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Minto Mine Constructed Wetland Treatment Research Program - Site Assessment

Prepared for

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1. Abbreviations and Definitions

CWTS(s) – Constructed Wetland Treatment System(s)

DNA (deoxyribonucleic acid) – is the hereditary material in humans and almost all other organisms. This can be used to identify organisms.

DO – dissolved oxygen

DSTSF – Dry Stack Tailings Storage Facility

Heterotroph – an organism deriving its nutritional requirements from complex organic substances.

Metagenomics – the study of genetic material recovered directly from environmental samples, related to a community rather than an individual organism.

Microbes – microscopic organisms which may be uni- or multi-cellular. This includes algae, bacteria, fungi, viruses, and yeast.

MPN (Most Probable Number Method) – a statistical value representing the viable population of microbes in a sample through use of dilution and multiple inoculations.

MVFE – Mill Valley Fill Extension

Oxidation – is the loss of electrons or increase in oxidation state by a molecule, atom, or ion. Can be catalyzed by microbes.

Redox – Oxidation-reduction potential, measured in millivolts

Reduction (chemical) – is the gain of electrons or a decrease in oxidation state by a molecule, atom, or ion. Can be catalyzed by microbes.

SeIV broth or agar – laboratory growth medium that contains selenite (SeIV) and indicates reduction of selenite to elemental selenium through a visible colour change.

Species – one of the basic units of biological classification and a taxonomic rank. Rank in the classification of organisms below genus and above strain.

SWD – Southwest Waste Dump

Taxonomy – classification, identification, and naming of organisms.

TSS – total suspended solids

YTS250 (Yeast, Tryptone, Starch, 250g each) – a type of laboratory growth medium used for environmental microbes.

2. Introduction

Constructed Wetland Treatment Systems (CWTSs) have been identified as a contingency method for improvement of the quality of site runoff water in the post-closure period for the Minto Mine (Minto Phase V/VI Expansion Project, YOR Project Number 2013-0100). Once established, wetlands can become self-sustaining ecosystems with the plants providing yearly renewal of carbon to fuel microbial activity. As such, they possess the desirable potential to remediate contaminated mine drainage for as long as it is generated. In order for the CWTS to be effective, they must be designed, piloted, optimized, implemented, and maintained in a site-specific manner.

At the request of Minto Explorations Ltd, Contango Strategies has undertaken a site assessment to inform the design of site-specific passive treatment technologies for the Minto site during post-closure. The overriding goal of the study was to identify natural water treatment processes that are currently occurring at the Minto site in areas receiving impacted waters, and how these could be optimized for passive water treatment in closure. As such, the focus of the research program reported here was on areas that currently receive impacted waters (Figure 1), with emphasis on the W15 monitoring station and upstream creek and wetland area that receive seepage and runoff from the Southwest Waste Dump (SWD). Additionally, preliminary information was gathered for the W37 area receiving seepage and runoff from the Dry Stack Tailings Storage Facility (DSTSF) and Mill Valley Fill Extension (MVFE) toe area. As was outlined in Appendix W – Preliminary Reclamation and Closure Plan Phase V-VI of the Phase V-VI expansion plan, the focus of work at this point is on the W15 area and SWD. At a future date, additional work will occur in the future down gradient of the DSTSF and within the footprint of the current water storage pond. The site assessment included two visits to the Minto Mine to gather information and samples. Sampling consisted of in situ measurements, collection of samples for physiochemical, geochemical, and microbiological analyses. General

feasibility of implementing passive treatment was also evaluated during the site visits.

It is our understanding that before this site assessment of the natural wetland area, there has been no monitoring of water quality along the creek between the W10 and W15 monitoring points, of the creek entering the W15 monitoring point, or the W15 runoff area entering the W15 monitoring point. Therefore, this study provides new information to inform decisions regarding the plausibility of water treatment by constructed wetlands at the Minto Site.

Copper is of greatest interest in this study, however, there are a number of other elements and parameters that can influence the biogeochemistry of a system and overall treatability of the copper. These include the presence of iron and sulphur, as well as the pH and oxygen content of the environment. Additionally, there are a few other elements (e.g., selenium) that will require some treatment in order to achieve discharge goals.

3. Site Visits

3.1. Information Gathering

Two site visits were conducted as part of the site assessment for the CWTS. Prior to the first site visit, preparations included gathering site-specific historical and predicted closure information including:

- Rates of seepage for known seeps (and interpretation as a function of dump area and catchments)
- References to analytical results about the site
- Rate of flow for W15 and W37 based on existing data
- Aerial photo interpretation or site personnel knowledge of areas with potentially acceptable granular soil materials, peat, limestone, and organic soils for subsequent on-site materials investigation, inspection and testing.
- Use of site-wide vegetation map to identify areas for possible vegetation borrow sources for the CWTS.
- Determine water quality parameters to be monitored and concentrations that will be used for pilot tests.

3.2. First Site Visit

The first site visit was conducted mid-August 2013 and had a primary goal of familiarizing Contango personnel with the site layout and water flows in order

to properly design sampling plans for assessment of existing natural treatment mechanisms occurring on site. Specific information gathered in the first site visit included:

- Visual inspection along the toe of SWD, Main Dump, DSTSF, and area down-gradient of W37 to identify and document primary seeps that have the potential to be intercepted along with an assessment of current fate of seepage surface flows.
- Identify conceptual options for intercepting and routing seepage into the CWTS.
- Selection of a significant seep(s) for further test work based on initial site reconnaissance. Evaluate potential path of seepage interception trench in comparison to seep location(s).
- Identify probable locations for CWTS. Investigation and evaluation of natural remediation processes that may be occurring on site as identified by redox and pH changes between surface water and plant roots, and choice of plant types to be tested in Contango's pilot CWTS tests.
- Identification of borrow sites for CWTS substrate (limestone, coarse sand/gravel, peat, plant sources for much, etc.)
- Identify ideal CWTS plant species based on site observations of vegetation.

Information collected prior to and during the first site investigation were reviewed and then used to refine the scope and direction of data collection in the follow up site visit. The refinement of scope and testing locations was conducted in consultation with ACG and staff from the Minto Mine.

3.3. Second Site Visit

A second site visit took place September 20-23, 2013 based on observations from the first site visit and historical and modeling data compiled to date. The purpose of the second site visit was to collect water, soil, plant, and microbiological samples from the natural wetland and creek area flowing between the W10 to W15 monitoring stations. This area begins with a creek that flows from off site through the W10 area and subsequently receives seepage and surface runoff from the SWD, surface runoff from the catchment area (including non-contact waters), and road runoff from a culvert entering the W15 collection pond. Samples were taken from potential borrow sources to assess suitability of the materials for constructing soils for the CWTS. Additionally, *Carex aquatilis* (Water Sedge) and *Sphagnum* (moss) plants were collected at the W10 site and transported back to Contango's CWTS research facilities in Saskatoon for use in the pilot-scale systems to be built specifically for testing and optimization of designs for the Minto site.

4. Experimental Setup

4.1. Sample and Analytical Data Collection

During the second site visit, fourteen different locations were sampled at the Minto Mine Site (Table 1 and Figure 2). Samples were collected starting at the most downstream location (W15) and progressing upstream to the W10 area over a period of 2 days. In all cases, water samples were collected first, then soil and plant samples. Water and soil samples were collected by grab sample, with soil sampled to a maximum depth of 5-7 cm. Water, sediment, and plant samples were submitted to Maxxam Analytics and analyzed for parameters listed in Table 2. For plants, only the above water (or above ground) portion was analyzed. For *Carex aquatilis*, *Equisetum* sp. and *Sphagnum* sp., the entire above ground (or water) portion was analyzed. For *Salix* sp., only the recent year's growth was collected. All micro samples were processed according to Sections 4.2 and 4.3.

4.2. Growth-based analyses (MPN)

The most-probable number (MPN) of bacteria was determined for all micro samples (Table 1). The test allows for estimation of the number of bacteria that can grow in a specific laboratory medium. The mediums tested for in this project were specific for bacteria that can reduce nitrate, selenite, and sulfate, respectively, as well as the total number of heterotrophic bacteria for comparative purposes (i.e., a growth-permissive test for all bacteria capable of growing on organic carbon as an energy source). MPN tests for sulphate (Active Standard ASTM D4412) were performed under anaerobic conditions, whereas selenite (Siddique et al., 2006) and nitrate (Nitrate Reduction Test, supplied by Sigma-Aldrich) were tested under aerobic conditions. Total heterotrophs (grown with YTS250 medium; Lefrançois et al., 2010) were tested for in both aerobic and anaerobic conditions. In brief, sediment samples were diluted 1:10 with a 0.1% peptone solution. This dilution was then used to perform a serial dilution along a sterile 96-microwell round-bottom plate containing the respective growth media. Sediment dilutions tested in the MPN plate ranged from 1/400 to 1/419,430,400. At a minimum, all tests were performed in duplicate. Wells were incubated at +30°C in a dark incubator and assessed for visible growth (formation of a bacterial pellet) and/or colour change specific to the type of media after 16 (aerobic) or 28 (anaerobic) days. A colour change to black or red/orange indicated sulphate or selenite reduction, respectively. Nitrate reduction was tested as per the kit manufacturer's protocol by addition of reagents to differentiate between reduction to nitrite or nitrogen gas. The most probable number of bacteria capable of each metabolism was then calculated as

described in the FDA Bacteriological Analytical Manual, Appendix 2: Most Probable Number from Serial Dilutions: (<http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm109656.htm>).

4.3. DNA-based analyses

DNA was extracted from all samples using the MO BIO Powersoil Powerlyzer DNA extraction kit. To identify the bacteria present in each sample, we performed targeted sequencing. The gene that is targeted by polymerase chain reaction (PCR) for bacteria is the v4 region of the 16S ribosomal RNA gene (Caporaso et al., 2011). Because the same gene is sequenced for all targeted organisms in a community, the relatedness of different microbes can be inferred based on sequence similarity. As such, the first step in the analysis of targeted metagenomics is clustering similar sequences into groups which are called Operational Taxonomic Units (OTUs). An OTU can essentially be described as a "species cluster", where all sequences present in an OTU are similar and presumed to represent the same or very closely related organisms. The threshold for choosing what is or is not considered the same organism is dependent on the region that is being targeted for sequencing. For example, bacteria are clustered together at 97% identity, as the v4 16S rRNA region that is targeted is known to have ~3% intra-species variation. Once the sequences have been clustered together, each OTU is assigned a taxonomy based on similarity with sequences from known organisms (using databases specific for each targeted region). The taxonomy classification is very dependent on the presence of that organism in the databases that are being used. For example, if a novel bacterium is sequenced, no taxonomy is assigned because no representative would be present in the database. Furthermore, different levels of taxonomy can be assigned based on how confident or similar the sequence is to known organisms. An OTU can therefore sometimes be classified as "Bacteria", or a second OTU as "Bacteria;Proteobacteria" as the software pipeline is not confident in the assignment of lower taxonomy levels, while a third OTU may be classified as "Bacteria;Proteobacteria;Epsilonproteobacteria;Campylobacteriales; Helicobacteraceae;Sulfurimonas" based on high similarity with other *Sulfurimonas* sequences found in the database. While we cannot always confidently assign names to each of these OTUs, we can be confident that they are different from one another, as we have already compared their sequences. Hence, even though in this example both the second and third OTUs belong to the *Proteobacteria* phylum, we are confident that they are in fact different organisms. Once taxonomy has been assigned, the abundance

of each OTU in each sample is determined. To compare the community profile of different samples, a multitude of statistical analyses were performed.

5. Results and Discussion

5.1. Site Assessment

The sections below correspond to results and discussion specific to each of the objectives that are reiterated here for ease of reference.

- Visual inspection along the toe of SWD, Main Dump, DSTSF, and area down-gradient of W37 to identify and document primary seeps that have the potential to be intercepted along with an assessment of current fate of seepage surface flows.
- Selection of a significant seep(s) for further test work based on initial site reconnaissance. Evaluate potential path of seepage interception trench in comparison to seep location(s).

Fourteen sites at the area receiving water from the SWD were selected for further testing (Table 1, Figure 2) and assessed for the parameters listed in Table 2. These include 3 seeps that are part of Minto's routine monitoring program (W32, W38, W39) as well as areas of the creek parallel to the seep (W38 Creek, W39 Creek, W32 Creek). Additionally, the W32 area had visible seepage between the seep and creek, and there were two distinctly different branches of the creek itself. Therefore, a total of four samples were collected at the W32 location. These are the W32 Seep, W32 Creek, W32 Mid (further seep between the toe seep and creek), and W32 Creek Red. The W32 Creek Red sample is a distinct branch of the W32 creek that was covered in red foam in August, and red precipitate on the soil in September (Figure 3). It should be noted that due to the parallel selection of sites and the direction of flow in the area between the seeps and creek, the water from seep W38 would never be present in W38 creek. We realize it is not possible to know the exact areas of entry of the seeps into the creek unless detailed hydrological studies and use of tracers were used; however, the purpose of this site assessment was to determine overall trends in water chemistry through the site and not a thorough hydrological investigation. During the first site assessment to locate priority sampling areas, the weather was hot (>+30°C daytime temperature) and dry, and therefore the seeps marked for routine monitoring (including W32, W38 and W39) were mostly dry with no visible standing or flowing water. However, these sites are routinely monitored and have a wealth of historical data relating to toe seepage from the SWD, and so they were identified as high priority areas. During the second site visit, snowmelt was causing a greater volume of seepage than in

the first visit. Water pools were present at all marked monitoring seepage points along the SWD toe, some with visible flows.

Runoff was observed directly from the road above via a culvert entering into the W15 collection pond, bypassing the creek and natural wetland area entirely (Figure 2). This "culvert runoff into W15" was sometimes clear, but at other times muddy, seemingly when the grader passed over the road on its routine schedule. While flow volumes and periodicity would need to be determined in order to calculate actual contribution to loading and concentrations, it is likely that water from the culvert (runoff into W15) is contributing to elevated concentrations of constituents of concern that have been observed to coincide with high total suspended solids (TSS) events at the W15 monitoring station at the far end of the pond (Figure 2).

Further geotechnical and hydrological assessments of the W15 area are planned to determine the depth of seepage, to ensure that the interception trench is adequately built such that seepage does not pass below it, and to delineate the extent of permafrost. This will be conducted under a separate scope of work.

The W37 area was inspected during the first site visit. However, as noted in the Phase V/VI project proposal, this area and the MVFE are likely to change with further development prior to closure. As such, they were not targeted as a key area for research at this time.

- Identify conceptual options for intercepting and routing seepage into the CWTS.

The W15 and W37 (Figure 1) areas are both in natural depressions and likely suitable for implementation of a CWTS, although this will also cause the depressions to receive non-contact runoff water from the general catchment area. Due to the multiple seeps along the toe of the SWD, an interception trench would be needed to collect the seepage and direct it towards the entry of the CWTS. Alternatively, it may be possible to construct several smaller CWTSs, each with their own entry point for seepage. These two options will be evaluated as information pertaining to flow rates and removal rates are refined through scientific testing and continued gathering of information from the site.

W37 area appeared to be amenable to future implementation of a CWTS. We recommend that studies be conducted on the sediments under the current water storage area behind the dam, in order to ensure there has been no accumulation of elements that might be released upon oxidation once the dam is breached upon closure.

- Identify probable locations for CWTSs. Investigation and evaluation of natural remediation processes that may be occurring on site as identified

by redox and pH changes between surface water and plant roots, and choice of plant types to be tested in Contango's pilot CWTS tests.

The W15, W37, and Main Pit have been identified as probable locations for CWTS in closure (Figure 1). The natural remediation processes occurring at the W15 site were investigated and evaluated in the second site assessment, with results and findings detailed in this report. We were unable to measure soil redox, however, as the water temperature was too cold for the reference probe, causing its internal buffer solution to crystalize (+0.5°C).

- Identification of borrow sites for CWTS substrate (limestone, coarse sand/gravel, peat, plant sources for much, etc.)

Overburden piles of sand and peat were located and tested for parameters relevant to their use as a soil in the CWTS. The samples of borrow material are suitable for use in a future CWTS. A source of slow release carbon (e.g., wood chips) is still yet to be identified.

- Identify ideal CWTS plant species based on site observations of vegetation.

Plant species and associated findings are discussed in section 5.3 below.

5.2. Water and Sediment Geochemistry

Dissolved chloride concentrations in the water can be used as a proxy to determine if concentration decreases in other elements can be attributed to dilution or putative treatment mechanisms. Comparison of changes in concentrations of elements of interest along the creek indicates fluctuations are similar to those of chloride, indicating dilution rather than treatment is occurring within the free flowing areas of the creek itself (Table 3). However, comparison of the water exiting and entering the W32 Creek Red site shows substantial treatment of copper and also some treatment of selenium and sulphur (Table 4). Comparison of the seeps with the creek also indicates significant treatment of copper, selenium and sulphur (Table 5). Overall, this indicates that active treatment of elements is occurring in the vegetated areas between the seep and the creek, but little or no treatment occurs in the creek itself. In this case, averages were used for the creek as the actual flow of seeps, and entry points into the creek are not known. More refined calculations cannot be made without detailed hydrological information for the area. Figures 4 and 5 show only small changes in chloride concentration through the system, yet large fluctuations in concentrations of other elements occur, especially near the seeps. Moreover, the chemistry of each seep varies greatly from one another, while the concentrations of elements in

the creek show more consistency (Figures 5-7), supporting the notion of treatment between the seep and creek, which evens out these fluctuations in chemistry by the time the water reaches the creek.

At the furthest upstream location sampled in this assessment, the W10 site has some of the highest copper concentrations in sediment (Figure 8), yet some of the lowest concentrations of copper in the water (Figure 6). This could reflect that the W10 site has been accumulating copper for a longer time span (i.e., in existence for more years, as it is a background location, upstream of the SWD), or perhaps the materials used to build the access road near the W10 contained copper and spread further than is currently visible due to overgrowth. This could also be caused by removal of copper from the water and into the sediment by binding to oxidized iron as suggested by the ratios of total vs. dissolved iron at that sampling area, with further stabilization (retention) of the copper promoted by conditions (redox, pH) in the sediment created by the combination of plants, microbes, and chemistry in the area.

The culvert runoff into W15 area has the highest copper concentrations in both sediment and water (Figures 6 and 8). The W15 runoff area has a separate and independent flow than the creek, entering the W15 collection pond via a culvert from the road above, bypassing the creek and natural wetland entirely (Figure 2). The culvert runoff into W15 appears to only flow during melt and rain events. This W15 runoff was sometimes clear (as in the time the sample for this study was taken), but at other times is muddy, seemingly when the grader passed over the road on its routine schedule. While flow volumes and periodicity would need to be determined in order to calculate actual contribution to loading and concentrations, it is likely that water from the culvert (W15 runoff) is contributing to elevated concentrations of constituents of concern that have been observed to coincide with high TSS events at the W15 monitoring station at the far end of the pond. This can be seen in Figure 9 as the W15 inflow (from creek) has a lower concentration of key elements (e.g., copper, selenium) than is routinely measured at the W15 monitoring point. The only visible source for this loading is the culvert runoff into W15. This is a key finding of this site assessment, as the routine sampling performed at the set W10 and W15 monitoring points would indicate that there is less treatment ongoing in the natural wetland area than is truly occurring. This is because of the extra loading of contaminants contributed by the culvert runoff into W15 mixing in the collection pond with the cleaner water exiting the natural wetland area before reaching the W15 monitoring point (Figures 6 and 7).

The W32 Creek Red site is a location between the W10 (upstream) and W15 (downstream) areas where the creek briefly splits into two streams at the W32 clearing. The W32 Creek Red sample has a high concentration of oxidized iron, indicated by a visible red precipitate (Figure 3). At the first site visit in August, the red substance was fluffy and floating in the water and

also on the sediment. In September, the red substance was entirely at the sediment interface. Moreover, the water exiting the W32 Creek Red site has the lowest concentration of copper in the water (at 1.92 µg/L, the only site sampled in this study with copper concentration below CCME guidelines). This finding, paired with moderate concentrations of copper in the sediment at the W32 Creek Red site compared to other locations (Figure 8), suggests that copper removal may be occurring via binding to oxidized iron at the W32 Creek Red site. It would be useful in the future to monitor sediment redox at this and other sites; however, the water temperature (+0.5°C) was too cold for the reference probes to be used at the time of the September sampling event. There were several other smaller areas similar in appearance to the W32 Creek Red site found along the creek, always where water was shallow and *Equisetum* or *Carex* present.

The W32 Seep has low ammonia (9.4 µg/L) and high nitrate (39.9 mg/L) concentrations in the water relative to other samples (Figure 7). It appears that the ammonia is being oxidized to nitrate as it seeps through the waste rock pile, which is consistent with the peaks of sulphur and selenium concentrations in the water at the W32 site, both of which could be released from the waste rock under oxidizing conditions. In contrast, the W32 Creek and W32 Creek Red water samples have higher concentrations of ammonia and lower concentrations of nitrate than other samples (measured as N, 0.11 and 0.18 mg/L ammonia and 3.02 mg/L and below detection limit (<0.02 mg/L) for nitrate, respectively; Figure 7). It is possible that there are greater concentrations of ammonia seeping from the SWD between the W10 site and W32 creek sampling point, or that the ammonia is being produced within the creek from decaying organic material. It is also possible that the ammonia is being produced by the abundant *Geobacter* bacteria at the W32 Creek Red site (see section 5.4), as it is a known iron-reducing organism that feeds on electrons from oxidized iron and can also respire nitrate to ammonia (Lovley & Phillips, 1988; Senko & Stolz, 2001).

As would be supported by redox and biogeochemical theory, there is a strong correlation between ammonia and total iron concentrations in water (Figure 10), while iron and nitrate show a negative correlation. This makes sense from a theoretical oxidation-reduction chemistry perspective, as ammonia will oxidize into nitrate, increasing nitrate concentration while the same oxidizing environment will cause iron to oxidize and precipitate from solution. Conversely, the sulphur, selenium, and copper concentrations in water are positively correlated to the nitrate concentration in water (Figure 10), as would be expected since sulphur and selenium are normally present as soluble compounds in oxidizing environments. Likewise, at increasingly

negative redox nitrate will be reduced to nitrogen gas, followed by selenate/selenite to elemental selenium and at an even lower redox, sulphate is reduced.

5.3. Plants

Sphagnum (Moss) was found to grow in nearly all areas of the site, except directly at the outlet of the creek to the W15 pond. *Salix sp.* (Willow) was found at all sites, except at sites in the W32 area, which appeared to have been cleared at some point. *Carex aquatilis* (Water Sedge) was dominant within the W32 area that was recently cleared (Figure 11), and also within the W10 area where the water was deeper. *C. aquatilis* was found to be spreading through the W10 area access road, being the first plant to colonize the road and even recent tire ruts (Figure 12), indicating that it will be a potentially good choice for quickly establishing the CWTS. However, at locations with shallower water, *C. aquatilis* and *Sphagnum* coexisted, and *Equisetum* was also present, growing through the *Sphagnum*. This suggests that if water depth is not maintained, *Sphagnum* and *Equisetum* will grow in succession.

Compared to the other plant species tested, *Sphagnum* showed significantly greater uptake of elements such as copper, uranium, and iron at all sites that *Sphagnum* was available (Figures 13 and 14). No detectable selenium was found in any of the plants except for *C. aquatilis* and *Sphagnum* at the W32 Seep site (0.7 mg/kg) and *Sphagnum* at the W32 Mid site (0.3 mg/kg). This is presumably due to higher amounts of selenium being present in water at the W32 Seep site (Figure 6). Uptake of copper and iron in all plants was negatively correlated with copper concentration (-0.904) or iron concentration (-0.994) in sediment at each location, respectively. These findings suggest varying bioavailability of copper at the different sites sampled, and when elements are being deposited into the soils, they are in forms (species) that are less bioavailable to the plants than when in aqueous form.

All cases of water channeling through the natural wetland area were in association with *Salix* plants (Figure 15). *Salix* species had the least uptake of elements compared to other plant species at most of the sites (Figure 13). The root structure of these plants appears to contribute to the creation of channelized creeks, which also corresponds with little or no treatment occurring in the creek area. *Salix* has not regrown in the W32 area that was previously cleared, and it is recommended that this area be surveyed yearly

to assess whether they will begin to reestablish in this area. It should also be determined when the W32 area was cleared. This information will be used to establish a maintenance schedule for the wetlands, as young *Salix* plants should be removed before becoming well established.

Although a greater water depth is one parameter that can be used in CWTS to create an anaerobic redox and decrease dissolved oxygen concentration in the water, the research conducted here has shown that *Sphagnum* can be an effective means of removing elements from the seepage through uptake, a finding supported by literature. As such, this potential succession of plants may occur, but is not expected to significantly impact the performance of the CWTS, and may even improve the performance via plant uptake in the *Sphagnum*.

5.4. Microbiology

Genetic profiling of the microbial communities indicate great diversity with hundreds of species, but for the purpose of discussion in this section, the top ten most abundant organisms are focused upon (Appendix I). When assessing the microbial genetic profiles of all sites, most samples have a very similar distribution of organisms (Figure 16). The outflow of the W15 creek was sampled in triplicate as a control for microbial variability within a given sampled site. All of the creek samples receiving seepage (W32, W38, W39, and the three W15 sites) cluster together, which indicates similar microbial profiles. All three W15 sites show nearly identical microbial distributions (Appendix I and II), while strong similarities are found for all creek sites. One common trend in all creek sites is the presence of the *Intrasporangiaceae* organism group (yellow in Appendix I). This group appears to be closely related to *Tetrasphaera*, which are known to be involved in phosphorus removal from wastewater. Conversely, the W10 and W10 *Carex* Root samples show distributions more similar to one another than to the downstream creek locations. A large distribution of the organisms at W10 and W10 *Carex* Root locations represents the order *Bacteroidales*, which are ubiquitous colonizers of a wide range of environments (e.g., soil, activated sludge, decaying plant material, compost, fresh water and marine samples, dairy products). However, the W10 *Carex* Root had a denser population of organisms than the W10 sample taken away from plants in the area (i.e., # of organisms per gram of sample; Figure 17).

Laboratory growth-based assays also tested for total heterotrophic, selenite-, sulphate-, and nitrate-reducing organisms at different sites (Figures 17 and 18). The population of total heterotrophs grown under aerobic conditions was

significantly greater than under anaerobic conditions (Figure 18). Interestingly, both the laboratory- and genetic profiling-based methods showed very few sulphate-reducing organisms at most of the sites, with the highest amounts found at the W10 *Carex* Root and W15 inflow sites (Figure 17). The W10 *Carex* Root sampling location also showed interesting sediment features with black color and smell at deeper depths, characteristic of sulphate-reducing organisms, but with red iron oxides visible at the water interface (Figure 19). Selenite-reducing organisms were found at all tested locations, while nitrate-reduction only occurred to completion at the W10 *Carex* Root, W10, W32 Creek Red, W38 Creek and W15 inflow locations (Figure 17). No correlation was found between the number of selenite-, sulphate-, or nitrate-reducing organisms and the water concentration of nitrate, selenium, and sulphate at each location. This is potentially due to the correlation analysis being performed on water concentrations instead of sediment concentrations for these compounds (which is where the reducing organisms would be found), as non-detectable selenium and nitrogen levels were found in most of the sediment samples. In the case of sulphur, the laboratory (Maxxam Analytics) did not perform the requested analysis on the soils, and discussions are ongoing to determine if new samples could be collected and analyzed at their cost to fill in this data gap.

In addition to the red precipitate at the W32 Creek Red site, this location has a unique microbiological profile (Figure 16 and Appendix I). A very dominant percentage of the microbial population at W32 Creek Red is a bacterium called *Geobacter*. This organism is known to seek out insoluble iron oxides, and establish direct contact and reduce them as part of its metabolism. As such, it is likely that the high abundance of *Geobacter* bacteria found at W32 Creek Red is actively reducing Fe(III) to Fe(II), which then reduces Cu(II) to Cu(I) through a coupled biogeochemical reaction. In addition to the interplay between iron-reduction and other elements, the W32 Creek Red site has much lower available phosphorus in the soil and total phosphorus in the water in comparison to the W32 Creek site which is water flowing just adjacent to the red precipitate area, also suggesting that increased microbial activity may be playing a role.

6. Summary and Conclusions

Collectively, these findings contribute greatly to the ability to design and operate a CWTS for effective treatment of waters at the site. The geochemical data indicate that substantial treatment of seepage is occurring on site within the natural wetland, validating the concept that a constructed wetland could be designed to function in the long-term closure condition at

the site, but with optimized treatment performance. Mechanisms for treatment of copper and selenium were identified in the natural wetlands receiving seepage from the SWD, including dissimilatory sulfate reduction followed by sulfide precipitation, dissimilatory selenium reduction, and iron co-precipitation.

It is clear that there is greater and faster treatment occurring within the natural wetland than would have been evident if calculated only using data collected by previously existing routine sampling programs. The additional loading of elements at the runoff/culvert into the W15 collection pond is likely a significant contributor to loading during rainfall and melt events, masking the treatment that is occurring through the wetland for seepage that is originating from the SWD toe.

It is evident that the seepage chemistry varies greatly, both between seeps and over time (this study and historical data gathered). This will need to be taken into consideration when evaluating options of constructing a seepage diversion trench compared with building multiple smaller wetlands. The sources and amounts (frequency and range in flows) can be coalesced or treated as separate flows with individual specifically designed wetlands. Collection of seepage by a diversion trench would allow mixing of the various seepage chemistries and more uniform composition and volume of inflow water to the wetlands, but individual wetlands may be favoured in the W15 area due to topography and seepage locations.

There appears to be sufficient soil on site for both hydrosol and berms for the CWTS. Analysis indicated that the chemistry of overburden soils is amenable to use as soil in the CWTS and to support plant growth, although slightly elevated in copper as is the entire background area. The overburden peat on site is also amenable to use in the soil as an amendment to the CWTS. However, additional sources of organic matter to create anaerobic conditions in the sediment and improve dissimilatory reduction pathways for sulfur and selenium will still need to be identified (such as wood chips, straw, and/or compost). Additionally, when an onsite demonstration-scale CWTS is built, there should be a mechanism developed to deliver seepage water to the CWTS rather than creek water, as substantial treatment is currently occurring between the seeps and the creek.

There is suitable vegetation present that is well adapted to the site conditions. The plants at site show a robust ability to grow in the range of water chemistries present in the area. Moreover, all sites sampled are host to a wide diversity of microbes with known capability of performing

favourable biogeochemical reactions, which will contribute to water treatment in a CWTS. This confirms that even at sites where no chemical evidence of treatment was found, there are microbes present that would be capable of these reactions if encouraged with the right environment (e.g., plant roots, shift in dissolved oxygen or redox, availability of electron donors). While organisms capable of favorable geochemical reactions were ubiquitous at the site, they were found in elevated numbers associated with *Carex* roots, enforcing our selection of this plant species for the CWTS.

CWTS designs are currently being piloted at Contango's year-round pilot facilities. These systems are planted with *C. aquatilis* from site, and peat from the selected borrow site is incorporated into the soils. Replicate pilot-scale CWTS experiments are in progress with and without *Sphagnum* added in order to assess whether the *Sphagnum* contributes positively or negatively to the treatment, and also whether succession from *C. aquatilis* to *Sphagnum* as the dominant plant can be prevented or enhanced through modification of water depth.

The pilot study will continue to integrate information and design decisions, as well as provide supplementary soil and water redox measurements to validate hypotheses about biogeochemical pathways in the CWTS. Further to this, pilot CWTS sediments will be subjected to leachability testing for elements of concern to confirm suitability and longevity of the designs. CWTS designs will continue to be optimized as closure water chemistry and volumes are refined.

7. Closure

We trust the information herein satisfies your present requirements. Should you have any questions, please contact the persons listed below. We appreciate the opportunity to provide the services detailed in this report, and look forward to discussing any comments you may have.

Respectfully submitted,

Contango Strategies Ltd

8. References

1. Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Lozupone, C. A., Turnbaugh, P. J., Fierer, N., and Knight, R. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proc. Natl. Acad. Sci. USA* 108 Suppl 1: 4516-22, 2011.
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3. Lovley, D. R. and Phillips, E. J. P. Novel mode of microbial energy metabolism: organic carbon oxidation coupled to dissimilatory reduction of iron or manganese. *Appl. Environ. Microbiol.* 54: 1472-80, 1988.
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9. Tables

Table 1: Summary of samples taken at the Minto Mine site

| Location | Water Sample | Sediment Sample | Micro Sample | Plant Sample |
|-------------------------|---------------------|------------------------|---------------------|---------------------|
| W10 * | Yes | Yes | Yes | Yes |
| W10 <i>Carex</i> Root | - | - | Yes | - |
| W32 Seep | Yes | - | - | Yes |
| W32 Mid | Yes | - | - | Yes |
| W32 Creek | Yes | Yes | Yes | Yes |
| W32 Creek Red | Yes | Yes | Yes | - |
| W39 Seep | Yes | - | - | - |
| W39 Creek | Yes | Yes | Yes | - |
| W38 Seep | Yes | - | - | - |
| W38 Creek | Yes | Yes | Yes | - |
| W15 Inflow 1 | Yes | Yes | Yes | Yes |
| W15 Inflow 2 | - | - | Yes | - |
| W15 Inflow 3 | - | - | Yes | - |
| Culvert runoff into W15 | Yes | Yes | Yes | - |

* *Carex aquatilis* and *Sphagnum* for the Minto Pilot 1 CWTS at Contango Strategies were obtained from this location

Table 2: Summary of water, soil, and plant analyses

| Water Analysis | Soil Analysis | Plant Analysis |
|---|---|--|
| Total Regulated Metals (Al, Sb, As, Ba, Be, Bi, B, Cd, Cr, Co, Cu, Fe, Pb, Li, Mn, Mo, Ni, P, Se, Si, Ag, Sr, Tl, Sn, Ti, U, V, Zn, Zr, Ca, Mg, K, Na, S, Hg) | Assessment ICP Metals (Al, Sb, As, Ba, Be, Bi, Cd, Ca, Cr, Co, Cu, Fe, Pb, Li, Mg, Mn, Hg, Mo, Ni, P, K, Se, S, Ag, Na, Sr, Tl, Sn, Ti, U, V, Zn, Zr) | Metals (Al, Sb, As, Ba, Be, Bi, B, Cd, Ca, Cr, Co, Cu, Fe, Pb, Mg, Mn, Mo, Ni, P, K, Se, Ag, Na, Sr, Tl, Sn, Ti, U, V, Zn) |
| Routine Water (Alkalinity, HCO ₃ , Ion Balance, Dissolved ions, Hardness) | Available Nitrogen, Phosphorous, and Potassium (NPK) | |
| Total Suspended Solids (TSS) | Cation Exchange Capacity (CEC) | |
| Chemical Oxygen Demand (COD) | Sodium Adsorption Ratio (SAR) | |
| Biological Oxygen Demand (BOD) | Total Organic Carbon (TOC) | |
| Total Kjeldahl Nitrogen (TKN) | Electrical Conductivity (EC) | |
| Total Organic Carbon (TOC) | Texture Analysis (Physical properties) | |
| Ammonia | | |

Table 3: Copper, selenium, sulphur, and chloride concentrations along the creek. The approximate fold change in concentration along the creek is provided in grey in parentheses.

| | W10 | W32 Creek | W38 Creek | W39 Creek | W15 Inflow |
|----------------------------------|------------|----------------------|----------------------|----------------------|-----------------------|
| Total Copper (µg/L) | 5.92 | 32.5 (5.5) | 10.6 (0.3) | 11.5 (1.1) | 19.7 (1.7) |
| Total Selenium (µg/L) | <0.1 | 0.71 (7.1) | 2.64 (3.7) | 2.53 (1.0) | 2.10 (0.8) |
| Total Sulphur (mg/L) | <3 | 9.7 (3.2) | 24.7 (2.5) | 23.0 (0.9) | 23.1 (1.0) |
| Dissolved Chloride (mg/L) | 1.2 | 1.8 (1.5) | 3.0 (1.7) | 3.1 (1.0) | 2.9 (0.9) |

Table 4: Copper, selenium, sulphur, and chloride concentrations at W32 Creek compared to W32 Creek Red location, and W32 Mid compared to W32 Seep. Approximate fold concentration difference between the two sites is provided in grey parentheses.

| | W32 Creek | W32 Creek Red | W32 Seep | W32 Mid |
|----------------------------------|----------------------|--------------------------|---------------------|--------------------|
| Total Copper (µg/L) | 32.5 (0.06) | 1.93 | 10.1 (1.5) | 15.1 |
| Total Selenium (µg/L) | 0.71 (0.3) | 0.21 | 19.6 (0.01) | 0.24 |
| Total Sulphur (mg/L) | 9.7 (0.3) | <3 | 110 (0.1) | 11.9 |
| Dissolved Chloride (mg/L) | 1.8 (2) | 3.5 | 5.5 (1.2) | 6.8 |

Table 5: Average copper, selenium, sulphur, and chloride concentrations in the creek (W32, W38, W39, and W15; i.e., not including W10), compared to each seep. Approximate fold concentration difference between the seep and average creek is provided in grey parentheses.

| | Avg. Creek | W32 Seep | W38 Seep | W39 Seep |
|----------------------------------|-----------------------|---------------------|---------------------|---------------------|
| Total Copper (µg/L) | 18.6 | 10.1 (0.5) | 102 (5.5) | 71.4 (3.8) |
| Total Selenium (µg/L) | 2.0 | 19.6 (9.8) | 7.59 (3.8) | 0.94 (0.5) |
| Total Sulphur (mg/L) | 20.1 | 110 (5.5) | 45.8 (2.3) | 46.0 (2.3) |
| Dissolved Chloride (mg/L) | 2.7 | 5.5 (2.0) | 5.5 (2.0) | 3.8 (1.4) |

10. Figures



Figure 1: Conceptual closure diagram of Minto mine (Appendix A, Minto Phase V/VI Preliminary Reclamation and Closure Adaptive Management Plan), indicating potential wetland locations and routine monitoring sites.



Figure 2: Sampling locations at the Minto mine. All routine monitoring points are indicated in white, while sampling locations unique to this study are shown in yellow. The blue arrows indicate the direction of water flow. The W32 Creek label corresponds to the location of both the W32 Creek and W32 Creek Red.



Figure 3: Minto Site W32 Creek (bottom in A; left in B) and W32 Creek Red (top in A; right in B) in A) August 2013 and B) September 2013. The pictures show the same site at two different dates.

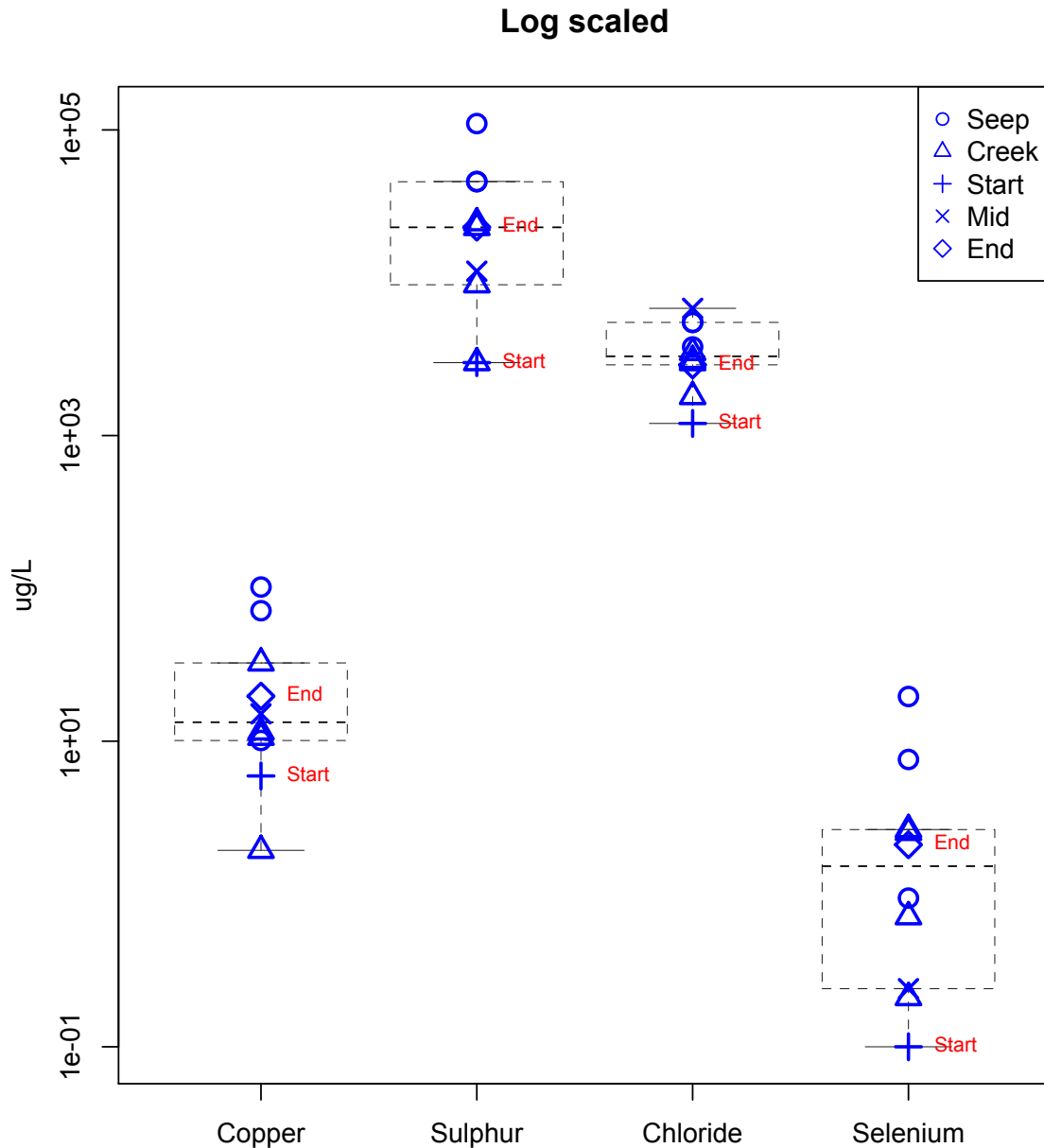


Figure 4: Comparison of copper, sulphur, chloride and selenium concentrations in the creek between the W10 and W15 areas, and seeps along the toe of the SWD. "Start" indicates the W10 location, while "End" indicates the W15 inflow. The W32, W38, and W39 Creek locations are shown as triangles, while all seeps are differentiated as circles. The W32 Mid site is depicted as a X. Note the spread of the copper, sulphur, and selenium plots is greater than that of chloride. The seeps demonstrate the highest concentration of copper, sulphur, and selenium, while chloride concentrations do not follow this trend.

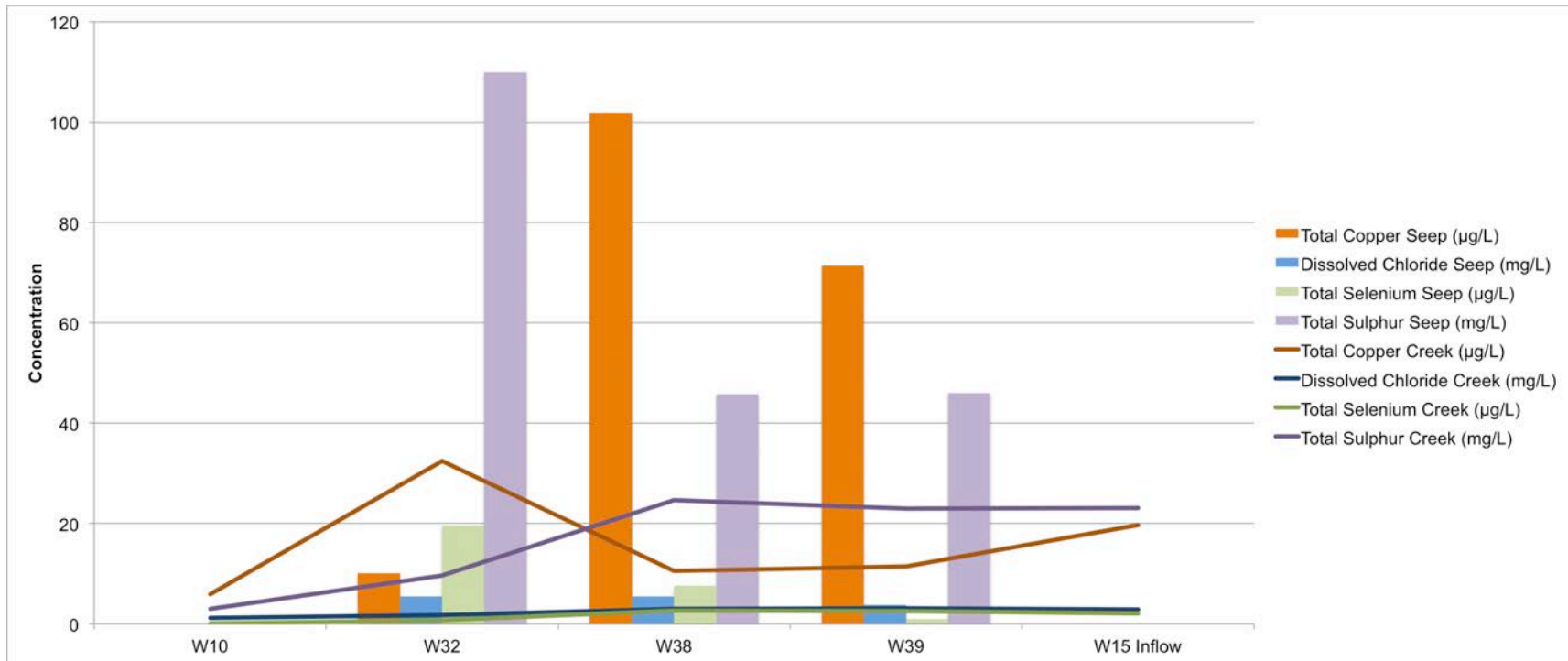


Figure 5: Copper, chloride, selenium, and sulphur concentrations in the creek and seep locations. Bars indicate seepage, while lines indicate the creek concentration of each element.

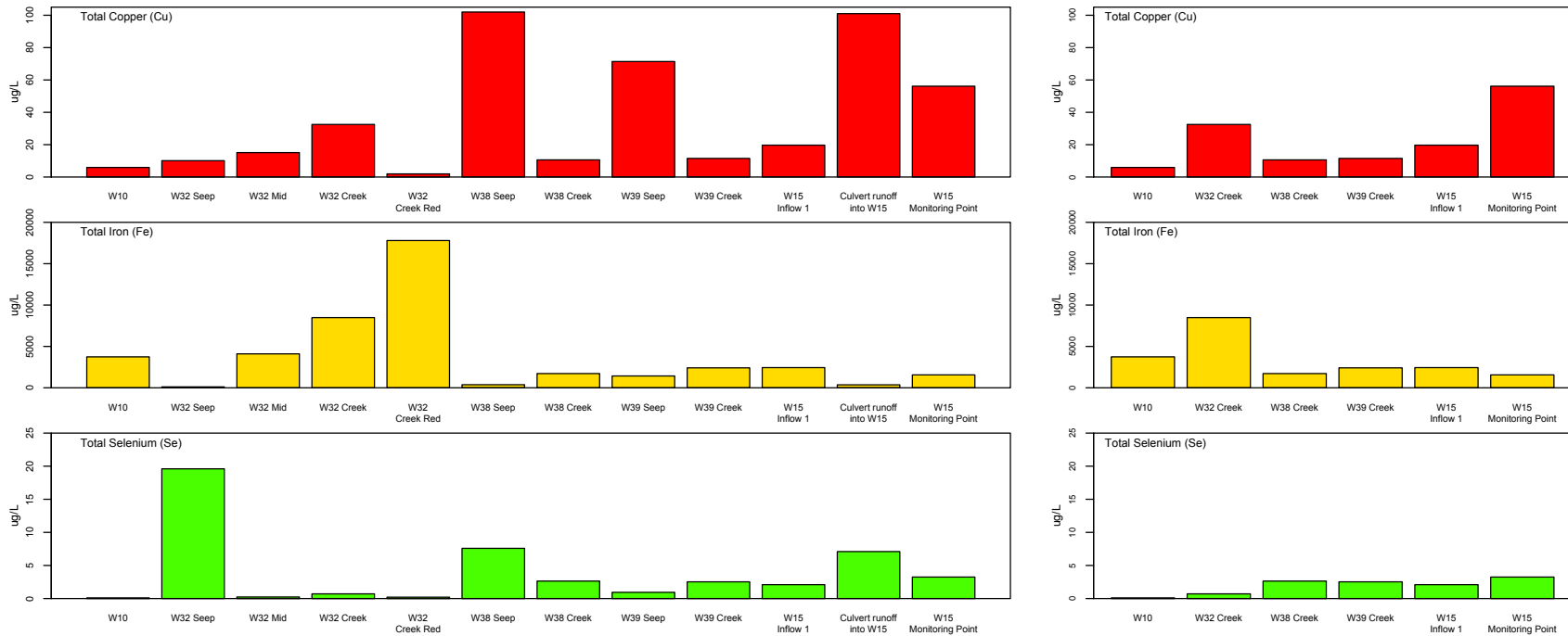


Figure 6: Copper, iron, and selenium concentration in water. The left plots show all sites, while the right plots show the distribution of elements along the creek. The W15 Monitoring Point represents the average of September 17th and September 28th, 2013, monitoring timepoints, which are the closest dates to the site visit when samples were obtained.

Minto Mine Constructed Wetland Treatment Research Program - Site Assessment

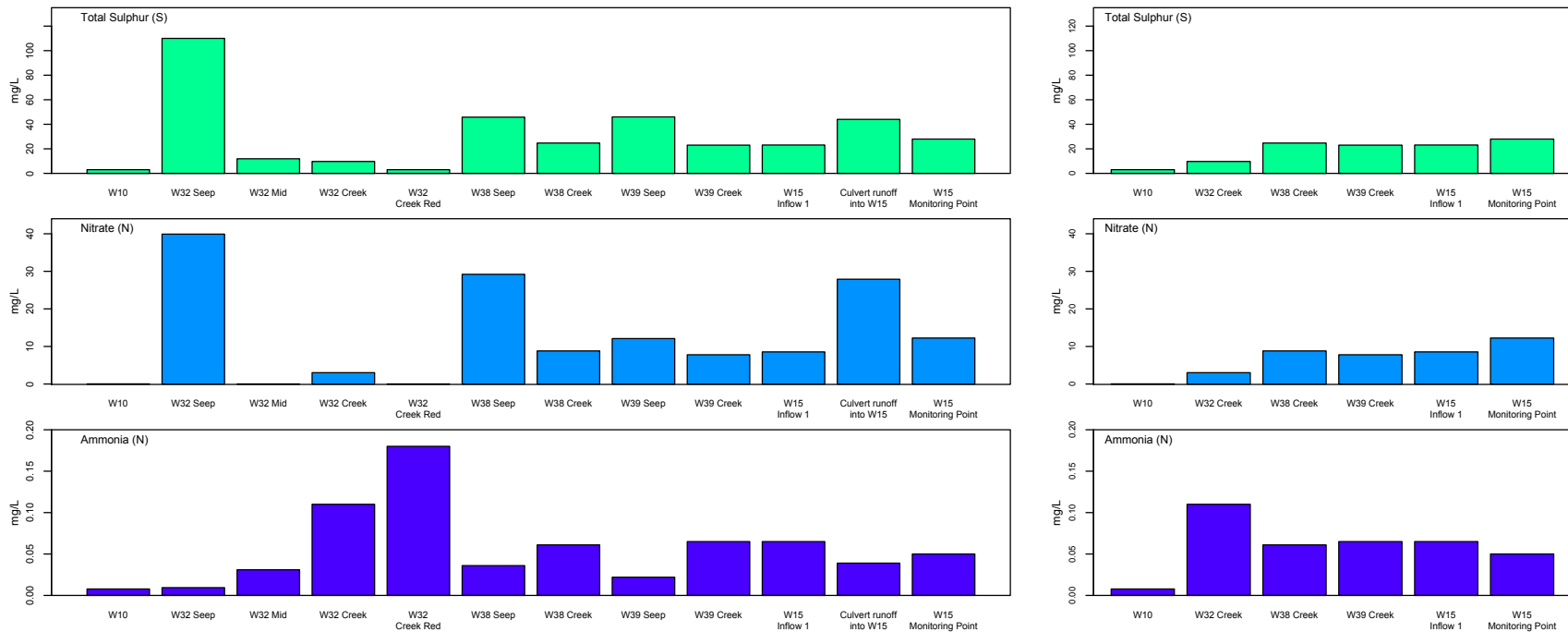


Figure 7: Sulphur, nitrate, and ammonia concentration in water. The left plots show all sites, while the right plots show the distribution of elements along the creek. The W15 Monitoring Point represents the average of September 17th and September 28th, 2013, monitoring timepoints, which are the closest dates to the site visit when samples were obtained.

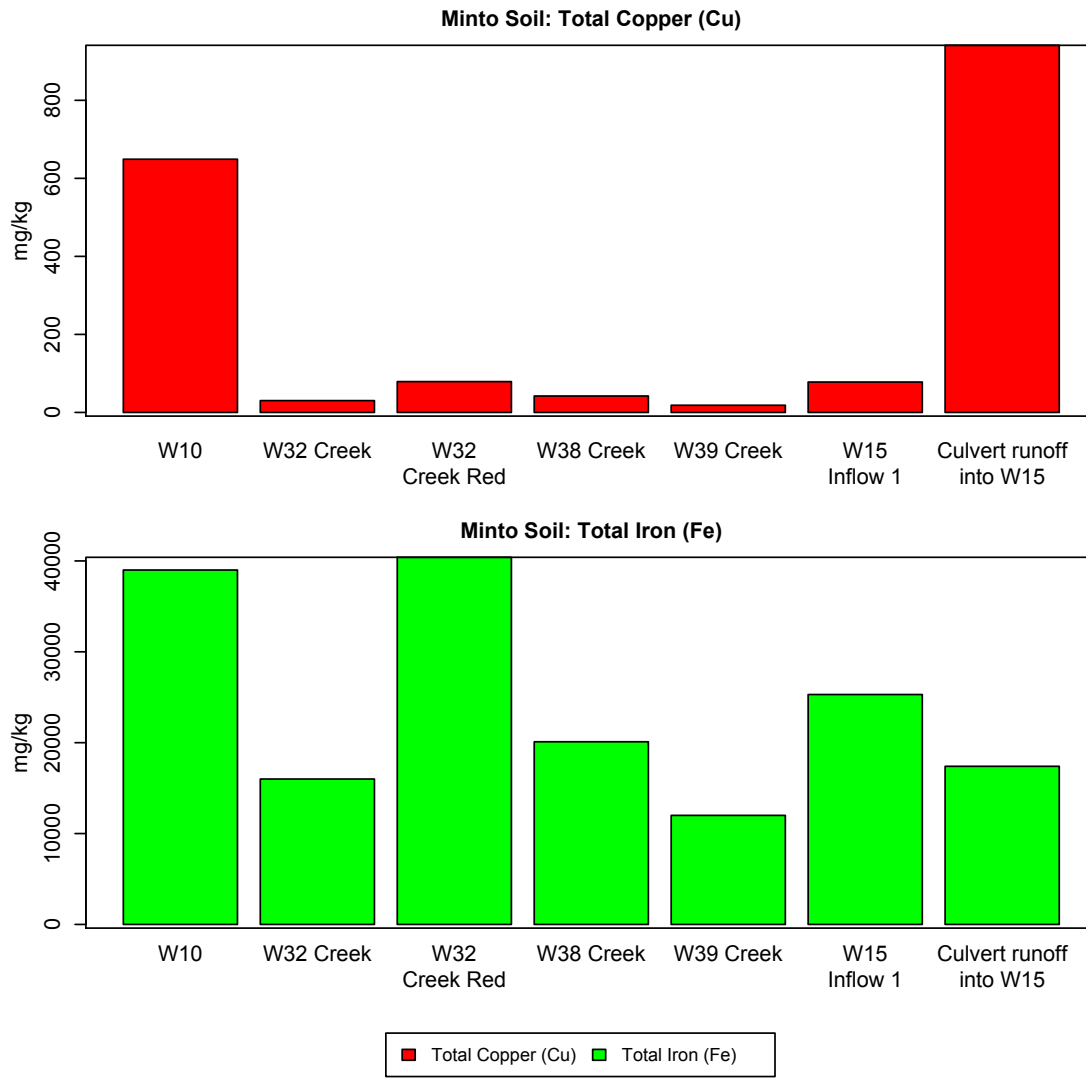


Figure 8: Copper and iron concentration in soil. Selenium was below the detection limit in sediment (<0.5 mg/kg), and is therefore not shown.

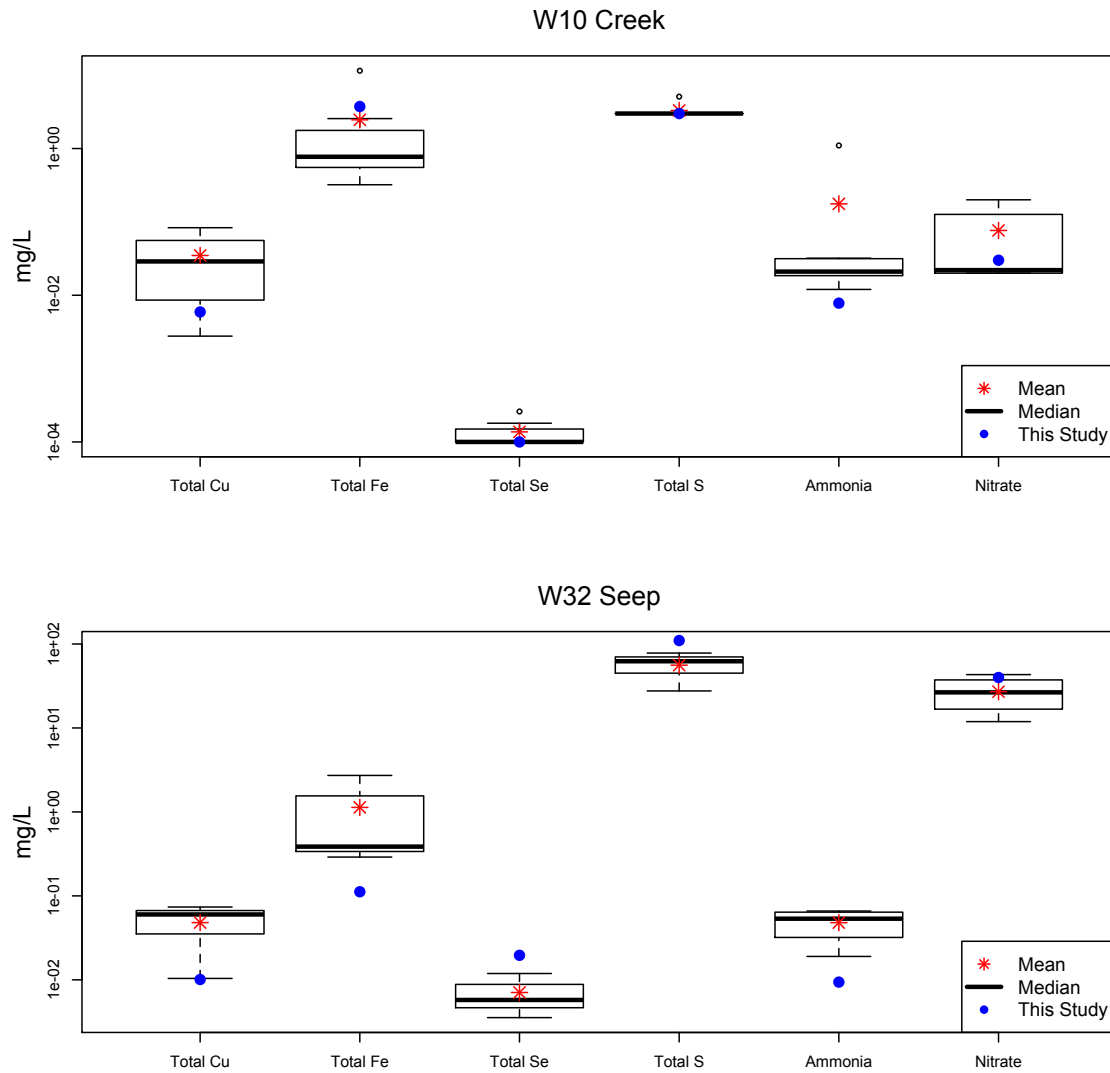


Figure 9: Concentration of select elements from all 2013 routine monitoring program measurements. Blue dot indicates concentration measured in samples collected as part of this study. Figure is continued on following page.

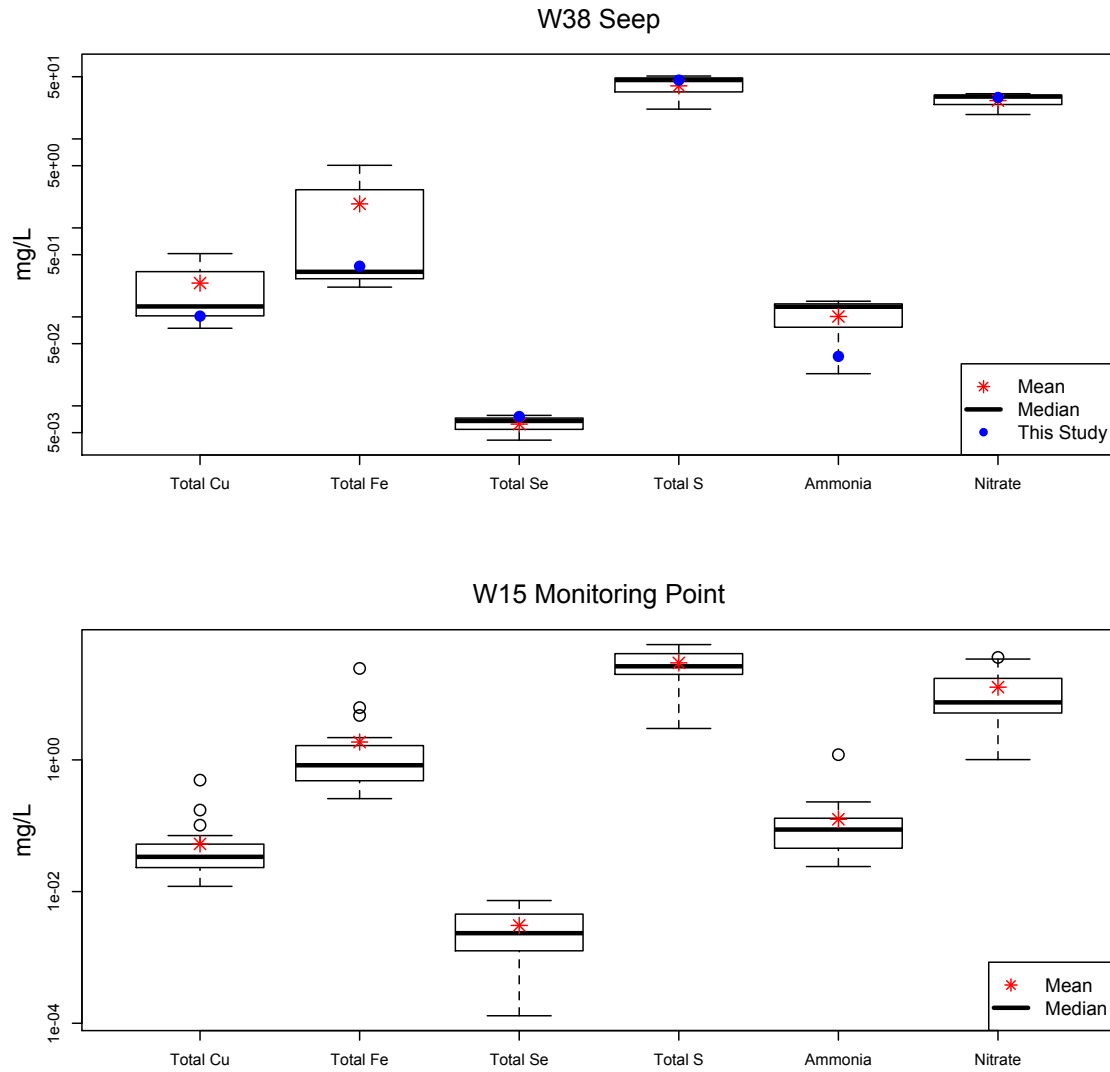


Figure 9: Concentration of select elements from all 2013 routine monitoring program measurements. Blue dot indicates concentration measured in samples collected as part of this study. Figure is continuation from previous page.

Minto water: significant geochem correlations (P<0.1)

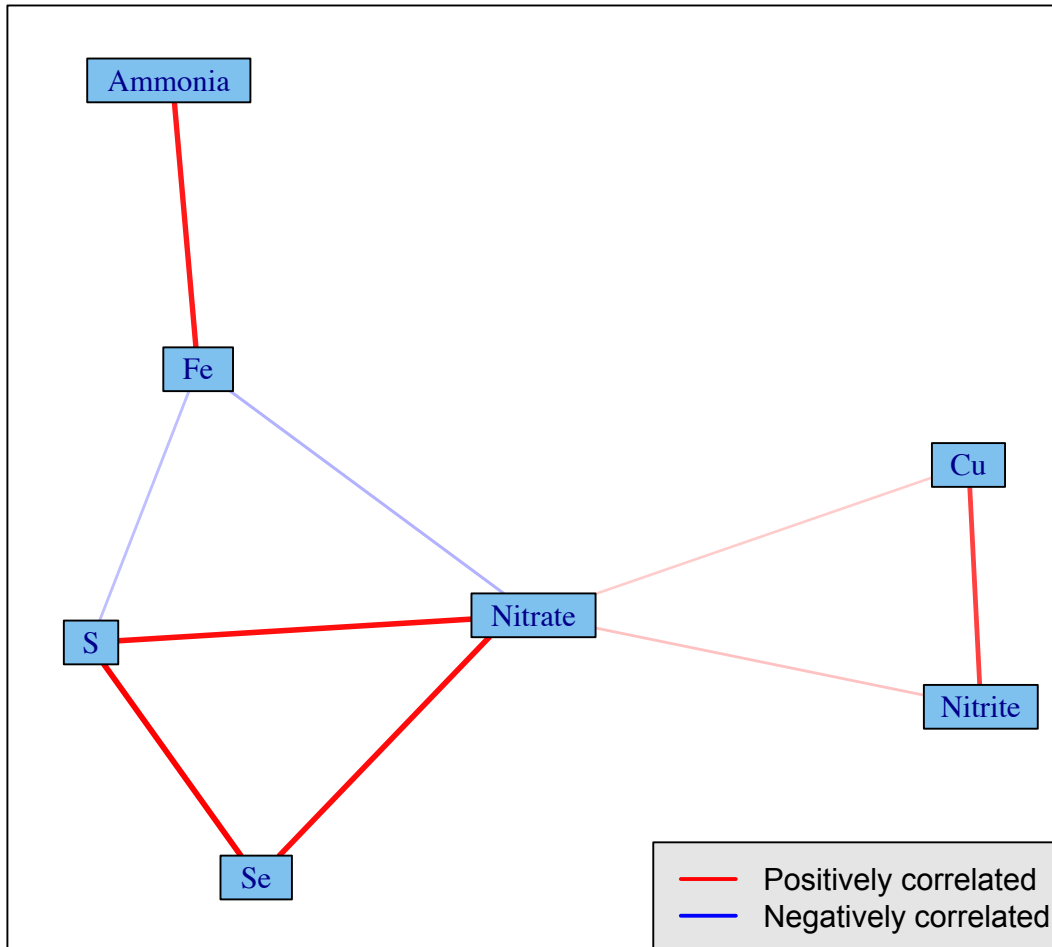


Figure 10: Correlation of various compounds in water. The strength of the line connecting different compounds indicates the strength of the correlation. For example, the thick line between Se and S corresponds to a Pearson correlation coefficient of 0.939, while the line connecting Cu, and Nitrate or Nitrite corresponds to a coefficient of 0.527 or 0.812, respectively. All Pearson correlations are significant at a level of P<0.1. Also of note, copper concentrations in soil were found to be significantly correlated with copper and nitrite concentrations in the water (not depicted in figure).



Figure 11: *C. aquatilis* and grasses are dominant at the W32 area that was recently cleared.



Figure 12: *C. aquatilis* spreading through the W10 area access road, being the first plant to colonize the road and recent tire ruts.

Minto plant data

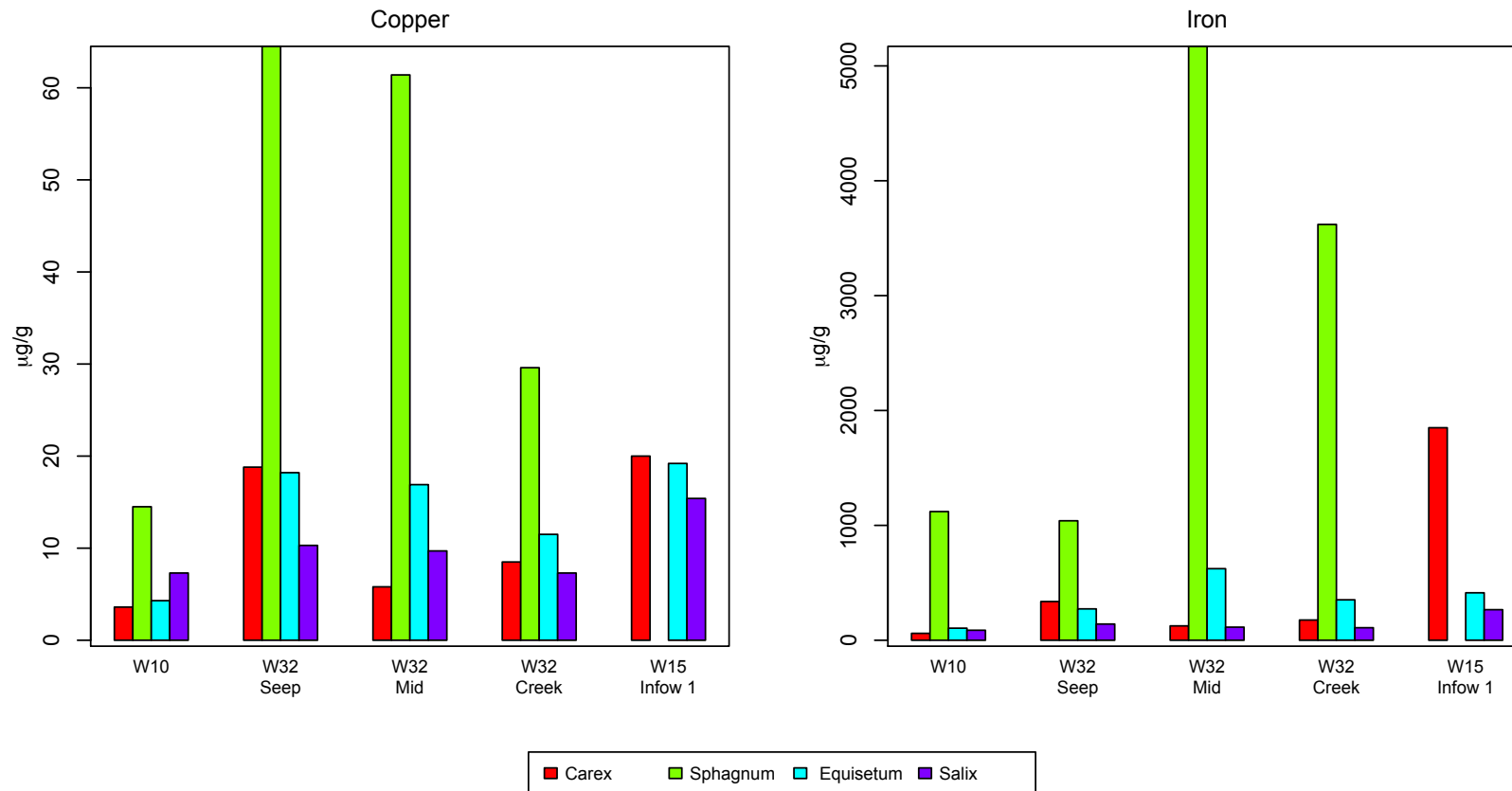


Figure 13: Concentration (µg/g) of dry plant matter for copper and iron in plants at the Minto Site. Note that there was no *Sphagnum* present at the W15 inflow site available for collection.

Minto-site: Enrichment factor for Copper, Iron and Uranium

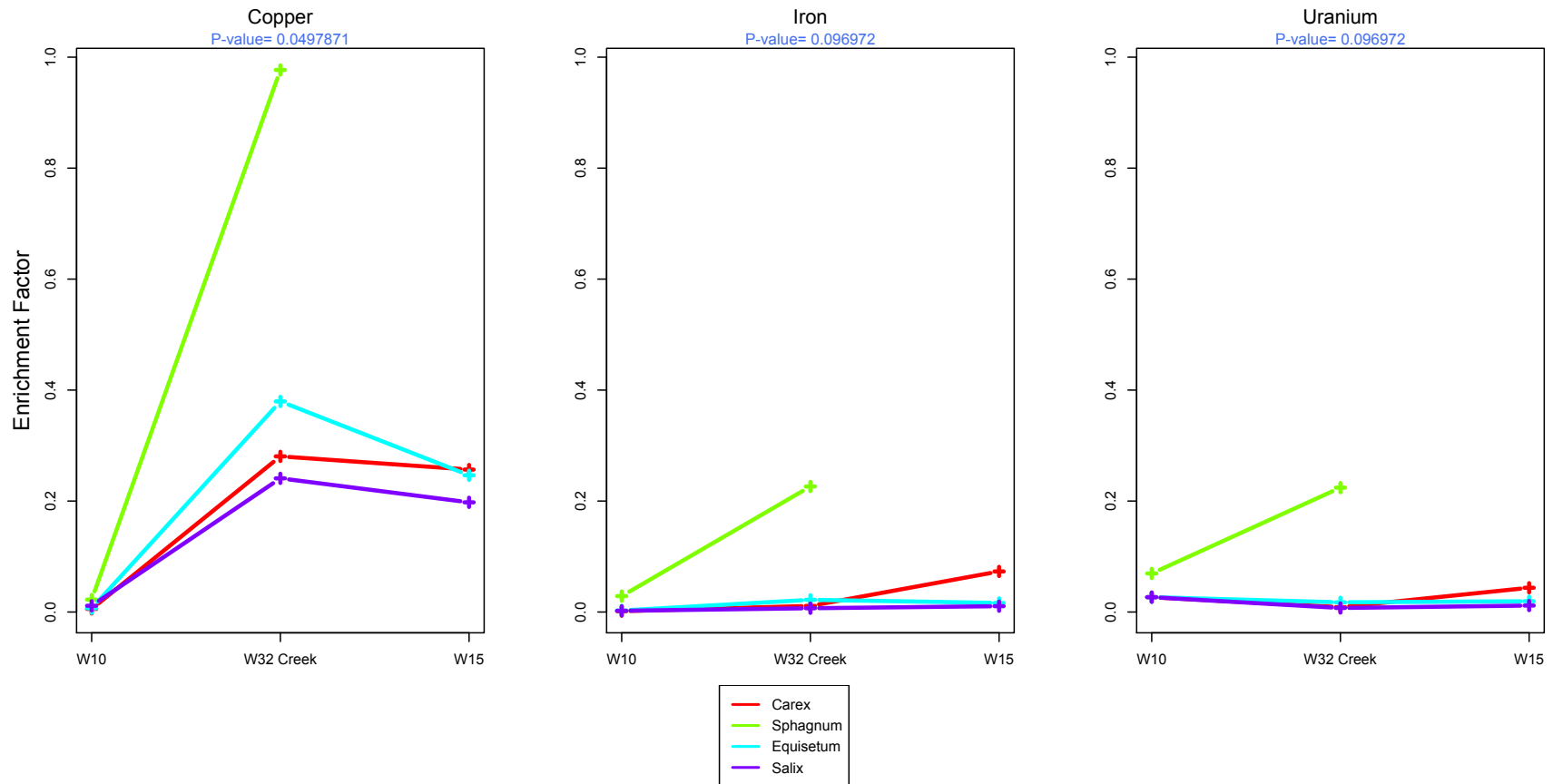


Figure 14: Enrichment factor for copper, iron, and uranium in *Carex*, *Sphagnum*, *Equisetum*, and *Salix*. A p-value <0.1 indicates there is a significant difference in the enrichment factor at different sites. Note that there was no *Sphagnum* present at the W15 inflow site available for collection.



Figure 15: Example of creek channeling through *Salix* plants.

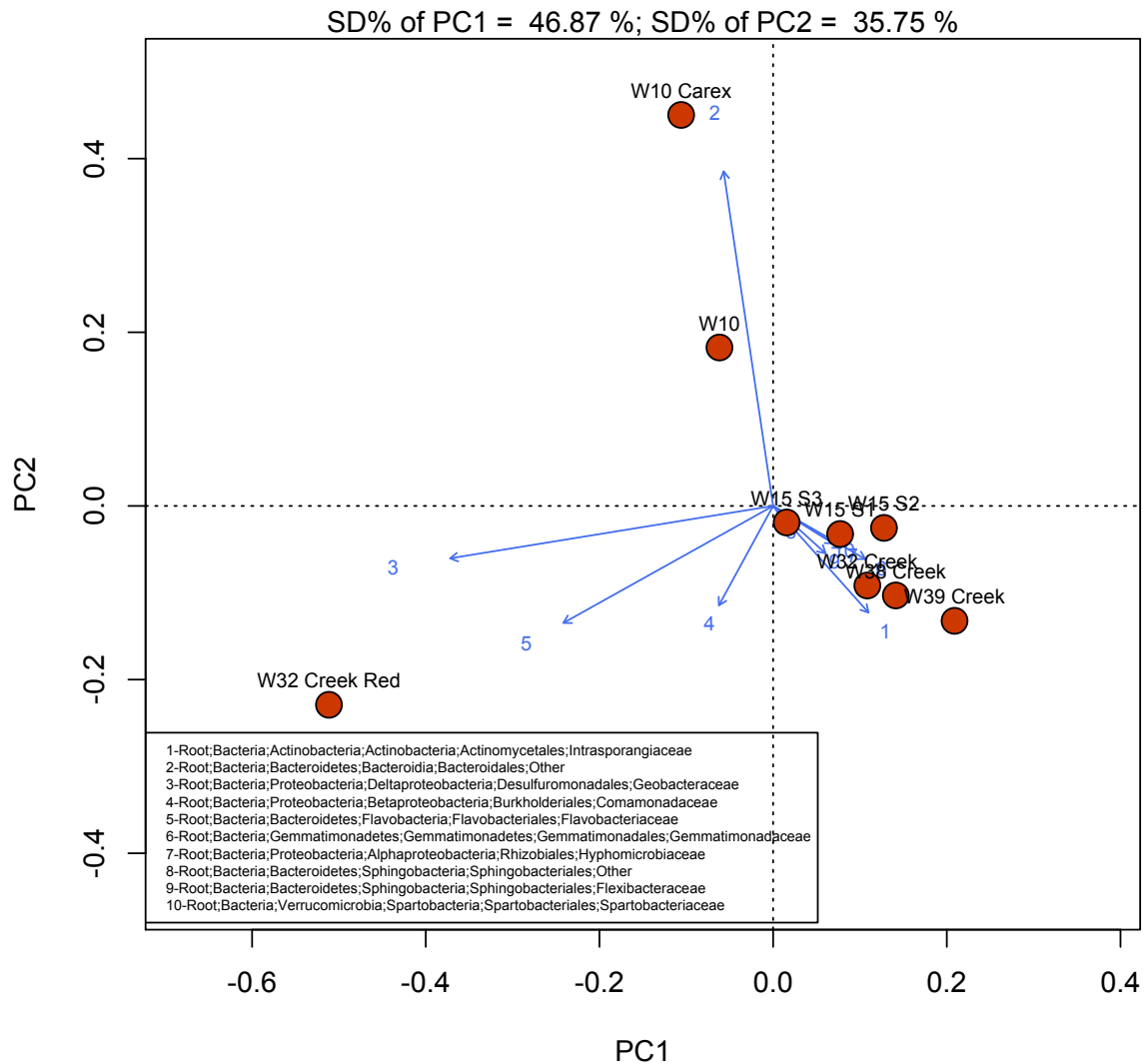


Figure 16: Principal component analysis of all sites based on microbial community profiles. All sites that cluster together have similar microbial profiles, while sites clustering on their own (e.g., W32 Creek Red) show a less similar profile. Each numbered arrow represents a group of microorganisms listed in the legend. Essentially, the direction and length of an arrow represents how abundant that group of microorganisms is in each sample. For example, W32 Creek Red would show a high abundance of *Geobacteraceae* (arrow #3) and *Flavobacteriaceae* (arrow #5), while W10 Carex shows a high abundance of *Bacteroidales* (arrow #2). All creek samples (W32, W38, W39, and W15) cluster together and show similar microbial profiles (high abundance of *Intrasporangiaceae*, arrow #1).

Minto MPN: SeIV, NO₂ production, N₂ production and SO₄

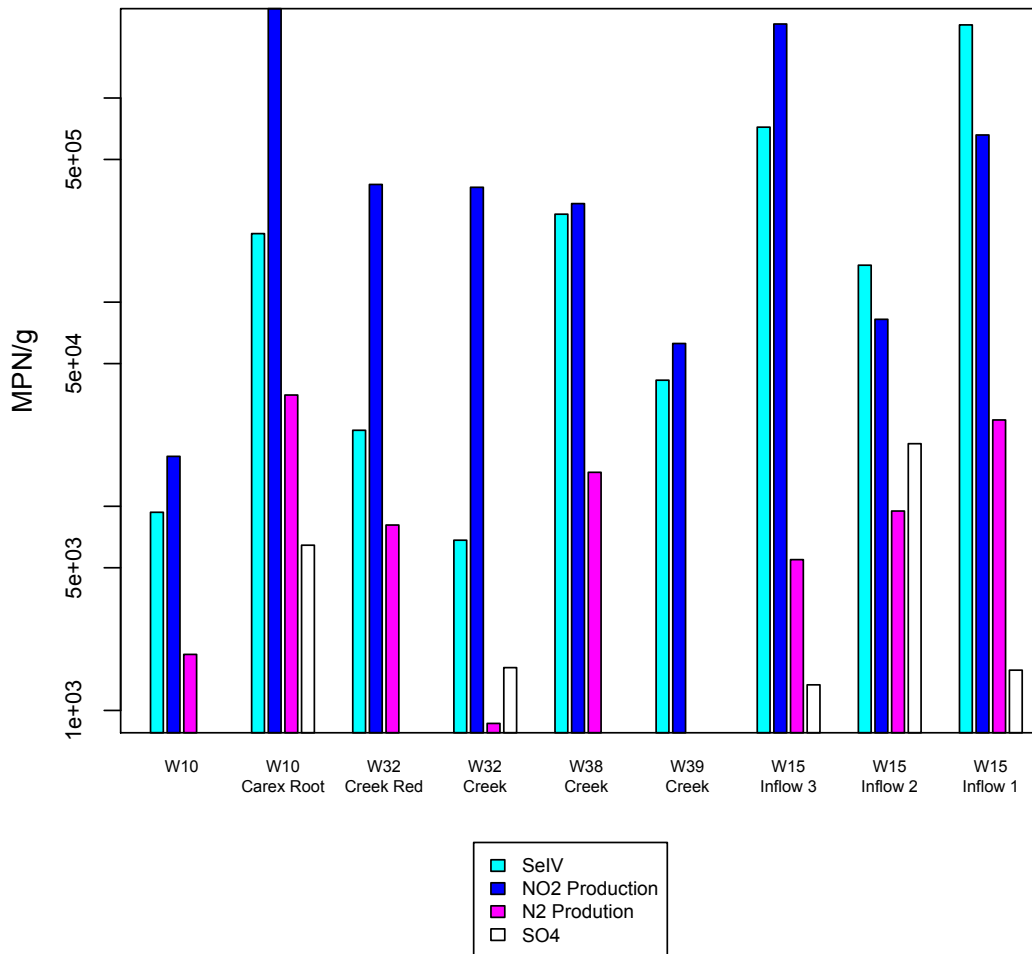


Figure 17: Predicted number of selenite, sulphate, and nitrate reducing bacteria found at various Minto sites, based on the most-probable number assay. The nitrate-reduction assay tests for microbes that can reduce nitrate to nitrite (i.e., NO₂ Production), as well as microbes that can further reduce nitrite to nitric oxide or nitrogen gas (i.e., N₂ Production).

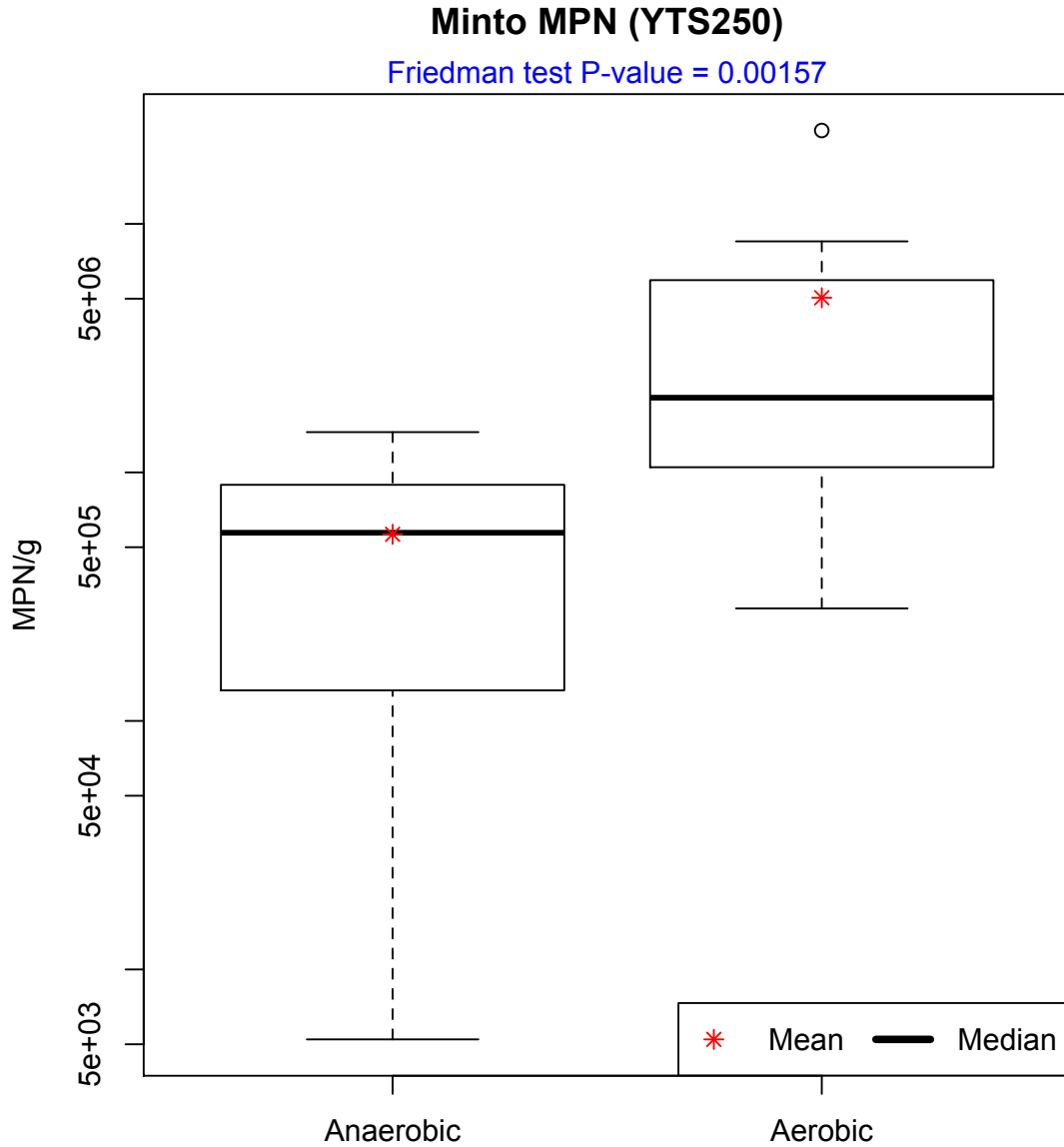
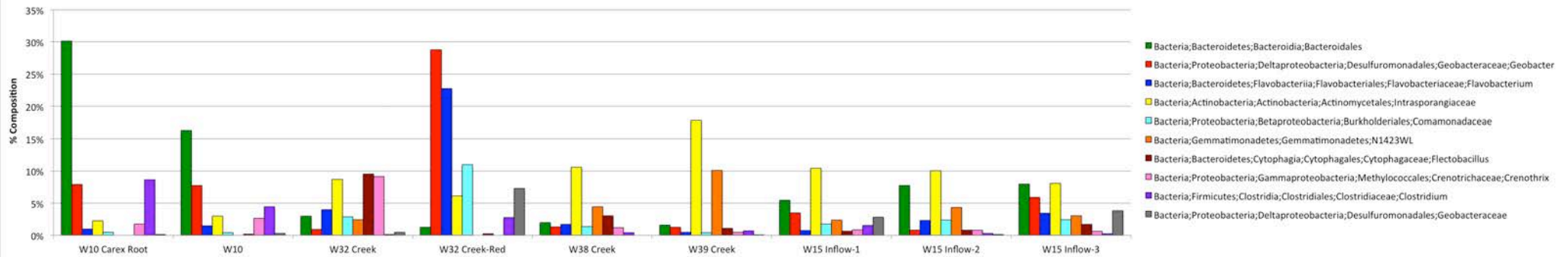


Figure 18: Most-probable number of total heterotrophic organisms at the Minto site. The number of heterotrophic organisms growing under aerobic (with oxygen) and anaerobic (no oxygen) conditions is shown. Significantly more total heterotrophs grew under aerobic conditions than under anaerobic, as demonstrated by a P-value < 0.05.



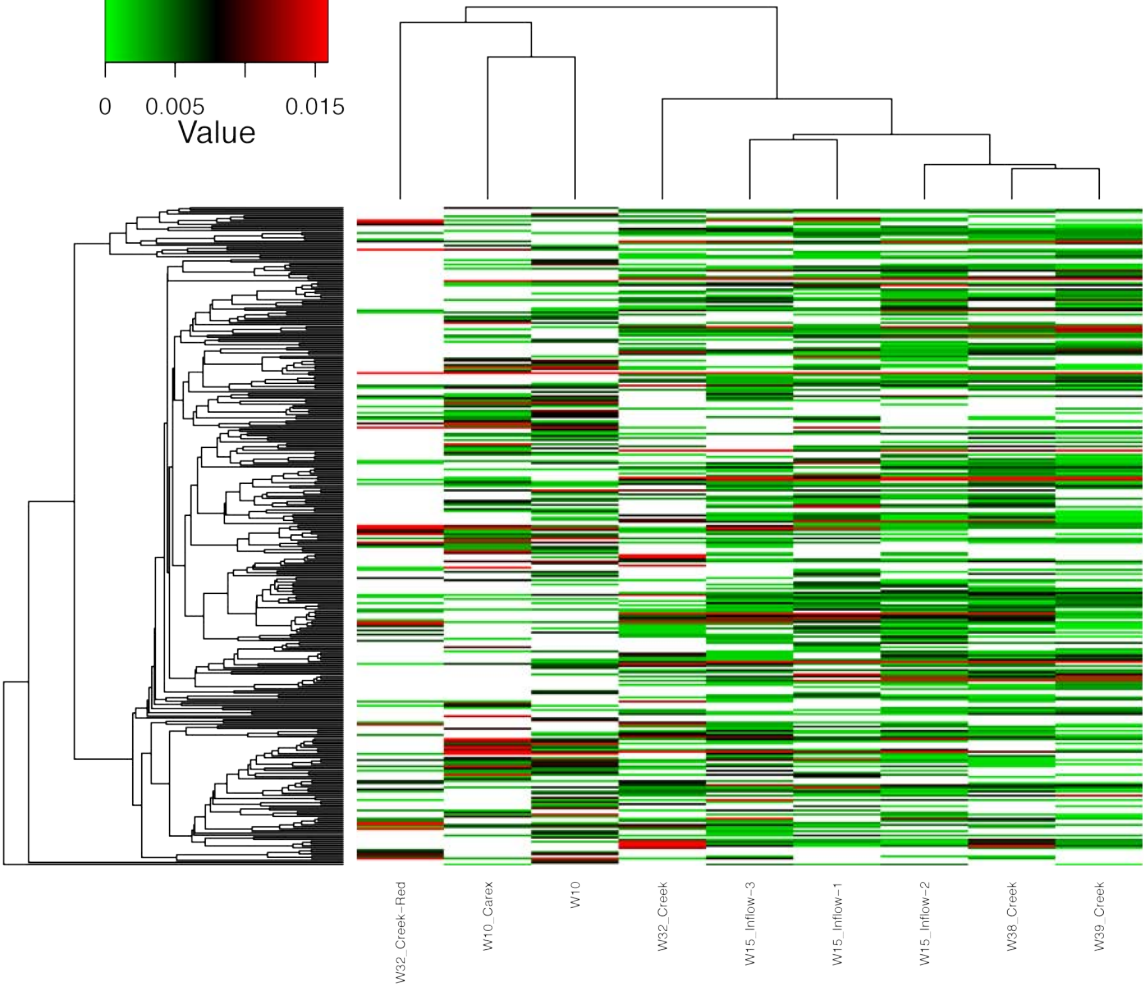
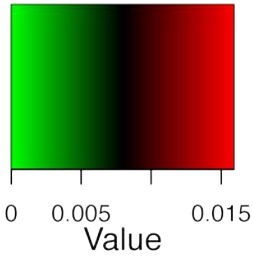
Figure 19: Color distribution of sediment surrounding *Carex* root at the W10 location.

Appendix I - Top 10 most abundant bacteria across all sites



Appendix II - Microbial Profile

Color Key



Heatmap showing the distribution of bacteria at the Minto Mine. Each column represents a sampling location, while each row represents an OTU (group of bacteria). The abundance of each group of bacteria is depicted through a heatmap: white indicates absent, and green through red indicates increasing abundance. Samples are clustered together based on the similarity of microbial communities, and indicated by the dendrogram at the top of the heatmap. The dendrogram on the left represents a phylogenetic tree of all OTUs.