	0.5747
	PVP High	0.36237	0.359	-0.2025	0.9272
	Ionic Low	0.14550	0.982	-0.4193	0.7103
	PVP Low	0.26850	0.677	-0.2963	0.8333
	Ionic Maximum	-0.05317	1.000	-0.6180	0.5117
Shoot Biomass Month 1	Weathered Low	0.07071	0.753	-0.0907	0.2322
	Uncoated High	-0.01789	1.000	-0.1793	0.1436
	Ionic High	0.01718	1.000	-0.1443	0.1786
	Sulphidized Low, 160 nm	0.03261	0.997	-0.1288	0.1941
	Uncoated Low	0.02472	1.000	-0.1367	0.1862
	Sulphidized Low, 120 nm	0.02327	1.000	-0.1382	0.1847
	PVP High	0.05211	0.934	-0.1093	0.2136
	Ionic Low	0.02102	1.000	-0.1404	0.1825
	PVP Low	0.07257	0.730	-0.0889	0.2340
	Ionic Maximum	0.05916	0.878	-0.1023	0.2206
Shoot Biomass Month 2	Weathered Low	0.23097	0.182	-0.0656	0.5275
	Uncoated High	0.04684	1.000	-0.2497	0.3434
	Ionic High	0.16258	0.528	-0.1340	0.4592
	Sulphidized Low, 160 nm	0.29515	0.052	-0.0014	0.5917
	Uncoated Low	0.20008	0.308	-0.0965	0.4967
	Sulphidized Low, 120 nm	0.10769	0.883	-0.1889	0.4043
	PVP High	0.00272	1.000	-0.2939	0.2993
	Ionic Low	0.21099	0.257	-0.0856	0.5076
	PVP Low	0.14771	0.631	-0.1489	0.4443
	Ionic Maximum	-0.00524	1.000	-0.3018	0.2913

Shoot Biomass Month 3	Weathered Low	0.02568	1.000	-0.4034	0.4547
	Uncoated High	-0.14127	0.927	-0.5703	0.2878
	Ionic High	-0.08989	0.996	-0.5190	0.3392
	Sulphidized Low, 160 nm	0.04734	1.000	-0.3817	0.4764
	Uncoated Low	-0.09521	0.994	-0.5243	0.3339
	Sulphidized Low, 120 nm	-0.15503	0.886	-0.5841	0.2740
	PVP High	-0.17100	0.826	-0.6001	0.2581
	Ionic Low	-0.17587	0.806	-0.6049	0.2532
	PVP Low	-0.01049	1.000	-0.4396	0.4186
	Ionic Maximum	-0.14475	0.918	-0.5738	0.2843
Shoot Concentration Month 1	Weathered Low	0.14665	0.998	-0.6436	0.9369
	Uncoated High	0.26509	0.920	-0.5251	1.0553
	Ionic High	0.59256	0.212	-0.1977	1.3828
	Sulphidized Low, 160 nm	0.90889*	0.019	0.1187	1.6991
	Uncoated Low	0.15440	0.997	-0.6358	0.9446
	Sulphidized Low, 120 nm	0.27315	0.907	-0.5171	1.0634
	PVP High	0.34881	0.746	-0.4414	1.1390
	Ionic Low	0.96167*	0.012	0.1714	1.7519
	PVP Low	0.33706	0.775	-0.4532	1.1273
	Ionic Maximum	1.21540*	0.001	0.4252	2.0056
Shoot Concentration Month 2	Weathered Low	-0.15664	1.000	-1.9728	1.6595
	Uncoated High	0.98658	0.538	-0.8296	2.8028
	Ionic High	-0.67770	0.868	-2.4939	1.1385
	Sulphidized Low, 160 nm	-0.99599	0.528	-2.8122	0.8202
	Uncoated Low	-0.81598	0.730	-2.6322	1.0002
	Sulphidized Low, 120 nm	-0.37465	0.996	-2.1908	1.4415
	PVP High	0.10458	1.000	-1.7116	1.9208
	Ionic Low	-0.19045	1.000	-2.0066	1.6257
	PVP Low	-0.84975	0.693	-2.6659	0.9664
	Ionic Maximum	1.72174	0.069	-0.0945	3.5379
Shoot Concentration Month 3	Weathered Low	-0.03510	1.000	-0.9422	0.8720
	Uncoated High	0.38971	0.769	-0.5174	1.2969
	Ionic High	0.43937	0.659	-0.4678	1.3465

	Sulphidized Low, 160 nm	-0.01542	1.000	-0.9226	0.8917
	Uncoated Low	-0.15315	0.999	-1.0603	0.7540
	Sulphidized Low, 120 nm	0.51558	0.489	-0.3916	1.4227
	PVP High	0.28290	0.945	-0.6242	1.1900
	Ionic Low	0.18072	0.997	-0.7264	1.0879
	PVP Low	-0.02217	1.000	-0.9293	0.8850
	Ionic Maximum	0.29234	0.935	-0.6148	1.1995
Root Concentration Month 1	Weathered Low	4.98579	0.333	-2.5951	12.5667
	Uncoated High	10.87036*	0.003	3.2895	18.4513
	Ionic High	24.57721*	0.000	16.9963	32.1581
	Sulphidized Low, 160 nm	2.34680	0.947	-5.2341	9.9277
	Uncoated Low	0.63222	1.000	-6.9487	8.2131
	Sulphidized Low, 120 nm	1.72671	0.992	-5.8542	9.3076
	PVP High	17.27504*	0.000	9.6941	24.8559
	Ionic Low	3.12179	0.803	-4.4591	10.7027
	PVP Low	1.02138	1.000	-6.5595	8.6023
	Ionic Maximum	100.60974*	0.000	93.0288	108.1906
Root Concentration Month 2	Weathered Low	6.20679	0.983	-18.1133	30.5269
	Uncoated High	18.21970	0.212	-6.1004	42.5398
	Ionic High	30.26851*	0.010	5.9484	54.5886
	Sulphidized Low, 160 nm	2.05081	1.000	-22.2693	26.3709
	Uncoated Low	0.96223	1.000	-23.3579	25.2823
	Sulphidized Low, 120 nm	1.31995	1.000	-23.0001	25.6400
	PVP High	30.32578*	0.010	6.0057	54.6459
	Ionic Low	3.35737	1.000	-20.9627	27.6775
	PVP Low	1.27698	1.000	-23.0431	25.5971
	Ionic Maximum	40.87459*	0.000	16.5545	65.1947
Root Concentration Month 3	Weathered Low	4.40714	0.999	-19.8945	28.7087
	Uncoated High	12.86217	0.566	-11.4394	37.1638
	Ionic High	26.49079*	0.028	2.1892	50.7924
	Sulphidized Low, 160 nm	1.67517	1.000	-22.6264	25.9768
	Uncoated Low	0.81979	1.000	-23.4818	25.1214

	Sulphidized Low, 120 nm	1.05585	1.000	-23.2457	25.3574
	PVP High	13.46698	0.516	-10.8346	37.7686
	Ionic Low	2.94267	1.000	-21.3589	27.2443
	PVP Low	1.02718	1.000	-23.2744	25.3288
	Ionic Maximum	267.77804*	0.000	243.4764	292.0796

Table D.92: T-test of Month 2 AWCD, CFU, DNA Extracted and Month 2 Shoot Silver Concentrations between the control and ionic maximum treatment

	t-test for Equality of Means					
	t	DOF	Significance (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
AWCD Month 2	-5.963	4	0.004	-0.30667	-0.44944	-0.16389
CFU	0.680	4	0.041	-15124292	-29299179	-949404
DNA Extracted	-2.979	4	0.041	-263.16676	-508.42866	-17.90485
Shoot Concentration Month 2	3.598	4	0.023	1.72174	0.39316	3.05031