"BUGS AND VEGGIES" AS TAILINGS MANAGEMENT TOOLS

Victoria Collins, MSc

Chibuike Chigbo, PhD

Amanda Schoonmaker, PhD

Heather Kaminsky, PhD

Andrea Sedgwick, MEng

Paolo Mussone, PhD

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EXECUTIVE SUMMARY

The objective of this research was to demonstrate the use of a plant mediated dewatering technology augmented with organic amendments and plant growth promoting bacteria for the purpose of improving consolidation and shear strength in oil sands tailings materials. This study evaluated two native boreal species (sandbar willow; *Salix interior* and slender wheatgrass; *Elymus trachycaulus*), two organic amendments (peat and hydrochar) and one bacterial culture enriched from tailings. An additional treatment of mulched willow roots was also added to some treatments to evaluate the potential of transferring beneficial rhizospheric bacteria from established willows to rooted seedlings. This project was executed in two phases. The objective of Phase 1 was to optimize a bacterial inoculum in the form of a biofilm grown on a substrate. Two cultures previously enriched from mature fine tailings (MFT) and two from the tailings used in the greenhouse phase were evaluated for metabolic activity and nitrogen fixation. The most active were evaluated for hydrocarbon degradation in scaled-up cultures. A series of substrate materials were also evaluated in combination with cultures to determine which material promoted the growth of biomass or metabolic activity. The objective of Phase 2 was to evaluate plants, amendments, and inoculum in combination in an outdoor greenhouse study and determine which treatment combination most effectively improved shear strength and removed water from tailings. Above and below ground biomass were measured as well as water use, shear strength, and solids content. The microbial community was also identified in a subset of these treatments; these data are discussed in detail in this report.

In Phase 1, the previously established enrichment cultures produced significantly more methane than tailings cultures established for this project suggesting the bacteria in these cultures were capable of metabolizing organic molecules (toluene and citrate) at a faster rate. Of these cultures, the previously established Canadian Natural culture also presented higher nitrogen fixing activity as determined by performing an acetylene reduction assay (proxy for nitrogen fixation). The Thickened Tailings culture also had a higher rate of acetylene reduction however, neither of these cultures reduced significantly more acetylene than other cultures. Of the three substrates and controls evaluated (activated carbon, fly ash, diatomaceous earth, and media alone), cultures grown on diatomaceous earth were significantly more active in both methane production and acetylene reduction. As such, Canadian Natural and Thickened Tailings cultures with diatomaceous earth were selected for scale-up. Scaled up cultures were evaluated for methane production to monitor culture development and assessed for toluene degradation. Of the two treatments, only Canadian Natural cultures showed significant methane production and hydrocarbon degradation.

Phase 2 was established in late May 2018 in an outdoor enclosure at the Center for Boreal Research in Peace River, AB. Of the amendments evaluated in this study, hydrochar significantly improved the development of leaf biomass suggesting this treatment is will improve plant mediated tailings dewatering. Bacterial amendments alone did not have a significant impact on plant growth however, treatments with bacterial inoculum and hydrochar in combination may have had a synergistic effect and merit further investigation. Willows were found to have a significant impact on increasing water removal, shear strength and solids content. Leaf biomass was strongly correlated with increased shear strength and increased solids content at depth.

The microbial community in these columns was driven by the presence of plants, oxygen, and tailings type rather than amendments or inoculum. Of these treatments, amendment with hydrochar increased the proportion of Bacilli and Clostridiales, which contain known nitrogen fixing and plant growth promoting species. Neither column treatment nor microbial population appeared to have a discernable effect on hydrocarbon concentrations.

The results of this study suggest that plant mediated tailings dewatering using native boreal species is feasible and the growth of native plants can be improved by using organic amendments, including hydrochar or potentially mined peat (in the case of the grasses). Bacteria may also have a synergistic effect given sufficient time for willow development however, additional work must be done to validate this hypothesis.

1.0 INTRODUCTION

Bitumen is extracted from mined oil sands ores using the modified Clark hot water extraction process. Hot water, caustic agents, dispersants, and diluents are employed to separate and recover bitumen from clays and other minerals (Chalaturnyk *et al.*, 2002). Following bitumen and diluent recovery, the remaining tailings slurry is pumped into settling ponds for water recovery and storage. These tailings are composed of water, sand, silt, clay, salts, and organic residues. Once in the ponds, the coarse sand quickly falls out of solution while the remaining materials form a metastable suspension that is known to persist for decades (Chalaturnyk *et al.*, 2002). Several treatment technologies have been developed to accelerate dewatering including the use of organic and inorganic flocculants and coagulants, centrifugation, and settling aids such as calcium sulfate (Allen, 2008). While these treatments are effective at reducing the volume of tailings, the economical consolidation of tailings into a trafficable dry landscape suitable for final reclamation remains a challenge.

A promising low-cost technology is the use of plants, which hold significant promise with early trial work demonstrating that many species are capable of reasonably healthy persistence on mine tailings (Silva 1999) and tailings sands (Woosaree and Hiltz 2011). In 1998, a detailed greenhouse study by Silva suggested that some plant species may be able to grow in tailings (Silva *et al.*, 1998; Silva, 1999). Moreover, these plants could dewater the materials while increasing the shear strength in the root zone. This approach provided several benefits such as increased bearing capacity, increased matrix suction and water release through evapotranspiration, potential degradation of organic acids, and CO_2 respiration at relatively low cost (Silva *et al.*, 1998; Silva, 1999). However, other plant species evaluated in Silva's study were adversely affected by highly saline conditions and efficacy was limited by the relatively shallow roots of grass species. Silva further suggested that native species may be more suitable because they tend to better tolerate lower overall nutrition and are well adapted to the difficult climate in NW Alberta. In fact, subsequent studies have demonstrated that a wide variety of native plants are capable of establishment and growth on various tailings substrates (Silva, 1999; Naeth and Wilkinson, 2002; Renault *et al.*, 2004; Wu, 2009, 2015; Yucel *et al.*, 2016; Schoonmaker *et al.*, 2018). In a 5-month long greenhouse trial per plant water use data was compelling; in slender wheatgrass (*Elymus trachycaulus*), daily transpiration in centrifuge tailings was estimated at 100+ mL of water per-plant (Yucel et al. 2016). For sandbar willow (*Salix interior*), daily transpiration averaged 25 mL per plant (Yucel et al. 2016). For context, if slender wheatgrass was established at a plant density of 25 plants m^2, this would equate to 25,000 L of transpiration per hectare, per day.

However, mine tailings can be limited in essential nutrients such as nitrogen (Collins *et al.*, 2016), which is required for plant growth. In addition, the successful establishment and productive growth of plants can be negatively affected by the presence of organic compounds (phenols, naphthenic acids [NAs], polycyclic aromatic hydrocarbons [PAHs], naphtha), and elevated levels of heavy metals, salts, alkalinity, and pH in tailings and associated oil sands process water. Conventional inorganic fertilizers or other organic, nutrient and carbon rich amendments have shown the potential to ameliorate some of the adverse effects and encourage plant development. Organic amendments such as peat (Renault *et al.*, 2004), biochar (Fellet *et al.*, 2011) and alfalfa pellets (Woosaree and Hiltz, 2011) have all been effectively used to enhance plant development for tailings sand stabilization. These amendments can enhance plant growth by supplying and/or retaining nutrients as well as improving the physical, chemical and biological properties of the tailings material.

Plant growth promoting microorganisms can provide nitrogen for plant growth, enhance survivability by degrading stress hormones associated with drought and salinity, produce growth hormones, and assist in the biodegradation of organic contaminants (Bashan, 1998; Vessey, 2003; Khan, 2005; Zhuang *et al.*, 2007; Ahemad and Kibret, 2014). Nadeem et al. (2013) found that inoculating wheat plants grown in naturally saline soil with cultured plant growth promoting bacteria increased seed germination, plant growth, yield, and overall nutrient content including nitrogen, phosphorous and potassium. The authors suggested the inoculated bacteria were capable of degrading 1-aminocyclopropane-1-carboxylate (ACC), the precursor to the plant stress hormone ethylene. Bacteria capable of degrading ACC have been found to promote root elongation and increase nutrient uptake in the presence of heavy metals (Belimov *et al.*, 2001), and hydrocarbon contamination when co-cultured with hydrocarbon degrading

bacteria (Khan *et al.*, 2013). Some bacteria including certain *Bacillus* sp. *Pseudomonas* sp. and *Rhizobiaceae* also produce indole-3-acetic acid (IAA), a plant hormone that influences growth. Grobelak et al. (2015) found inoculating grasses with IAA producing bacteria increased shoot and root elongation. Root elongation provides multiple benefits. Rhizodeposition of root exudates increases with root biomass development, thereby feeding the microbial community and expanding the bacterial population in root zone (Juhanson *et al.*, 2007). Enhanced root growth (thereby greater root surface area for nutrient absorption) may be advantageous in nutrient deficient tailings.

Bacteria for bioremediation or bioaugmentation have been well studied and are commercially available, however bacterial amendments applied *in-situ* have traditionally been prone to failure or unpredictable results (Thompson et al., 2005, and references therein). Challenges such as high salinity, heavy metal content, pH, nutrient availability, competition, and toxins present in the amended materials can inhibit microbial growth and nullify potential benefits. Bacteria well adapted to environmental conditions can be employed to overcome these obstacles. Endogenous bacteria can be enriched from the source materials (Salem *et al.*, 2003), or enrichment cultures from materials with similar environmental conditions and known functions can be developed (Nasseri *et al.*, 2010). However, enrichment conditions are highly controlled and environmental shock can still be a concern, therefore the development of a biofilm-based inoculum is desirable. Biofilms are highly resilient and serve to protect the microbial community from environmental factors and enable environment dependent metabolic activities. This includes anaerobic nitrogen fixation in the presence of oxygen (Lee *et al.*, 2014; Wang *et al.*, 2017). Growth matrix substrates play a key role in promoting biofilm development. For example, bacteria attach more rapidly to non-polar surfaces due to interactions with the cell wall. This activity has been observed on hydrophobic materials such as plastic and Teflon (Donlan, 2002). Biofilms have also been cultured on biochar as a potential alternative to sand and soil wastewater filters (Dalahmeh *et al.*, 2018). Porous or rough surfaces also increases bacterial colonization due to the reduction of shear forces and higher surface area (Donlan, 2002). Substrates such as activated carbon and wood-based fly ash have unique properties including: hydrophobicity and high surface area, with previous studies suggesting activated carbon promotes biofilm formation and bioremediation (Koch *et al.*, 1991; Scott *et al.*, 1995; Caldeira *et al.*, 1999). Diatomaceous earth is another potential carrier substrate as it has high surface area, low cost, and silica based composition as sand is used as a matrix for biofilm development in wastewater treatment (Dalahmeh *et al.*, 2018).

The overall objective of this project was to demonstrate the integrated use of native boreal plant species, organic amendments and selected indigenous bacteria cultures capable of degrading residual hydrocarbons and organic acids while fixing nitrogen in the soil as tools to further accelerate dewatering and transformation of treated tailings material (thickened tailings/centrifuge cake) into a reclaimed soil. The study is one of the first to use bioaugmentation of indigenous plant growth-promoting-microorganisms enriched for hydrocarbon degradation, an approach selected to overcome the common adaptation problems to adverse environmental conditions. This project was divided into two phases where the goal of the first phase was to optimize production of bacteria, grown on solid carriers, capable of growing in oil sands tailings and that are able to achieve one or more of the following: (i) degradation of hydrocarbons, or (ii) capacity to fix nitrogen. These characteristics were anticipated to result in the improvement of the rhizosphere environment thereby enhancing plant development and growth. The second phase of this study evaluated the growth of two native plant species (*Elymus trachycaulus* and *Salix interior*), with or without inoculation of bacteria, in treated tailings, where the following questions were asked:

1. Does inclusion of an organic amendment improve plant establishment and growth? And does the type of organic amendment matter?
2. Does inclusion of bacteria inoculum improve plant growth?
3. Is there a synergistic effect when combining organic amendments with bacteria in terms of improving plant growth?
4. How does the inclusion of an organic amendment affect the bacterial community?
5. Does establishment of native plants lead to improvements in the geotechnical properties of tailings?
6. Does inoculation with bacteria result in measurable reductions in hydrocarbons in tailings?
7. Does the type of tailings impact the growth of plants?

2.0 MATERIALS AND METHODS

This study was articulated into two phases. The purpose of Phase 1 was to identify a metabolically active bacterial source capable of nitrogen fixation that could persist in oil sands tailings and evaluate a set of solid substrates (activate carbon, fly ash and diatomaceous earth) as vehicles to inoculate the tailing-plant root rhizosphere. The most active microbial community cultured on carrier substrate from this investigation were utilized for Phase 2 activities. Phase 2 comprised a multi-factor growth trial with two native species (slender wheatgrass and sandbar willow) grown in treated tailings obtained from Imperial Oil's Kearl mine and CNRL's Albian mine.

2.1 Phase 1

2.1.1 Chemicals, materials and gasses

Acetylene (scientific grade), 30% CO_2/70% N_2 (scientific grade), and all other gasses (certified standard 4.8 or higher) were purchased from Praxair (Mississauga, Ontario). All chemicals were reagent grade or higher from Fisher Scientific (Toronto, Ontario). Thickened tailings and centrifuge cake were provided by Imperial Oil and Canadian Natural, respectively. Syncrude and Canadian Natural media based enrichment cultures were established using methods previously described (Collins *et al.*, 2016) to increase the proportion of bacteria capable of hydrocarbon degradation and nitrogen fixation in tailings (unpublished data). Substrates diatomaceous earth and activated carbon were purchased from Red Lake Earth (Kamloops, BC), and Alfa Aesar (#3330236), respectively. Fly ash substrate was obtained from a pulp and paper mill in Alberta and was composed primarily of volatile carbon and silicates. Substrates were selected based on the literature, which suggested non-polar, porous, and high surface area substrates promote biofilm development (Donlan, 2002).

2.1.2 Primary bacterial culture and growth substrate evaluation

This two-factor study examined bacterial cultures and growth substrates in combination. Cultures originated from previously developed enrichment cultures (Syncrude, Canadian Natural) or were developed through enrichment of the tailings under evaluation in this study (centrifuge cake, thickened tailings). Substrates included activated carbon, diatomaceous earth, fly ash, and methanogenic media alone. Sterile negative controls for substrates were established without bacterial inoculum, and sterile controls for inoculum were established in media alone without substrate. All treatments were prepared in triplicate.

Cultures were prepared in 125 mL bottles (DWK Life Sciences Wheaton, #219755) with open top butyl septa caps (DWK Life Sciences Wheaton, #240680). Due to the varying densities of substrates, culture composition was established by volume to include 35 mL of each substrate. Substrates were as follows; 20 g diatomaceous earth, 25 g of ground fly ash, and 11 g of activated carbon powder. Media was added to a final volume of 75 ml. Bottles with media and substrate were autoclaved, sealed, exposed to vacuum to remove air, then flushed for 5 min with 30% CO_2 balance N_2 prior to addition of reducing agent. Cultures were then inoculated with 1 mL tailings or enrichment culture.

Nitrogen deficient methanogenic culture media was prepared as described in Collins *et al*., (2016) where a methanogenic media recipe containing salts, trace metals, phosphate buffer, reducing agent, and anaerobic indicator (Fedorak and Hrudey, 1984; Holowenko *et al.*, 2000) was prepared with nitrogen containing components either substituted or omitted. The following modifications were made: the final concentration of the anaerobic indicator, resazurin, was 0.5 mg L^{-1} while 1 g L^{-1} cysteine was used as the reducing agent in lieu of sodium sulfide. Media and substrates were prepared and autoclaved aerobically before anaerobic preparation described above. Following inoculation and two days incubation in the dark, cultures were amended with 230 mg L^{-1} toluene and 500 mg L^{-1} filter sterilized citrate (777.6 mg L^{-1} trisodium citrate).

2.1.3 Evaluation N_2 Fixation Potential

The reduction of acetylene to ethylene was evaluated as a proxy for N_2 fixation (Hardy *et al.*, 1973). Secondary cultures were established for the acetylene reduction assay as acetylene can negatively impact the metabolism of methanogens (Sprott *et al.*, 1982). For active cultures, 10 mL volume was extracted via needle and syringe at day 55 and transferred to a sterile 16 mL Hungate tube (Chemglass Inc, #420801) that had been previously sealed and flushed with sterile 0.22 µm filtered CO_2/N_2 gas. Cultures were amended with an additional 100 mg L^{-1} citrate to promote metabolism and sterile filtered acetylene was added to the headspace for a final concentration of 1.5 vol% (~4 µmol). Ethylene was measured at day 0 and day 19.

2.1.4 Solid Carrier Substrate Characterization

Specific surface area was determined by using the Brunauer–Emmett–Teller (BET) single point method on a Monosorb Quantachrome surface area analyzer. Samples were degassed under heat and inert gas flow (30% He balance N_2 gas mixture: 15-20 psi, 100% N_2: 5 psi, glass sample cells). Surface area was measured in triplicate and corrected for ambient temperature and pressure. To determine the particle size of diatomaceous earth, samples were dispersed in water, sonicated and analyzed on a Horiba LA-950 laser diffraction particle size instrument.

Conductivity and pH were determined by combining substrates with water in centrifuge tubes at the ratio present in cultures (47 vol%; see Primary Cultures) and shaken for 20 min at speed 6 on a VWR standard analogue shaker. Solids were removed by centrifuge (7,000 x g, 20 min) and liquids were analyzed for conductivity (EC) and pH using Barben 551 analytical liquid conductivity sensor and Mettler Toledo seven compact meter, respectively.

SEM samples were prepared by adhering substrate to carbon adhesive tabs (Pelco tabs, 12 mm OD.; Ted Pella, 16084-1) on mounting stubs (pin mount Al 12. 7x11 mm, 8 mm pin; Ted Pella, 16111) and sputtering with gold-palladium alloy. Images were taken on a Tescan Vega3 scanning electron microscope with a 30 kv high voltage power supply using a secondary electron detector. Images were adjusted in Microsoft Powerpoint to increase image clarity (contrast, brightness) and assure 20 µm white bars accurately reflected scales.

2.1.5 Scale-up cultures for phase 2

Scale-up cultures were established in brown 1.1 L glass bottles (33-430) closure fitted with open top butyl septa caps. Bottles were filled with 500 g diatomaceous earth (Red Lake Earth, Kamloops, BC) and autoclaved on 30 min gravity cycle. Nitrogen deficient methanogenic media was prepared as previously described and added to each culture bottle (600 ml). The headspace was then removed from the bottle using a vacuum line attached to a needle and the cultures were flushed with N_2/CO_2 for 10 min. Toluene (200 mg L^{-1}) and sterile trisodium citrate (100 mg L^{-1} citrate) where added to cultures, and 1 ml culture volume from each of the three replicates in Phase 1.1 were added to each 1 L culture for a total of 3 ml inoculum per scale-up culture. Day 0 samples were stored at -20°C and cultures were incubated at 35°C in the dark.

Both Canadian Natural and Thickened Tailings cultures were scaled up in parallel for comparison in preparation for use as inoculum in Phase 2. Cultures were evaluated for methane production and hydrocarbon degradation (toluene) to assess culture development and metabolic activity prior to inoculation. The microbial community was also evaluated at the end of the experiment.

2.1.6 Chemical Analyses

The headspace pressure in cultures were unexpectedly high, likely due to the formation of CO_2 from dissolved calcium carbonates that may be present in the diatomaceous earth product (Hadjar *et al.*, 2008, and references

therein). The high pressure resulted in pressure loss when the septa was punctured to remove samples, and excess pressure was vented periodically to reduce strain on the culture bottles and caps. As such, methane production was determined in percent rather than moles.

Methane, ethylene, and toluene were analyzed using gas chromatography. Phase 1 gas chromatography analyses were performed using an Agilent Technologies 7890A GC System equipped with CTC autosampler and heating block, flame ionization detector (FID) and thermal conductivity detector (TCD). Moles of methane were determined by injecting 0.5 mL culture headspace into a 10 mL headspace autosampler vial and analyzed on GC-FID (column: DB-VRX, 1.2 mL min^{-1} He). Ethylene concentration was analyzed similarly using 0.2 mL headspace from secondary cultures or samples on GC-TCD (column: HP-PLT/Q, 5 mL min^{-1} He). Headspace pressure was monitored using a digital pressure gauge (DPG1000B ± 15.00PSIG-5, MOD-TRONIC Instruments Limited, Brampton, ON) equipped with luer-lock fitting and needle. All gas chromatography data were quantified using external standards, and sterile controls were used as blanks to account for substrate induced background noise.

To determine toluene concentration, 1 ml culture volume was extracted via needle and syringe and shaken with 5 ml hexanes (Optima, HPLC and GC grade) for 5 min. The aqueous phase was discarded and 0.5 g sodium sulfate was added to remove water. The solution was swirled gently for 5 seconds and the remaining solution was filtered (0.45 um) into a 2 ml GC vials (Fisherbrand, 0339118). The vials were sealed without headspace and run the GC-FID system described previously. Culture pH was determined using pH strips (VWR BDH35312.607, BDH35310.601).

2.1.7 Molecular Analyses

Samples from Phase 1 were collected following 55 days of incubation in the substrate trials, and 50 days of incubation in the scale-up cultures (10 days prior to inoculation in Phase 2). Phase 2 samples were collected at the conclusion of the greenhouse study during column destruction. All DNA samples were stored at -20°C until DNA extraction. DNA was extracted using the MP Biomedicals FastDNA SPIN Kit for Soil (MP Biomedicals, 116560200) according to manufacturer instructions with the following modifications. In lieu of a fastprep homogenizer, a Qiagen vortex adapter (13000-V1-24) and vortex were employed. Tubes were shaken for 10 min at maximum speed. Genomic DNA was shipped on ice to the Laurentian Forestry Centre (Natural Resources Canada, Quebec, QC) and amplified for Illumina Miseq V3 (2 x 300 bp) sequencing using primers 515F-Y (5′-GTGYCAGCMGCCGCGGTAA) and 926R (5′-CCGYCAATTYMTTTRAGTTT) to produce a 351 bp fragment (Parada *et al.*, 2016). Data was processed using the MetaAmp bioinformatics pipeline (Dong *et al.*, 2017), which incorporates Mothur (v. 1.39.5), Usearch (v. 9.0.2132) and the SILVA 16S rRNA database (v. v132). Sequences were processed using default settings and trimmed to 255 bp with a maximum 10 bp overlap error. Samples were grouped into OTUs with 0.03 cutoff.

2.1.8 Data Analysis for Phase 1

Statistical significance was determined using single and two factor ANOVA as noted in captions with Tukey HSD post-hoc test. Assumptions of equality of variance and normality were checked using skew and kurtosis, and Levene's test. Data was analyzed in Real Statistics Resource Pack software (Release 5.4). Copyright (2013–2018) Charles Zaiontz. www.real-statistics.com.

2.2 Phase 2

2.2.1 Tailings characterization

Two types of tailings were used in this study: thickener underflow (thickened tailings) and centrifuge cake.

The thickened tailings supplied by Imperial Oil were collected March 18, 2018 between 12:30 and 12:45 pm from the K1+FFT barge with an expected density of 1330kg/m^3. Since the material in the barrels had settled significantly during transport, the released water was decanted off. Homogenization was conducted in three batches by emptying the sediment from three barrels into a large tub and mixing it with a handheld drill equipped with a paint mixer attachment.

The centrifuge cake was supplied by Canadian Natural. This sample was dug out of a centrifuge cake deposit at Jackpine mine at the beginning of November 2017. The samples were sent to Peace River in two specially insulated totes on November 7, 2017 and were kept outside over the winter. In late May 2018, the totes were opened for sampling and the centrifuge cake was found to be completely frozen. The lid was removed and the tote allowed to partially thaw in the sun. To facilitate the thawing and to homogenize the cake the frozen chunks were broken up through the application of mechanical force and the cake was mixed to homogenize it prior to being used to fill the columns.

For both samples, solids content measurements were taken from the bottom, middle and top of each column as they were filled. The results of tailings homogenization can be seen in **Figure A1**. In addition, three larger subsamples were taken for each batch of tailings that were used for filling the columns; one at the start of fill, one half way through the column fill for a given batch and one near the end of the batch. These larger samples were taken back to Edmonton and analyzed at the Centre for Oil Sands Sustainability for bitumen, solids and water content using the Dean & Stark analysis (Dean & Stark, 1920; **Table 1**). Particle size distribution by laser particle size analysis (COSIA FMWG Fines Method, 2016), methylene blue index (Kaminsky, 2014), and Atterberg limits (ASTM D4318 – 17) were also performed (**Figure A2**).

2.2.2 Experimental design and setup

Plants were established outdoors in one meter deep (10 cm diameter) clear-polycarbonate columns (Plastifab Industries) and grown from June to September 2018. This study utilized the following treatments (**Table A1**):

1. **Species:** native grass (*Elymus trachycaulus*) and shrub (*Salix interior* (willow)); these species are native to Alberta and have demonstrated a high level of growth and tolerance in previous mine tailings trials (Yucel *et al.*, 2016; Schoonmaker *et al.*, 2018).
2. **Amendment:** Peat, and hydrochar (the product of the hydrothermal carbonization of meat and bone meal feedstock). Hydrochar was applied at 0.2% of total material weight, while 80 g of peat was added in columns. The hydrochar was under evaluation in a different remediation study and was well characterized. There was evidence to suggest that including a secondary, carbon rich source in soils will facilitate a higher level of microbial activity, further enhancing phytoremediation activities.
3. **Bacteria inoculation**: Type 1 (inoculum developed in Phase 1), and Type 2 (root mulch from willows previously established on centrifuge cake). Bacteria was introduced on diatomaceous earth (as detailed in Phase 1) and incorporated into tailings prior to plant establishment.

Each species, amendment, or bacteria inoculation was established in either centrifuge cake (Albian mine) or thickened tailings (Kearl mine). Forested soil and reclaimed soil (from a recently reclaimed airstrip (Canadian Natural lease holder) located approximately 50 km NE of Peace River, AB) were used as reference soil treatments

for species growth observations. Six replicates of each treatment combination were evaluated resulting in a total of 228 columns experiment-wide. Prior to filling columns, each tailings type, forest soil, and reclaimed soil was homogenized to create a consistent initial solids content. Clear columns (diameter x height = 10 cm x 100 cm) were filled with either centrifuge cake (CC) or thickened tailings (TT) and solids content was subsampled at bottom (65-100 cm), middle (35-65 cm) and top (0-35 cm) of tailings while filling. Following column filling, two seedlings of *Salix interior* were hand planted, and *Elymus trachycaulus* was hand seeded into specified columns. For *Salix interior*, each of the seedlings had been propagated either from seed or from a hardwood stem cutting (one of each propagation type was established in a column; **Table 2**) for a 3-month period prior to establishment in this trial (refer to **Table 3** for summary statistics on the seedling stock). Urea (46-0-0) fertilizer (0.24 grams per column) was sprinkled on the substrate surface of vegetation columns as these tailings were deficient in nitrogen. Columns were watered to sustain plant growth over the course of the study. Water data were recorded and used for subsequent data analyses.

2.2.2 Plant measurements

Plant measurements were conducted in September 2018. Seedling maximum height was measured and recorded using a measuring tape to the nearest 0.5 cm. Plants were harvested with hand clippers in order to remove the aboveground biomass from the CC or TT surface; a subsample of leaves (for determination of leaf area index (LAI)) were stored in plastic bags at -4°C to preserve leaf shape prior to analysis. Vegetation samples were oven dried at 70°C for 48 hours or until constant weight. Aboveground plant biomass was determined to the nearest 0.1 g, but in cases where the sample was very small (< 1 g), the weight was measured to the nearest 0.0001 g. For determination of LAI, individual leaves from each column were placed on a flatbed scanner and WinFOLIA™ software (basic 2012) was used to estimate the leaf area from the scanned image. The scanned leaves were then dried at 70°C and weighed to the nearest 0.1 g. The ratio of leaf area: leaf mass was used to estimate leaf area of each column where above-ground leaf mass had been harvested.

2.2.3 Total root mass

Tailings root sample core was obtained with a 5 cm diameter soil auger at 10 cm depth intervals from the tailings surface and stored at -4°C to preserve roots until processing. Root separation from sample cores follows Schoonmaker *et al*. (2018) where tailings cores were soaked in a container of water overnight to allow the tailings to soften, easing separation of the roots from the tailings and reducing root breakage and fragmentation. Following soaking, the whole sample was mixed by hand to break up clods of tailings. Roots were then separated from the sample by washing through a series of soil sieves (#18 [1.0 mm opening], #60 [0.25 mm opening] and #120 [0.12 mm opening]). Root samples were oven dried at 70°C for 24 hrs and weighed to the nearest 0.0001 g.

2.2.4 Geotechnical measurements

Consolidation (amount of settlement) was measured after harvesting where a measuring tape was used to measure the distance between the top of the column and the surface of the tailings to the nearest 0.5 cm. Soil strength was measured using a hand shear vane (Geonor, no 136, Osteras, Norway), which was carefully pushed into each column to minimize disturbance. The shear strength was analyzed by rotating the hand vane at a uniform rate of approximately 0.1˚/s using the torque application handle until the tailings failed. The maximum torque required to obtain tailings failure was measured, and the vane was continuously rotated for at least three complete turns to record residual torque. This measurement was repeated every 10 cm through to a depth of 90 cm.

Atterberg limits from the hydrochar/bacteria/willow treatments were measured post dismantling to check for changes as these samples had the largest change in texture of all the samples in the study. No change in the Atterberg

limits were found for these samples and therefore the Atterberg limits from before treatment were assumed to hold true for all after treatment samples.

After plant harvesting, solids content was measured by taking a representative tailings sample at depths of 0-35 cm (top), 35-65 cm (middle), and 65-100 cm (bottom). Samples were immediately weighed, dried at 105˚C for 48 hours, and re-weighed to determine dry mass. Solids content (%) was calculated as dry mass divided by wet mass and multiplied by 100. The geotechnical water content was taken as the difference in wet mass and dry mass divided by dry mass and multiplied by 100.

The liquidity (I_L) index and predicted soil strength (C_{wr}) (Locat and Demers, 1988) for all samples were calculated using the equations:

$$I_L \ \ (Index\ of\ Liquidity/Liquidity\ Index) = \frac{water\ content\ of\ sample\ -Plastic\ Limit}{(Liquid\ Limit - Plastic\ Limit)} \qquad (1)$$

$$C_{wr} = (19.8/IL)^{2.44} \qquad (2)$$

The soil strength prediction is used as an approximation for the expected undrained soil strength based on the solids content measured at the end of the trial. Strength in excess of this value can be attributed to strength from roots.

2.2.5 Microbial community analyses

Sample preparation, DNA extraction, sequencing, and data processing were carried out as outlined in Phase 1. Because sequencing replicates per treatment was cost prohibitive, composite samples were used. As such, statistical significance between individual treatments could not be determined and significance was instead evaluated by treatment combination. Data were evaluated using subsampled Bray-Curtis HOMOVA (homogeneity of molecular variance) testing.

2.2.6 Statistical analysis

Effects of different tailings, amendments, and bacteria on the aboveground biomass, root mass, leaf area index, root: leaf ratio of species, and the effect of either (i) *Elymus trachycaulus*, or (ii) *Salix interior*, on tailings (CC and TT) solids content, consolidation, percentage of tailing under water, evapotranspiration, and water use were analyzed using the statistical program R 3.4.1 (R Core Team 2018). Analysis of variance was performed by fitting linear-mixed effect models, with the *lme()* function from the *nlme* library (Pinheiro *et al.* 2017). Replicate blocks were treated as a random effect. Tukey adjusted multiple mean comparison test was used to identify and separate significant treatment effects at $P < 0.05$. The post hoc analysis and the calculation of least squares means was completed using the *lsmeans* package (Lenth, 2018). Principal components analysis was also utilized the visually depict the variation in bacteria community data, a graphical display of principal components 1-4 were illustrated utilizing the ggbiplot function (Vu 2011; **Table 4**).

3.0 RESULTS AND DISCUSSION

3.1 Phase 1

3.1.1 Primary bacterial culture and growth substrate evaluation

The objective of Phase 1 was to enrich nitrogen fixing and hydrocarbon degrading bacteria from oil sands tailings as a biofilm on a substrate suitable for inoculation in Phase 2. Based on the results from the Phase 1 culture and substrate evaluation, one enrichment culture and one tailings culture combination were selected for scale-up and further evaluation. These were the Canadian Natural enrichment and Thickened Tailings cultures, both grown on diatomaceous earth. These culture combinations demonstrated the highest metabolic activity based on measurements of methane production, and the highest mean average for potential nitrogen fixation (acetylene reduction) of the previously enriched cultures and tailings cultures (**Figure 1-3**). Sequencing data could not be obtained for diatomaceous earth cultures in this stage of work, however DNA results from media cultures (**Figure 4-6**) suggested Canadian Natural and Thickened Tailings cultures may contain a higher proportion of nitrogen fixing Archaea as compared to other Phase 1 inoculates, as well as potential nitrogen fixing, hydrocarbon degrading bacteria from the family *Clostridiaceae*.

3.1.1.1 Microbial metabolism in substrate cultures

Bacteria grown on diatomaceous earth produced significantly more methane compared to all other treatments by day 62 suggesting this substrate promoted the highest metabolic activity. Canadian Natural and Syncrude treatments established from enrichment cultures also produced methane faster than tailings inoculum, Thickened Tailings and Centrifuge Cake (**Figure 1**). Methane concentrations present in the headspace of these cultures were significantly higher at day 54.

Toluene concentration was also monitored to determine if cultures were metabolizing toluene to methane, but data were inconsistent across treatments and no trends could be established (data not shown). However, biogeneic methane production from the degradation of toluene and citrate is well established in the literature. Both Collins *et al*. (2016) and Li (2010) clearly demonstrated the metabolism of citrate to methane in oil sands tailings communities alongside with the absence of methane production in control experiments carried out without citrate. Similar observations have been reported for toluene amendments in tailings cultures (Laban *et al.*, 2015).

To confirm the presence of nitrogen fixing activity, an acetylene reduction assay was conducted. The acetylene molecule is similar in structure to N_2, which allows the nitrogen fixing enzyme, nitrogenase, to bind and reduce acetylene to ethylene. Fly ash cultures were omitted due to low metabolic activity, growth inhibiting conductivity and high pH. Ethylene production was significantly higher in diatomaceous earth cultures compared to media ($p = 0.0015$) and activated carbon treatments ($p = 0.0009$; **Figure 2a**). Amongst culture inoculum sources, the Canadian Natural culture had the highest mean average for nitrogen fixing potential, however the difference was not statistically significant ($p > 0.05$) (**Figure 2b**).

3.1.1.2 Substrate characterization

Despite the lower surface area (compared to other substrates; **Table 5**), diatomaceous earth promoted higher microbial metabolism in cultures. Although fly ash and activated carbon provided rough and porous surfaces suitable for biofilm development, the visibly diverse array of porous, textured structures representing the fossilized remains of diatoms in diatomaceous earth may have provided more suitable anchor points for cell attachment (**Figure 3**). High electrical conductivity (EC) and pH in fly ash cultures may have inhibited bacterial growth on the substrate compared to diatomaceous earth, which had a pH of approximately 7 in culture media. Although EC could not be accurately measured in activated carbon, a pH 8 would not have negatively affected growth as process water in tailings ponds is slightly alkaline (Allen, 2008). Therefore, enhanced metabolic activity observed in diatomaceous

earth relative to activated carbon is less clear but may have been related to surface chemistry. Activated carbon has a more complex surface chemistry relative to the SiO_2 and calcium bentonite based diatomaceous earth used in this study (Li *et al.*, 2002). Essential culture components such as organic carbon sources, nitrogen fixed by the initial inoculum community, and trace nutrients, could have been sorbed to the active surface limiting nutrient access and decreasing overall growth. Calcium bentonite in diatomaceous earth may have also played a role in promoting metabolism as the literature suggests calcium may be an essential nutrient for cellular processes and biofilm formation (Herbaud *et al.*, 1998; Dominguez, 2004; Patrauchan *et al.*, 2005; Das *et al.*, 2014).

3.1.1.3 Microbial community analysis

The microbial community composition for composite samples was determined by Illumina MiSeq sequencing the 16S rRNA gene, this gene is highly conserved between prokaryotes and is used to identify the groups of Bacteria and Archaea that make up the microbial community. DNA was extracted from both media and diatomaceous earth cultures but could not be recovered from activated carbon cultures. Due to the presence of PCR inhibitors, the diatomaceous earth samples could not be amplified for sequencing at the time of sample submission and will undergo additional clean-up steps prior to resubmission. Results from DNA samples extracted from media based cultures were compelling (**Figure 4-6**). At day 0, the proportions of Archaea in nearly all samples were very low (≤1%; **Figure 4**). This population increased to 7% of the microbial community in Canadian Natural and Thickened Tailings cultures over 55 days of incubation. The Archaea were dominated by hydrogenotrophic methanogens of the family *Methanobacteriaceae* (**Figure 5**) which utilize H_2 to produce methane. These data suggested the observed methane production in Canadian Natural media cultures (**Figure 1**) primarily resulted from the oxidation of H_2 produced during the fermentation of organics (citrate and toluene; Morris *et al.*, 2013). Methanogens, including *Methanobacteriaceae* have been also implicated as nitrogen fixers in soil (Bae *et al.*, 2018), and may have played a role in the higher mean average of acetylene reduction in Canadian Natural and Thickened Tailings cultures (**Figure 2**).

Over the course of 55 days of incubation, the bacterial community in media cultures shifted from primarily proteobacteria in all cultures to predominantly Firmicutes. The groups *Clostridiaceae* and *Carnobacteriaceae* together made up more than 70% of the microbial population present in all media cultures with the exception of Syncrude, which did not contain *Carnobacteriaceae*. These families are primarily fermentative. Members of the *Carnobacteriaceae* family are known to ferment citrate (Stams *et al.*, 2009), and *Clostridiaceae* play a syntrophic role as a key toluene degrader in methanogenic cultures (Fowler *et al.*, 2012). Fermentative bacteria metabolize organic compounds to smaller molecules and acids as well as CO_2, acetate and H_2. The H_2 produced by these bacteria is then metabolized by the hydrogenotrphic methanogens to produce methane as observed in the Canadian Natural media cultures. Unlike *Carnobacteriaceae*, the family *Clostridiaceae* also contains many known nitrogen fixing species (Chen *et al.*, 2001), and likely contributed to the nitrogen fixing activity observed in these cultures.

3.1.2 Scale-up cultures for Phase 2

Canadian Natural and Thickened Tailings cultures grown on diatomaceous earth were selected for scale-up based on the primary bacterial culture and growth substrate evaluation. Of these two treatments, Canadian Natural cultures exhibited the highest metabolic activity and was found capable of toluene degradation. Canadian Natural cultures also contained high proportions of bacterial groups previously identified as nitrogen fixers (Spirochaetes, *Peptococcaceae,* and other *Clostridiales*) and hydrocarbon degrading bacteria (*Geobacter, Peptococcaceae,* and other *Clostridiales*). These cultures were used to inoculate tailings columns in Phase 2.

3.1.2.1 Microbial metabolism in scale-up cultures

Methane production was significantly higher in Canadian Natural cultures as compared to Thickened Tailings cultures by day 33 ($p = 8.33 \times 10^{-5}$; **Figure 7**). This relationship is aligned with the methane production results

observed in the culture and substrate evaluation work (**Figure 1**). Toluene degradation was also monitored over the course of the incubation (**Figure 8**). By day 42, significantly less toluene was present in Canadian Natural cultures as compared to Thickened Tailings cultures or sterile cultures, suggesting that toluene may have been metabolized to methane in these cultures. This hypothesis is supported in the literature where both the metabolism of citrate (Li, 2010; Collins *et al.*, 2016) and toluene (Fowler *et al.*, 2012; Laban *et al.*, 2015) have been reported under methanogenic conditions.

3.1.1.1 Microbial metabolism in substrate cultures

DNA extraction and sequencing of samples from both Canadian Natural cultures and Thickened Tailings cultures were carried out prior to Phase 2. However, Thickened Tailings samples could not be amplified for sequencing at the time of sample submission. As such, only results for the Canadian Natural scale-up cultures used as inoculum in Phase 2 are available at this time.

Predominantly bacteria were detected with only 0.4% of the total population identified as belonging to an archaeal group (100% *Methanosarcinaceae*; *Methanosarcina*). Members of this genus are capable of both hydrogenotrophic and acetotrophic methanogenesis as well as nitrogen fixation (Leigh, 2000). These methanogens are able to produce methane from both H_2 and acetate resulting from the degradation of organics such as toluene and citrate used in this study. However, this percentage is low when compared to the community composition of methanogenic cultures in other studies (Penner and Foght, 2010; Fowler *et al.*, 2012; Siddique *et al.*, 2012; Yergeau *et al.*, 2012; Ramos-Padrón, 2013; Collins *et al.*, 2016). The low percentage of observed methanogens may be due to primer bias as methanogens and other Archaea may be underrepresented in the sequencing data when using 16S rRNA universal primers (Eloe-Fadrosh *et al.*, 2016; Raymann *et al.*, 2017). Alternatively, methanogens may have been actively metabolizing organic ligands to methane but slow to produce biomass due to limitations in bioavailable nitrogen required for growth (Touratier *et al.*, 1999).

Bacterial sequences were dominated by Firmicutes (53%) followed by Deltaproteobacteria (27%), Spirochaetes (12%), and Bacteroidetes (4%; **Figure 9)**. The majority of the bacterial sequences fell into the groups, *Geobacter* (*Geobacteraceae*), *Veillonellaceae*, *Desulfitobacterium (Peptococcaceae)*, and *Spirochaetaceae*. Members of the *Veillonellaceae* family are known to ferment citrate (Ogg and Patel, 2009), whereas Spirochaetes have been found to ferment carbohydrates and proteins into acetate, ethanol, and H_2 in anaerobic, hydrocarbon contaminated aquifers suggesting these organisms play an important role in recycling dead biomass. Spirochaetes are also known to contain free living nitrogen fixing species, including those found in the rhizosphere of cordgrass (Lilburn *et al.*, 2001). Both groups likely support methanogenesis through the production of methanogenic ligands, H_2 and acetate. Interestingly, *Desulfitobacterium* have the potential to fix nitrogen, and dehalogenate organic molecules (Kim *et al.*, 2012). *Peptococcaceae* have also been implicated in toluene degradation in methonogenic cultures (Fowler *et al.*, 2014).

Based on the data from Phase 1, members of the phylum Firmicutes were likely responsible for the observed toluene degradation in the Canadian Natural scale-up cultures. It is reasonable to suggest that based on the abundance of this phylum in both the culture and substrate evaluation, and the scale-up cultures, Firmicutes metabolized toluene and citrate to acetate and H_2, which were in turn metabolized to methane by *Methanosarcinaceae* and potentially *Methanobacteriaceae*. This process was supported by nitrogen fixation in the microbial community. While methanogens only composed a small percentage of the population in the media cultures, nitrogen fixing methanogens have been found to represent a disproportionate fraction of the nitrogen fixing population (Collins, 2019 unpublished data) and likely contributed to sequestering nitrogen to support microbial metabolism alongside bacteria from the order *Clostridia* in these cultures.

3.2 Phase 2

The objective of Phase 2 was to determine the effect of organic amendments and bacterial culture on the growth and dewatering ability of native boreal plant species. A series of treatment combinations including an organic amendment, either peat or hydrochar, and a bacterial inoculum established in Phase 1 were evaluated in combination with slender wheatgrass (*Elymus trachycaulus*) and sandbar willow (*Salix interior*). Root mulch from willows previously grown on tailings was also added as a treatment for columns with willows with the objective of augmenting the rhizosphere with beneficial microorganisms from an established plant. Overall, willow growth was found to increase shear strength in centrifuge cake. Moreover, treatments with hydrochar increased above ground biomass in willows, which contributed to increased strength and solids content. Treatments with bacteria alone did not show significant improvements in plant growth or tailings dewatering however, treatments with bacteria and hydrochar in combination tended towards higher shear strength and solids content. Additional studies to evaluate a number of different microbial inoculates with longer growth times are needed to fully assess the potential of plant growth promoting bacteria for the bioaugmentation of plant mediated, tailings dewatering technology.

3.2.1 Did inclusion of organic amendments improve plant development and growth?

Slender wheat grass

Both species evaluated in this study demonstrated some level of persistence when grown on either centrifuge cake or thickened tailings, regardless of the organic amendments (**Appendix D**). However, total aboveground biomass and root biomass of slender wheatgrass was substantially lower than the willow experiment-wide. Grasses were hand seeded into the columns and warm and windy conditions were recorded during initial week of the experiment when the grass was sown, resulting in the tailings surface drying out and inhibiting seed germination. The hard, crusted surface was subsequently broken up with a fork and seeds were re-sown a second time approximately 3 weeks later but this delay resulted in a ~ 4 week lag in growth.

Incorporation of peat did not significantly improve growth (maximum height, leaf or root biomass, leaf area and LAI) of slender wheatgrass (**Figure 10**) relative to the control treatment. Root biomass estimates were highly variable and there was no systematic difference between treatments (**Figure 10**); this may have been the result of subsampling the column (rather than extraction of roots from the entire column). However, the addition of hydrochar appeared to have negatively impacted aboveground development of slender wheatgrass compared with the control treatment (**Figure 10**). This difference was primarily due to poor emergence and slower initial growth in the hydrochar treatment. In a parallel short-term growth study, the authors did observe that poor emergence and growth was rate dependent with a modest decline in growth when hydrochar was applied >0.1% and substantial decline at a concentration >0.5% (**Figure B1**). In the current study, the application rate was 2.7 grams of hydrochar per column which is equivalent to 0.35% on a weight basis; since this material was surface applied and then stirred in (to a depth of ~25 cm), the effective concentration was likely much greater. Others have also found that hydrochars can be inhibitory to initial germination of plants and have suggested that depending on the type of feedstock used for production, hydrochar may contain compounds that inhibit seed emergence (Fang et al. 2015; Puccini *et al.*, 2018).

Sandbar willow

In contrast with slender wheatgrass, amending with hydrochar significantly increased the leaf biomass of willow compared to the control, while peat incorporation treatments were not significantly different (**Figure 11**). It is notable that the treatment effect with hydrochar did not correspond with an increase in LAI (**Figure 11**); it is likely that this may be reflective of a change in leaf morphology where the leaves in the hydrochar treatment were potentially thicker (though not effectively larger in surface area) or carried higher concentrations of non-structural carbohydrates. In contrast, the root mass, and root to leaf ratio of willow were unaffected by amendment type

(**Figure 11**). Leaf nitrogen concentration for willows amended with hydrochar was also higher compared with the control and actually comparable to leaf N concentration observed in the natural forest soil grown plants (which were grown in parallel throughout the study as a secondary environmental control group). This increase in N concentration correlates with the observed increase in leaf biomass as well as visibly deeper green color in leaves (refer also to example image in **Appendix D**). The hydrochar used in this study contained ~8.5% nitrogen and may have contributed nitrogen directly to the willows, or increased nitrogen availability in tailings leading to increased leaf biomass production. Research suggests hydrochar may immobilize bioavailable nitrogen following initial amendment (Gajić and Koch, 2012; Bargmann *et al.*, 2014) and this nitrogen is then slowly released and made available to plants resulting in enhanced growth in some plant species (Bargmann *et al.*, 2014). Hydrochar may also contain phytotoxic compounds such as polyphenols (tannins) and volatile fatty acids, which are naturally attenuated with time (Puccini *et al.*, 2018), This may explain findings that support pre-incubation of soil following hydrochar amendment for up to three months to alleviate phytotoxic effects (as reviewed by Kambo and Dutta, 2015).

Interestingly, studies have also found hydrochar addition can increase root colonization by plant growth promoting mycorrhizal fungi (Kambo and Dutta, 2015), or decrease the abundance of arbuscular mycorrhizae and increase root nodulation (George *et al.*, 2012). This indicates that hydrochar can also have a substantial effect on the plant microbial community, which could be another factor in the observed increase or decrease in plant germination and growth.

3.2.2 Did inclusion of bacteria improve plant development and growth?

Overall, there were no significant changes in grass or willow growth between treatments with bacteria alone (both type 1 developed in Phase 1, and type 2 from mulched willow roots) and no treatment (**Figure 10-11**). One previous study by Lefrançois *et al*. (2009) found that Alders treated with the endophytic, nitrogen fixing bacteria *Frankia*, exhibited improved plant establishment on oil sands tailings after 1.5 years. The current greenhouse study used non-endophytic bacteria and only ran for 3.5 months suggesting that more time may be required for the inoculum to have an effect. It is also possible that endophytic bacteria have a greater effect on plant systems.

3.2.3 Is there a synergistic effect when combining organic amendments with bacteria in terms of improving plant growth?

Slender wheat grass

Inoculation with bacteria into treatments with organic amendments present (hydrochar or peat) did not result in higher levels of growth (whether above or belowground) in slender wheatgrass (**Figure 10**).

Sandbar willow

Bacteria inoculated into columns with organic amendments did not significantly affect leaf biomass or LAI relative to treatments that only contained the organic amendment (**Figure 11**). Root biomass was highly variable with bacteria inoculation in combination with an amendment showing no statistical difference from amendments alone (within each amendment treatment) though root mass was on average lower with bacteria in the hydochar but higher when inoculated with peat (**Figure 11**). As mentioned previously, at least some of the variability in root biomass was likely associated with the sampling method. In future studies, it may be more appropriate to take sections of column for root determination rather than core sampling as root distribution was heterogeneous.

3.2.4 Does establishment of native plants lead to improvements in the geotechnical properties of tailings?

Slender wheat grass

In this study, grasses were grown outdoors and in columns with small surface area (10 cm diameter), which together resulted in surface crusting due to warm and windy conditions in the first week of the trial. Seeds caught in the crust failed to germinate and the crust had to be broken and reseeded three weeks after initiation of the study (in late June). This substantially reduced growth period (2.5 months) in combination with anticipated slower growth in outdoor conditions are the primary reasons for limited aboveground growth observed for this species. As such, there was no significant increase in solids content, shear strength or consolidation in columns planted with grass relative to columns without plants in this study. Despite the limited leaf area development, there were some modest indications that seasonal water use was on average, higher for columns with grasses relative to those without, significantly so for the hydrochar + bacteria treatment (**Figure 17**). Nevertheless, the limited growth observed in this trial should not eliminate this species as a candidate as slender wheat grass has been grown on oil sands tailings in greenhouse studies (Wolter and Naeth, 2014; Noah, 2017; Wu 2009; Wu et al., 2010), and has been used successfully in tailings dewatering studies at NAIT (Yucel et al. 2016, Schoonmaker et al. 2018). In Yucel et al (2016), individual grass plants were found to remove up to 70 mL of water from centrifuge cake per day through evapotranspiration in a 5-month greenhouse trial.

Sandbar willow

Willow growth consistently increased the solids content in the top 35 cm of the centrifuge cake columns across all treatments as compared to columns without willows (**Figure 12**). Even in the middle measurement point (35-65 cm), there was a consistent and often significant increase relative to unplanted columns (**Figure 12**). Similarly, solids content for willows grown in thickened tailings followed the same general trend as that observed for centrifuge cake though the difference between planted and non-planted columns (within an amendment treatment) was not always significant (**Figure 13**). One explanation for the difference in solids content between the two tailings types may be increased water infiltration due to the higher sand content of the thickened tailings material. The coarser nature of the thickened tailings makes them easier to re-wet whereas the centrifuge cake does not allow for substantial water infiltration due to the high density of the fine particulates.

Consolidation increased, on average, in presence of willows though there was no statistically significant effect in centrifuge cake (**Figure 14**), but both consolidation and % of tailings column under water significantly improved in thickened tailings columns (**Figure 15**). On average, willows improved consolidation by ~5.3cm (58%) and decreased water levels by ~19.1 cm (24%) compared to non-vegetated columns. These results should be treated conservatively however, as several precipitation events preceded final measurements which may have decreased the apparent efficacy of willow treatments in both tailings types. Evapotranspiration (**Figure 16**) and seasonal water usage (**Figure 17**) were also significantly higher in centrifuge cake columns with willows reducing tailings column water by an additional ~21% compared to columns without willows. Willows grown in thickened tailings followed a similar pattern in evapotranspiration and water use though the results were not significantly different from unplanted columns (**Figure 16-17**).

Mean undrained shear strength was consistently higher, for both types of tailings, in columns planted with willows compared with unplanted columns and this difference was measurable to a depth of 50 cm or more (**Figure 18, 20**). The highest mean undrained shear strength was found in the centrifuge cake treatment with willow and both hydrochar and bacteria (**Figure 18**), which exceeded predicted shear strength based on solids content (**Figure 19**). In general, there was good agreement between predicted and measured shear strength, for both types of tailings (**Figure 19, 21**) suggesting the gain in strength was largely attributable to increased solids content. When the measured strength exceeded predicted strength, this indicated that root stabilization may have further contributed to strength gain. This was observed in centrifuge cake planted with willow for the hydrochar + bacteria, peat +

bacteria and no-amendment treatments (**Figure 19**); it was also observed in thickened tailings with willow for the hydrochar, peat + bacteria and no amendment treatments (**Figure 21**). Root-induced soil stabilization is a well-documented phenomenon (Wu and Watson, 1998) and the technique is utilized for hill slope stabilization (Xu *et al.*, 2009). Modelling of this behavior for river bank stabilization has suggested that the greatest gains in shear strength come from a small number of larger roots rather than an abundance of small roots, though the authors also suggested that grasses with strong, dense roots may also offer equally effective stabilization (Simon and Collison 2001).

Overall, leaf biomass correlated with consolidation in centrifuge cake (**Figure 22**). Strong correlations ($p<0.05$) were also observed between leaf biomass and shear strength in centrifuge cake throughout the 1 m column, and solids content in the 35-65 cm region of the column (**Figure 23**). The lack of observed correlation in the top 35 cm of the column suggests that all willow treatments grew to a similar extent (or extracted water) at the surface however, those with higher leaf biomass increased solids content in the middle of the column, likely because these plants had greater transpirational demands, forcing roots to explore greater depths in the column. Increased shear strength also correlated with leaf biomass but not with solids content in the bottom (65-100 cm) of the columns (**Figure 23-24**). This indicates that the plants with more leaf biomass were able to increase solids content in the middle of the column through evapotranspiration, whereas roots were directly increasing shear strengths at the bottom of the column (65-100 cm). Although there were no statistically significant trends in these parameters observed between willow leaf biomass and geotechnical properties, the general patterns were often similar to that observed in centrifuge cake (**Figure 25-27**).

In the present study, a correlation between root biomass and shear strength was not observed, in fact, the treatment with the highest shear strength in centrifuge cake (hydrochar with bacteria, **Figure 11**) had significantly less root biomass than untreated willows; this was an unexpected result and may have been due to root sampling methods used in the columns (as described above) rather than a true treatment effect.

3.2.5 How does the inclusion of an organic amendment affect the bacterial community?

Microbial community analysis revealed a large bacteria population with Archaea only contributing < 1% of the population. Methanogens are known to abound in mature fine tailings (Penner and Foght, 2010; Fowler *et al.*, 2012; Siddique *et al.*, 2012; Yergeau *et al.*, 2012; Ramos-Padrón, 2013; Collins *et al.*, 2016), however the absence of Archaea both in the day 0 centrifuge cake sample and other samples from this experiment suggests that either the tailings were too fresh to have allowed for community development of a methanogenic population, or that the homogenization process combined with observed algal growth in the clear columns severely reduced the population of the strictly anaerobic archaea (Rother, 2010). The possibility of primer bias (Eloe-Fadrosh *et al.*, 2016; Raymann *et al.*, 2017) and the presence of other, more energetically favorable electron acceptors are also possible explanations that will require additional investigation. Of the remaining archaeal sequences, Crenarchaeota and Thaumarchaeota were the dominant groups.

As mentioned above, the bacterial population was diverse (**Figure A5-A6**), with the top four richest samples containing peat from the top of the column. Peat is known to contain an array of microorganisms (Dedysh *et al.*, 2006), including bacteria known to colonize plant rhizospheres and fix nitrogen (Belova *et al.*, 2006). The composition of peat also likely would affect the microbial population by providing a different physiochemical environment from the tailings (Andersen *et al.*, 2010). These combined factors likely accounted for the increased richness observed in these treatments.

Proteobacteria were dominant in all treatments ranging from 46-77% of the total population (**Figure A5-A6**). These were primarily from the groups *Betaproteobacteriales*, now within the Gammaproteobacteria, and *Pseudomondaceae*. Many of these species are known promote plant growth through nitrogen fixation and other

functions (Chen *et al.*, 2003). Of the amendments evaluated, the inclusion of hydrochar appeared to alter the microbial population overall in the top of the columns as compared to unamended columns in the absence of plants (**Figure 28C**).

Overall, the community composition was different (Bray-Curtis, $p<0.05$) between centrifuge cake and thickened tailings (**Figure 29)**. This observation was supported in a study by Yergeau *et al.*, (2012), which found microbial communities varied between tailings pond sediment and other sediment in the Athabasca region due to the composition of the material. The microbial communities from the middle section of the columns were also different from the top of the columns in both tailings types (**Figure 28D**). This is explained by the potential for oxygen infiltration, which would have reduced the presence of strict anaerobes such as *Desulfuromonadaceae*. Willow treatments also contained a substantially different microbial population in the top of the column compared to non-vegetated treatments across both tailings types (**Figure 29B**). This suggests that roots exhibited stronger selective pressures on the community than amendments or inoculum. Roots are known to secrete organic acids, sugars, amino acids, and other desirable molecules, many of which benefit the microbial community in proximity to the roots (Ahemad and Kibret, 2014). The continuous secretion of these compounds likely drove the community structure in this region.

3.2.6 Does inoculation with bacteria result in measurable reductions in hydrocarbons in tailings?

Hydrocarbons in the F2 and F3 fractions were below detection levels in the majority of the thickened tailings samples (**Figure A7**). On average, centrifuge cake samples contained a higher concentration of hydrocarbons than thickened tailings. This is likely due to the higher observed bitumen content in this tailings material (**Table 1**). There were no trends amongst treatments however, this may be due to lack of replication. Samples were taken from the last set of replicate columns that were not disassembled at the end of the plant growth phase, as such, only a single sample was evaluated for each treatment.

3.2.7 Does tailings type impact the growth of plants?

There was no significant difference in root biomass or root to leaf ratio between centrifuge cake and thickened tailings columns over the course of this study (**Figure 10**, **Figure 11**). However, grass and willow height (**Figure 10**), and willow leaf area index (**Figure 11)** were higher when grown in centrifuge cake as compared to thickened tailings suggesting preferential growth on this material.

4.0 CONCLUSION

This results from this work suggest plant mediated tailings dewatering may be feasible for certain applications. Hydrochar is also a beneficial plant amendment as it has been found to increase leaf biomass, which is strongly correlated to increased shear strength and solids content at greater depths. This amendment could potentially decrease the growth time required or increase the efficacy of this potential technology. While bacterial treatments did not prove significantly effective on their own, there is promise in the combination of bacteria and hydrochar treatments which may have become significant with increased growth time. As such, bacteria treatment in combination with hydrochar amendment merits further investigation.

5.0 RECOMMENDATION AND FUTURE WORK

In order to assess the scalability of this technology to field application, it is recommended that larger volumes of tailings (barrels or totes) are treated over longer periods of time. This will help eliminate edge effects when

measuring shear strength thereby providing results more similar to what could be expected with *in-situ* application. Plant material should also be analyzed for contaminant accumulation (PAHs, salts, metals etc.) to determine if plant material will require additional disposal considerations.

Although we did find some evidence that incorporation of hydrochars was beneficial to plants, the authors cannot confirm if this was due to a specific combination of properties unique to hydrochar or whether it was simply an additional source of nitrogen. Future studies to further optimize this technology, additional bacterial inoculum and hydrochar amendments, and their application rates, should be screened for efficacy. A wide range of plant growth promoting bacteria are available commercially in addition to those that can be cultured from plants currently thriving on tailings deposits. One of these selections may provide greater benefits to native boreal species. Hydrochars with different surface chemistry and physical properties can be synthesized from feedstock ranging from meat and bone meal products, sugars, and lignin. The optimization of these amendments could decrease the time required for plant growth and potential tailings dewatering. Hydrochar and bacteria treatments should also be evaluated as compared to varying rates of fertilizer addition to determine if sufficient nutrients are present or can be generated by the amendments to support plant growth, or if these benefits are negated at higher concentrations of fertilizer.

Additionally, the microbial community of the plant roots including the fungal population should be evaluated. By comparing data between effective treatments, it could be determined if bioaugmentation efforts for the purpose of promoting plant growth should be focused on culturing entophytic microorganisms rather than free-living organisms. Ideally, these results would be compared with the root communities of related plant species growing on tailings deposits in the field.

In addition to the work outlined above, the basic chemical analyses on the tailings materials will be completed in the coming weeks for use in the publication of these experiments. These include electrical conductivity, pH, anion, and cation analysis.

The commercial costs for field scale application of this technology has not been assessed. A full cost assessment is recommended taking into account the commercial availability of hydrochar and plant growth promoting bacteria at scale once this technology is further optimized.

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Table 1. Characterization results on tailings used in the study. All batch samples were run in triplicate and the average is presented for each batch.

	Centrifuge Cake				Thickened Tailings			
Characteristics	Batch 1	Batch 2	Batch 3	Average	Batch 1	Batch 2	Batch 3	Average
Solids	45.1%	45.8%	44.3%	45.1%	61.9%	62.6%	64.9%	63.1%
Water	52.9%	51.2%	52.8%	52.3%	37.6%	37.4%	34.7%	36.5%
Bitumen	0.03%	0.02%	0.05%	0.04%	0.01%	0.01%	0.02%	0.01%
Yield stress (Pa)	72	104	67	78	36	34	54	41
MBI	11.7	11.9	11.6	11.8	5.2	5.4	5.8	5.5
% passing 44 microns	95.3	96.6	96.8	96.3	71.9	87.0	74.9	77.9
D50	5.5	5.3	5.4	5.4	11.9	7.6	10.8	10.1
Plastic Limit (Geotechnical Water)	17.9	16.9	17.6	17.5	12.4	11.8	11.6	12.0
Liquid Limit (Geotechnical water)	64.2	63.9	63.7	64.0	25.0	25.1	26.9	25.7
Geotechnical water content of initial sample	117.15	111.84	119.22	116.07	60.68	59.82	53.38	57.96
Liquidity Index of Initial Sample	2.1	2.0	2.2	2.1	3.8	3.6	2.7	3.4

Table 2. Arithmetic mean (± SE, n = 10 for seedling, n=13 for cutting) of leaf biomass, total shoot biomass, root biomass, and maximum height of stock *Salix interior* (willow), prior to planting in in centrifuge cake and thickened tailings. Propagation type denotes the method of plant introduction into the treatment column from a rooted seedling (either from seed [seedling] or hardwood cutting [cutting]).

Plant type	**Propagation type**	**Biomass (g)**						**Maximum height (cm)**	
		Leaf		Total shoot		Root			
		mean	range	mean	range	mean	range	mean	range
Willow	Seedling	0.10±0.02	0.02-0.18	0.24±0.03	0.09-0.41	0.35±0.07	0.08-0.82	14.65±1.13	9.50-22
	Cutting	0.41±0.06	0.18-0.78	1.62±0.37	0.25-4.62	0.67±0.15	0.17-1.89	34.80±3.08	19.50-60.00

Table 3. Arithmetic mean (± SE, n = 6) of aboveground biomass and maximum height of *Elymus trachycaulus* (grass) and *Salix interior* (willow) planted in forest and reclaimed soils. Propagation type denotes the method of plant introduction into the treatment column from seed or from a rooted seedling (either from seed [seedling] or hardwood cutting [cutting]).

Plant type	Soil type	Propagation type	Biomass (g)				Maximum height (cm)	
			Leaf		Shoot			
			mean	range	mean	range	mean	range
Grass	Forest soil	Seed	1.51±0.87	0.21-2.19	-		38.75±27.40	30.5-47
	Reclaimed soil	Seed	0.89±0.44	0.4-1.71	-		27.00±13.50	18.5-35.5
Willow	Forest soil	Seedling	1.10±0.64	0.37-2.08	0.98±0.57	0.51-1.7	48.50	35-62
		Cutting	0.96±0.68	0.38-1.53	3.13±1.81	0.43-6.2	44.75	7.5-82
	Reclaimed soil	Seedling	0.47±0.27	0.27-0.79	0.78±0.45	0.52-1.14	34.00±17.00	27-42
		Cutting	0.16±0.09	0.01-0.35	1.26±0.73	0.78-2.14	53.00±30.60	38-81

Table 4. Summary statistics for PCA 1-4 illustrated in **Figure 28** and **Figure 29**.

Importance of components:								
	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
Standard deviation	2.199	1.5003	1.3289	1.2349	1.05934	0.97529	0.8764	0.78743
Proportion of Variance	0.3224	0.1501	0.1177	0.1017	0.07481	0.06341	0.05121	0.04134
Cumulative Proportion	0.3224	0.4724	0.5902	0.6919	0.76667	0.83008	0.88129	0.92263
	PC9	PC10	PC11	PC12	PC13	PC14	PC15	
Standard deviation	0.59186	0.52199	0.45715	0.41496	0.3806	0.10869	3.38E-10	
Proportion of Variance	0.02335	0.01816	0.01393	0.01148	0.00966	0.00079	0.00000	
Cumulative Proportion	0.94598	0.96414	0.97808	0.98956	0.99921	1.0000	1.0000	

Table 5. Candidate bacteria carrier substrate physical properties. SD indicates one standard deviation of the mean (n=3 for BET surface area analyses, BET: Brunauer, Emmett and Teller).

BET	Mean Specific Surface Area (m^2 g^{-1})		SD
Diatomaceous Earth	28.3		1.1
Fly Ash	51.3		1.8
Activated Carbon	>200		-
	pH		**EC**
	Substrate	Culture	(ms cm^{-1})
Diatomaceous Earth	5.4	7	1.7
Fly Ash	12.7	13	62.4
Activated Carbon	9.0	8	-
Particle Size	Mean (µm)		SD
Diatomaceous Earth	10.2		7.4

Table 6. Leaf biomass, total nitrogen, and nitrogen pool of *Elymus trachycaulus* (grass) and *Salix interior* (willow) planted in centrifuge cake and thickened tailings (H+B1= hydrochar + bacteria (type 1); H= hydrochar only; P+B1= peat + bacteria (type 1); P= peat only; B1= bacteria only (type 1); N= no amendment or bacteria inoculation). Results are from one treatment block (block 5).

Species	Treatment	Centrifuge cake			Thickened tailings		
		Leaf biomass (g)	N concentration (w/w %)	N-pool (%)	Leaf biomass (g)	N concentration (w/w %)	N-pool (%)
Grass	Hydrochar + bacteria (type 1)				0.033	2.470	0.082
	Hydrochar only	0.042	2.930	0.123	0.026	2.130	0.055
	Peat + bacteria (type 1)	0.460	1.370	0.630	0.828	1.055	0.873
	Peat only	0.744	1.120	0.833	1.039	1.020	1.060
	Bacteria only (type 1)	0.091	1.750	0.159	0.684	1.250	0.855
	No amendment	0.023	2.730	0.062	0.268	1.390	0.373
	No amendment (forest soil)	2.140	2.830	6.056	0.300	2.570	0.771
Willow	Hydrochar + bacteria (type 1)	1.573	2.340	3.680	2.955	2.210	6.530
	Hydrochar only	1.824	1.970	3.592	2.448	2.270	5.557
	Peat + bacteria (type 1)	1.700	1.450	2.465	1.110	1.530	1.698
	Peat only	1.167	1.600	1.868	1.174	1.040	1.221
	Bacteria only (type 1)	0.975	1.740	1.697	1.367	1.260	1.722
	Bacteria only (type 2)	1.353	1.530	2.070	0.807	1.310	1.058
	No amendment	0.994	1.410	1.402	0.844	1.460	1.232
	No amendment (forest soil)	2.380	2.200	5.236	2.220	2.070	4.595

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Figure 1. Mean methane production in cultures incubated for 62 days. **DE**: Diatomaceous earth. Treatment Canadian Natural Media refers to Canadian Natural bacteria enrichment culture in media without substrate. Treatments (fly ash, activated carbon, Syncrude media, thickened tailings media, tailings cake media, and heat killed sterile controls) with methane yield below 20 µmol by day 62 are not shown. Error bars represent 95% confidence interval (n=3), line indicates approximate theoretical methane production from citrate and toluene (372 and 377 µmol, respectively; Symons and Buswell, 1933). Statistical significance determined at day 34 and 54 using single factor ANOVA due to low or absent methane in other substrate cultures. No significance was observed at day 62. Within each time point, different letters between treatments means indicate a statistically significant difference ($\alpha < 0.05$).

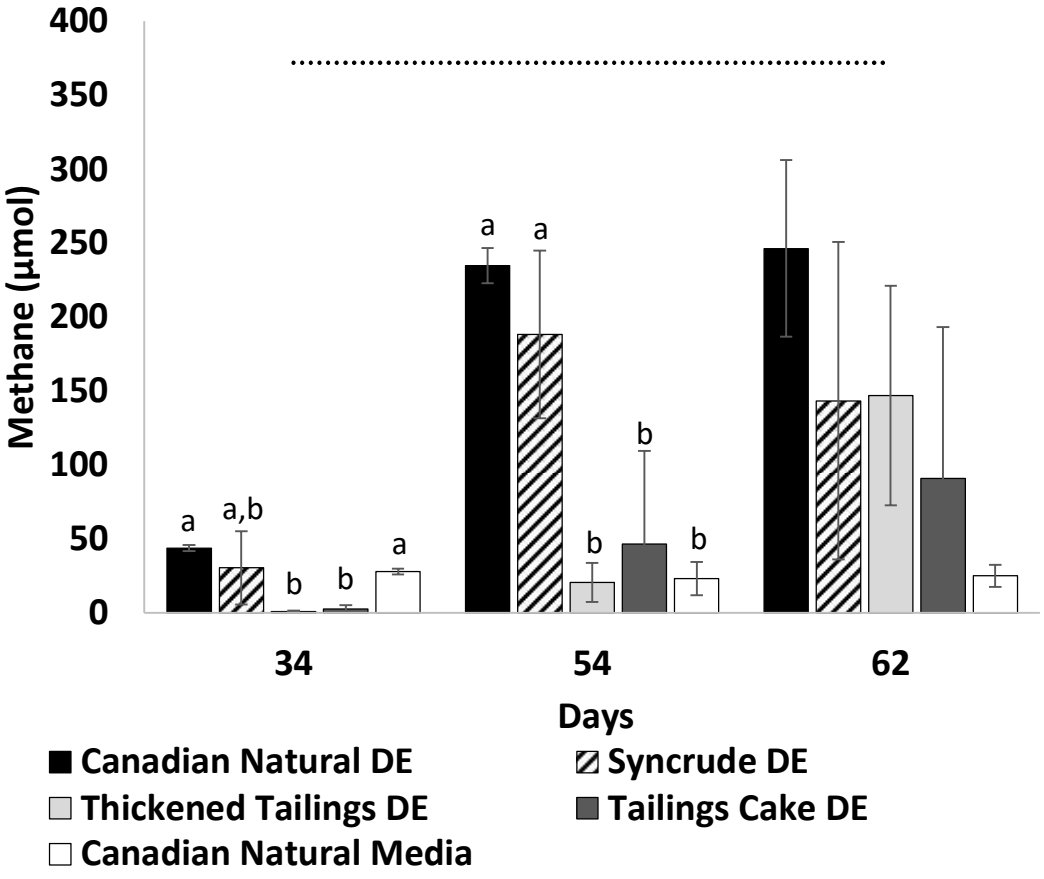


Figure 2. Mean acetylene reduced to ethylene by **A**: substrate material and **B**: bacteria source. Treatments that did not produce measurable quantities of ethylene were not shown. Error bars represent 95% confidence interval (n=3). Statistical significance determined using two factor ANOVA and post-hoc test, no significant differences were observed amongst bacteria sources. Different letters between treatments means indicate a statistically significant difference ($\alpha < 0.05$).

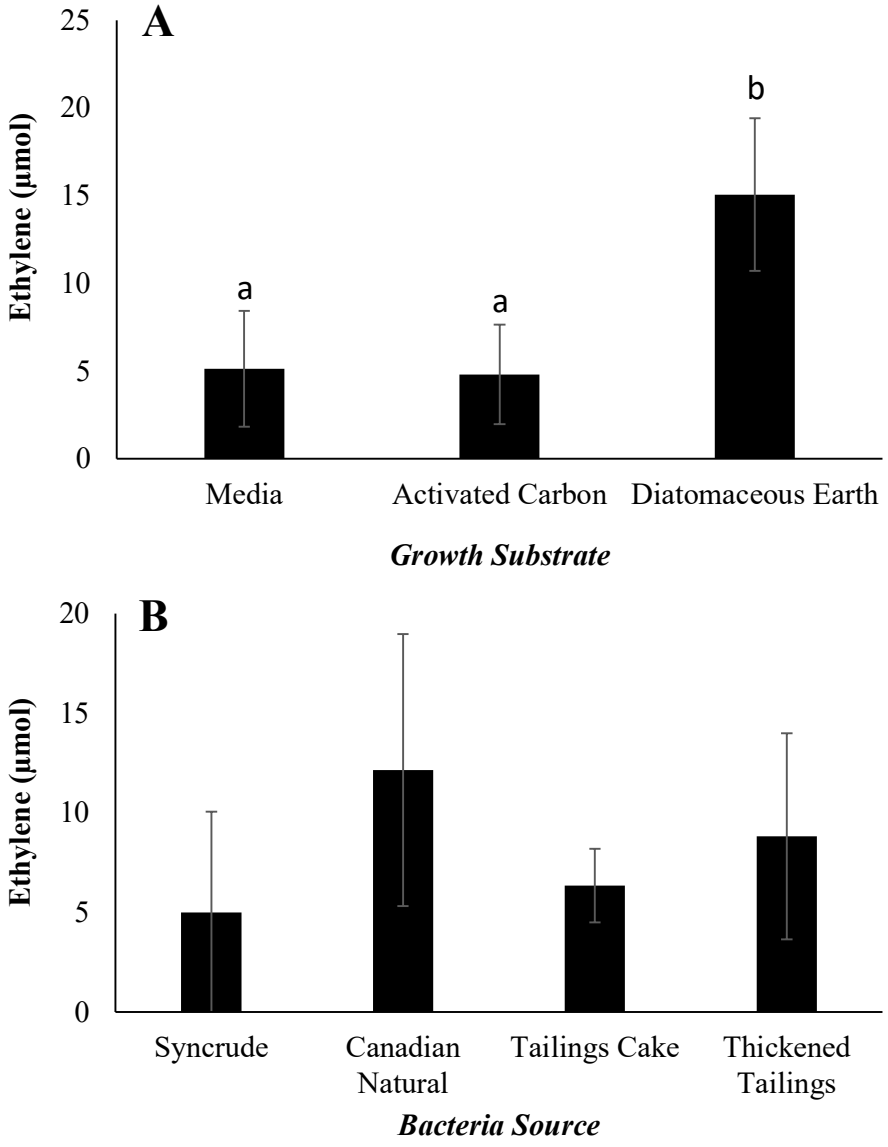


Figure 3. Scanning electron micrograph of substrate materials. A: Diatomaceous earth, B: Fly ash, C: Activated carbon. White bar represents 20 µm.

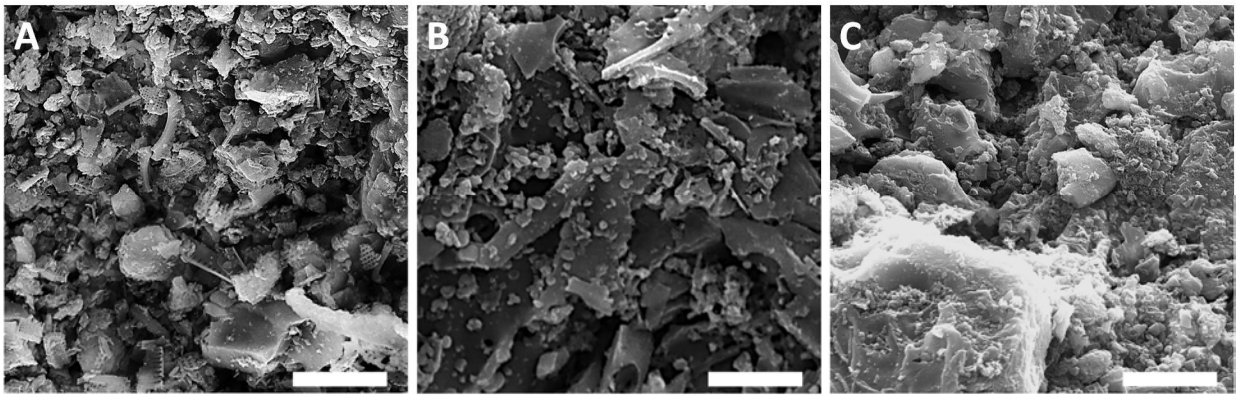


Figure 4. Community composition indicating percentage of Archaea and Bacteria based on 16S rRNA sequencing of composite samples and alignment using the SILVA database. D0 indicates day 0 samples prior to incubation, end point samples were taken following 55 days of incubation. CN and Syncrude indicate media cultures established using 1 ml of Canadian Natural or Syncrude innoculum from previously established enrichment cultures. CC and TT indicate cultures established using 1 ml tailings centrifuge cake or thickened tailings, respectively.

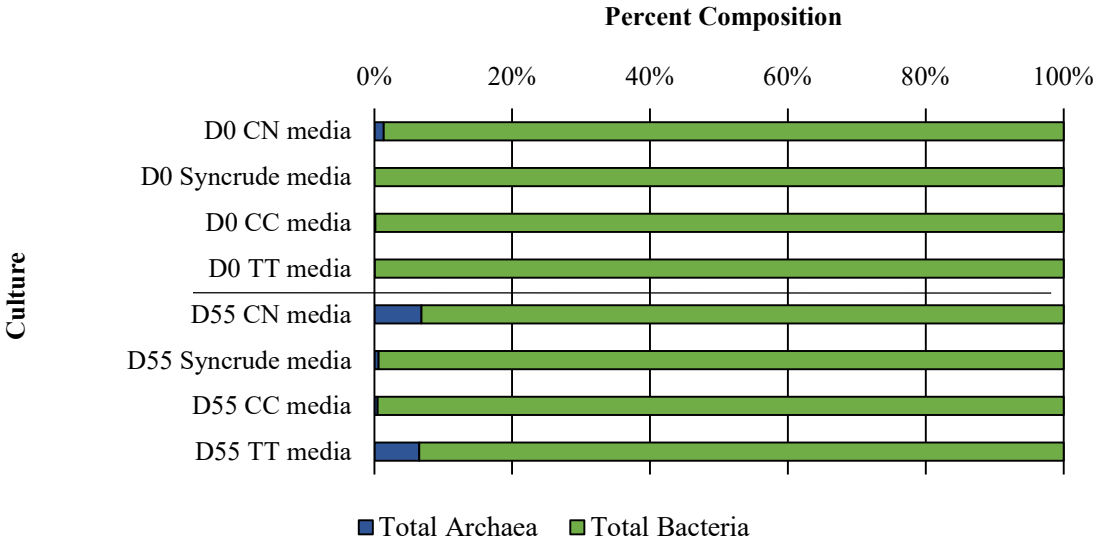


Figure 5. Community composition indicating percentage of Archaea based on 16S rRNA sequencing of composite samples and alignment using the SILVA database. D0 indicates day 0 samples prior to incubation, end point samples were taken following 55 days of incubation. CN and Syncrude indicate media cultures established using 1 ml of Canadian Natural or Syncrude innoculum from previously established enrichment cultures. CC and TT indicate cultures established using 1 ml tailings centrifuge cake or thickened tailings, respectively**.** Members of the class Crenarchaeota are represented in yellows and orange, hydrogenotrophic methanogens Methanobacteriaceae, are represented in gray, and acetoclastic methanogens Methanomicrobiales and Methanosarcinales are depicted in green and blue, respectively.

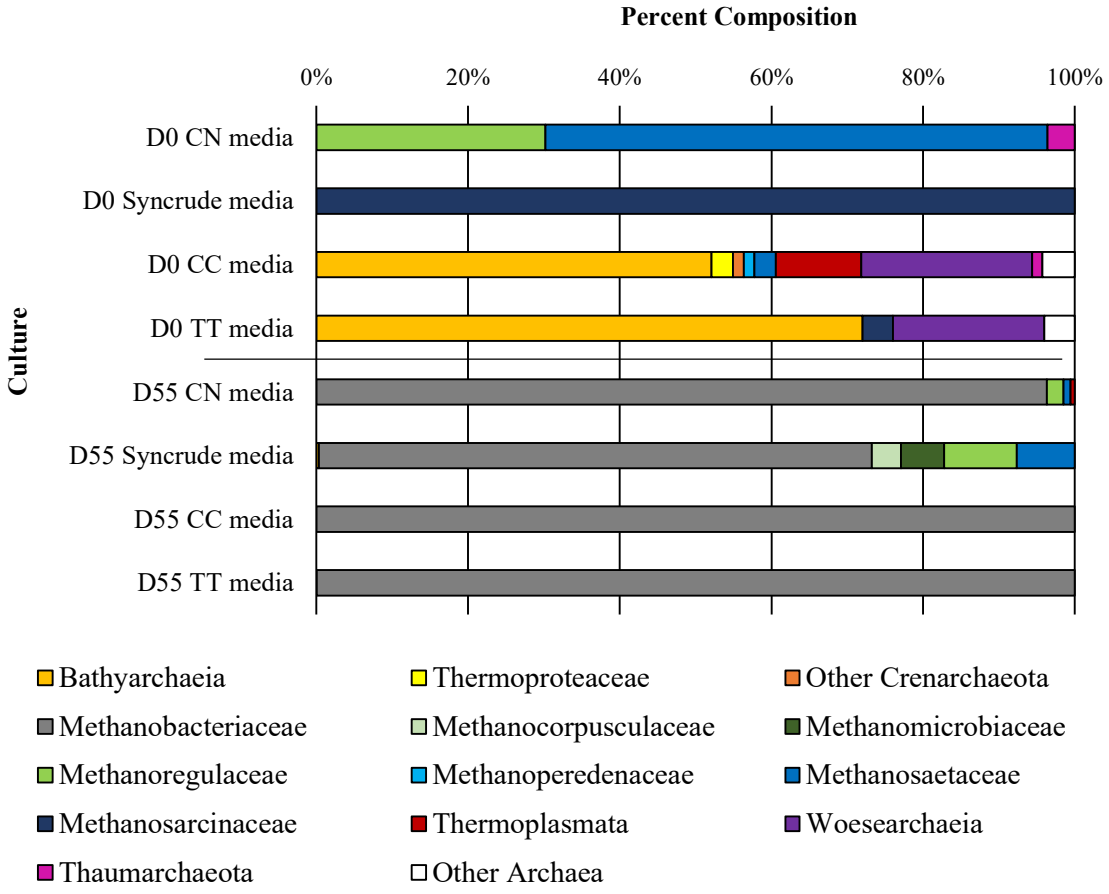


Figure 6. Community composition indicating percentage of Bacteria based on 16S rRNA sequencing of composite samples and alignment using the SILVA database. D0 indicates day 0 samples prior to incubation, end point samples were taken following 55 days of incubation. CN and Syncrude indicate media cultures established using 1 ml of Canadian Natural or Syncrude innoculum from previously established enrichment cultures. CC and TT indicate cultures established using 1 ml tailings centrifuge cake or thickened tailings, respectively. The Class Bacteroidia is represented in brown, Acidobacteriia are shown in black and white, Lactobacillales are gray, Clostridiales are green, Rhizobiales are red, and Betaproteobacteriales and the remaining Gammaproteobacteria are depicted in yellow and pink, respecively.

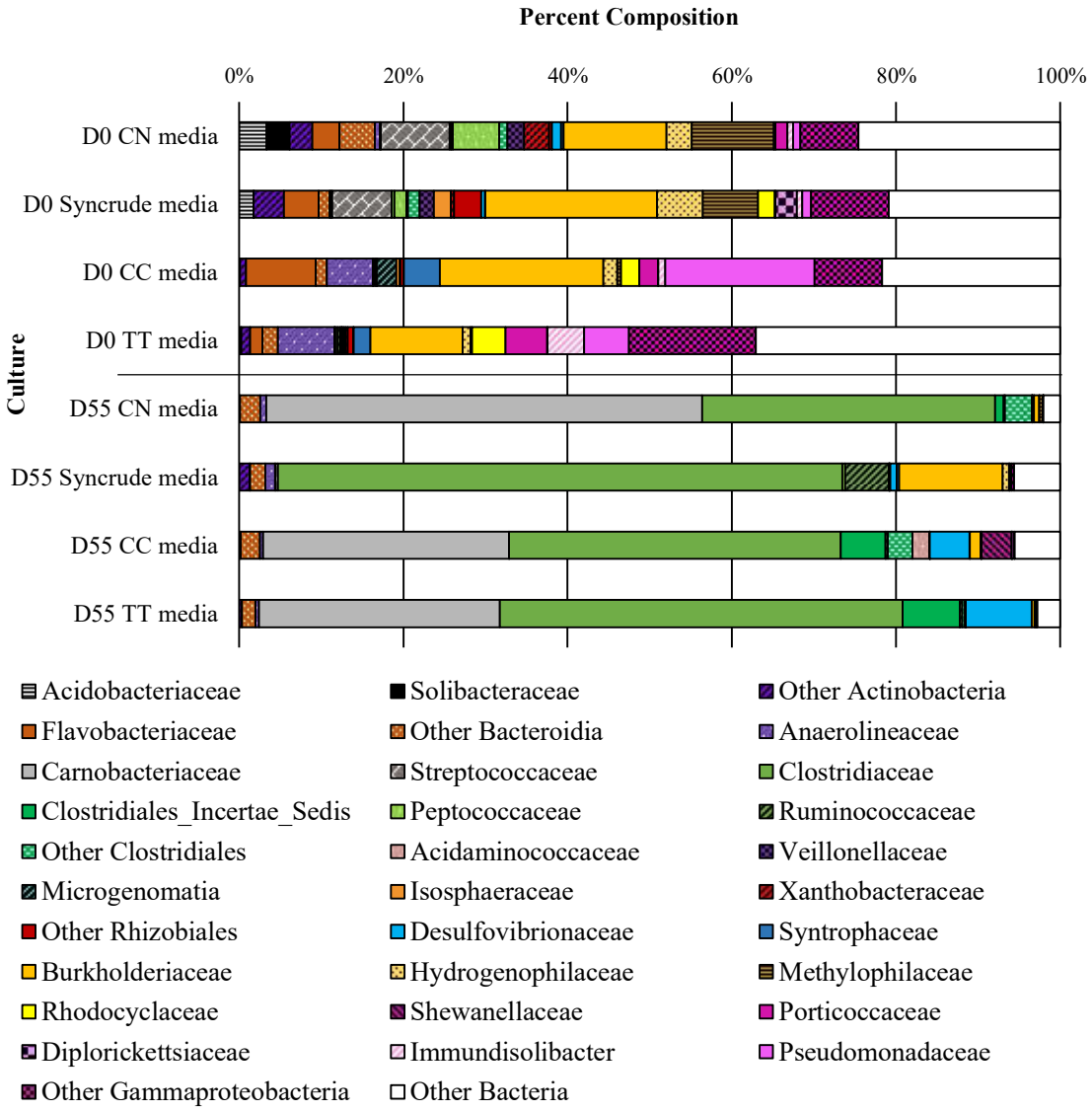


Figure 7. Mean methane production in cultures grown on diatomaceous earth and incubated for 41 days. Error bars represent 95% confidence interval (n=3). Theoretical methane production from citrate and toluene in 1 L culture volume; approximately 992 and 5024 µmol, respectively; (Symons and Buswell, 1933). Statistical significance determined using single factor ANOVA and Tukey HSD post-hoc test. Different letters between

treatments means indicate a statistically significant difference ($\alpha < 0.05$). Significant methane production only observed in Canadian Natural cultures.

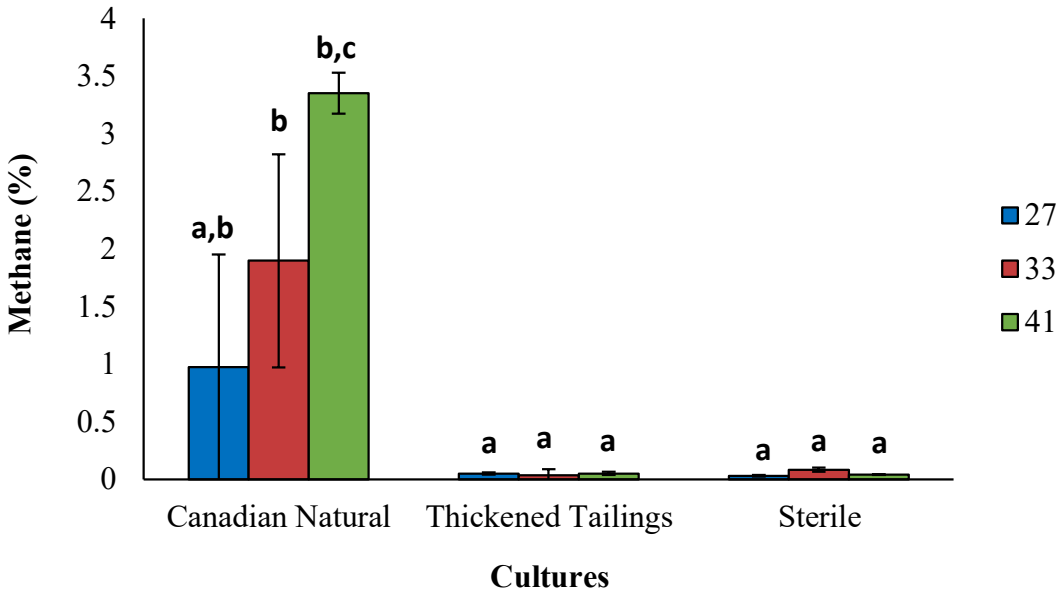


Figure 8. Mean toluene degradation in cultures grown on diatomaceous earth and incubated for 42 days. Error bars represent 95% confidence interval (n=3). Statistical significance determined using single factor ANOVA and Tukey HSD post-hoc test. Different letters between treatments means indicate a statistically significant difference ($\alpha < 0.05$).

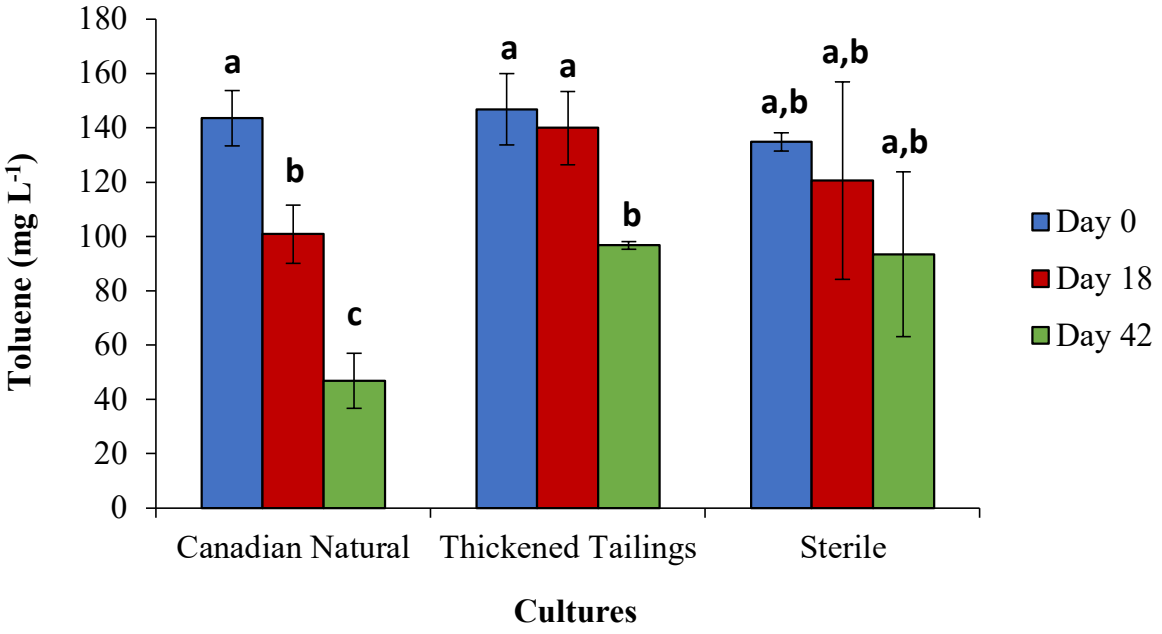


Figure 9. Community composition indicating percentage of Bacteria based on 16S rRNA sequencing of composite samples and alignment using the SILVA database. Samples were taken following 50 days of incubation prior to Phase 2 inoculation. CN indicates diatomaceous earth cultures established using 3 ml of Canadian Natural inoculum from Phase 1.1 cultures. The Order Clostridiales is represented in green.

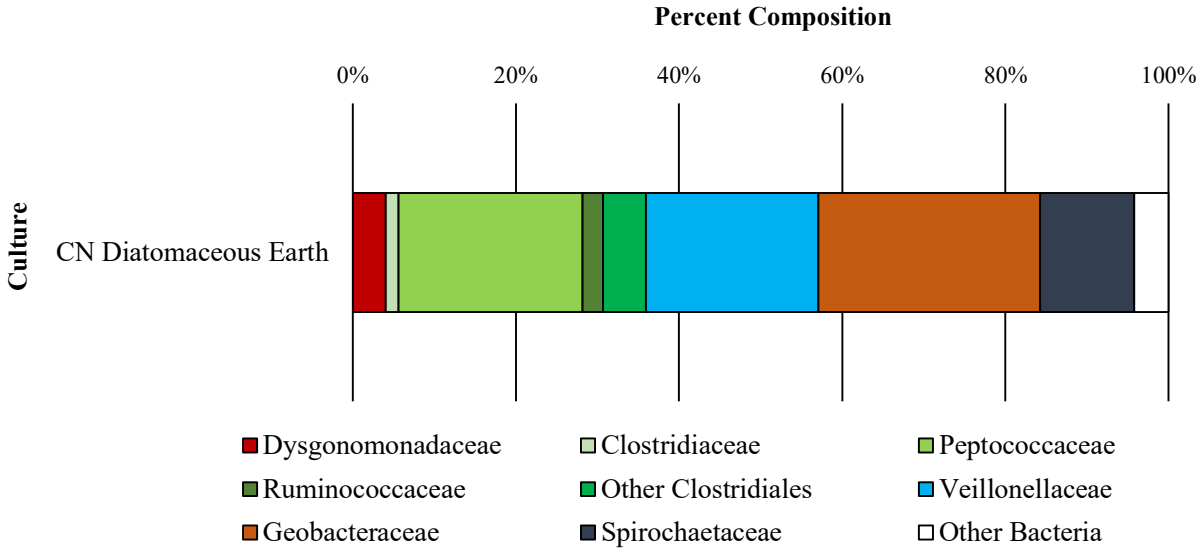


Figure 10. Maximum shoot height, mean leaf biomass, root mass, leaf area index (LAI), and root: leaf ratio *Elymus trachycaulus*, planted in centrifuge cake and thickened tailings with different amendments and bacteria inoculation (H+B1= hydrochar + bacteria (type 1); H= hydrochar only; P+B1= peat + bacteria (type 1); P= peat only; B1= bacteria only (type 1); N= no amendment or bacteria inoculation). Means (± SE, n = 6) followed by different letter(s) are significantly different ($p < 0.05$) from each other among amendments and tailings type.

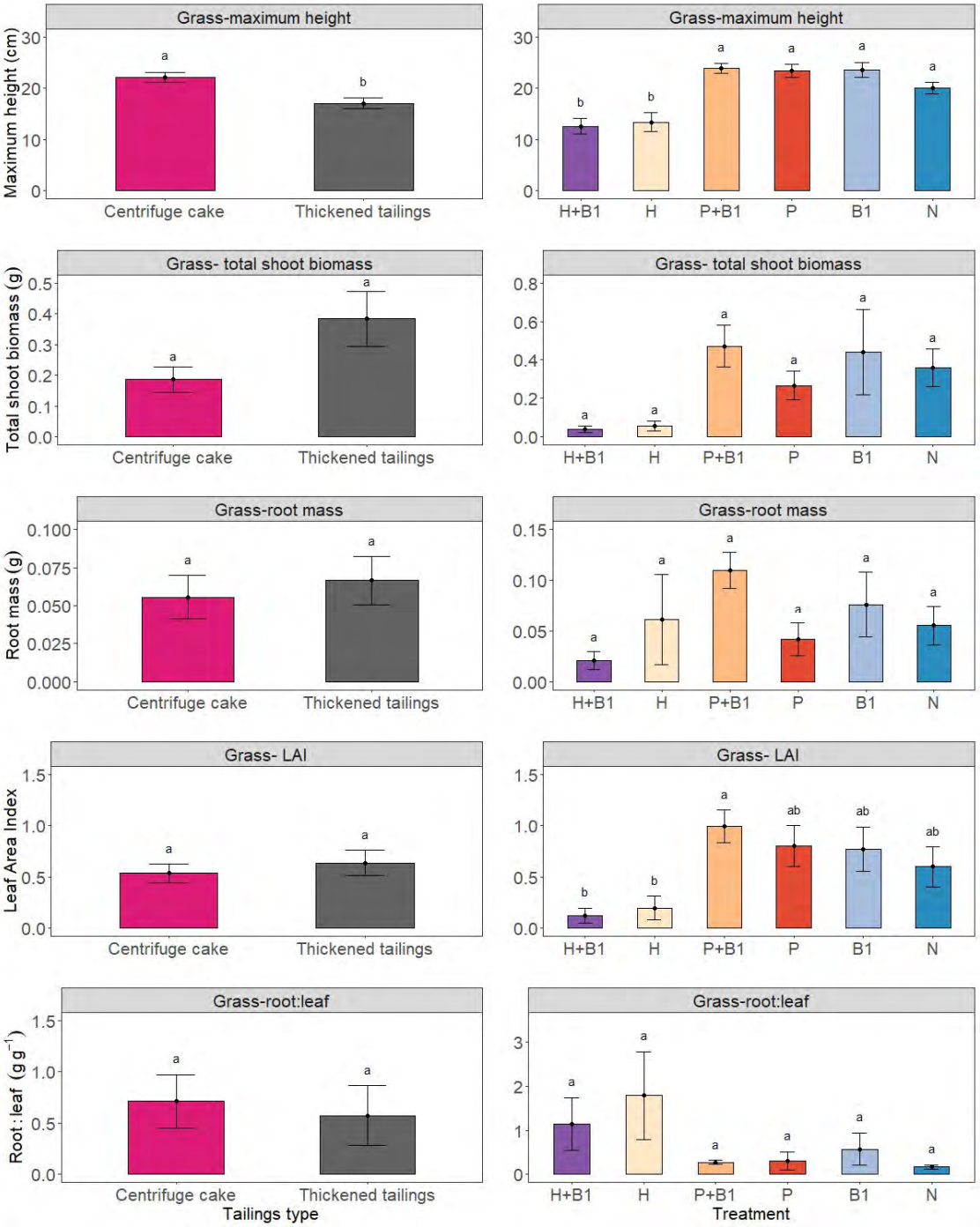


Figure 11. Mean shoot height, leaf biomass, root mass, leaf area index (LAI), and root: leaf ratio of *Salix interior* (willow), planted in centrifuge cake and thickened tailings with different amendments (H+B1= hydrochar + bacteria (type 1); H= hydrochar only; P+B1= peat + bacteria (type 1); P= peat only; B1= bacteria only (type 1); B2= bacteria only (type 2); N= no amendment or bacteria inoculation). Means (± SE, n = 6) followed by different letter(s) are significantly different ($p < 0.05$) from each other among amendments and tailings type

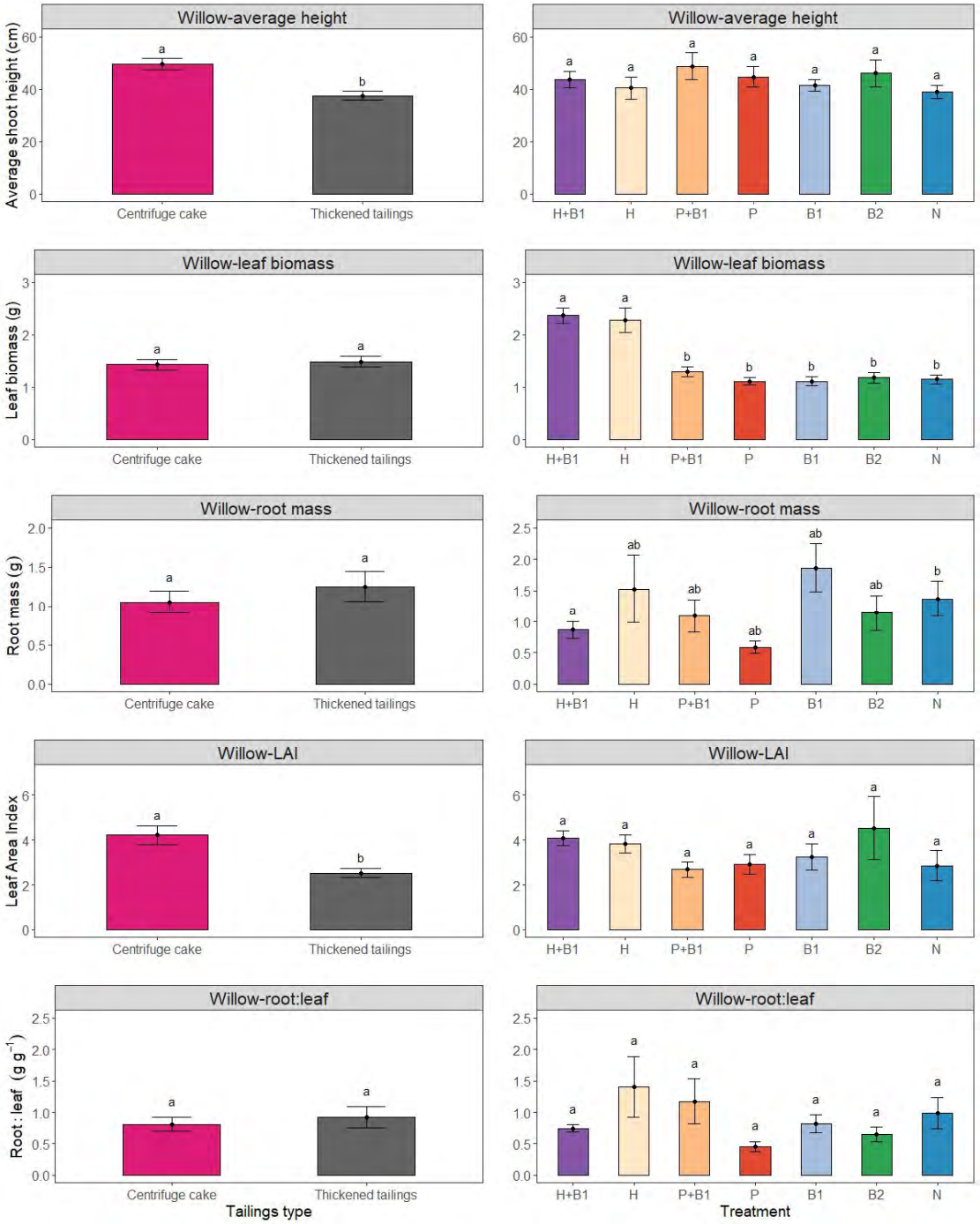


Figure 12. Mean solids content (%) for centrifuge cake planted with *Elymus trachycaulus* (grass) and *Salix interior* (willow), and with no plants under different amendments (H+B1= hydrochar + bacteria (type 1); H= hydrochar only; P+B1= peat + bacteria (type 1); P= peat only; B1= bacteria only (type 1); B2= bacteria only (type 2); N= no amendment or bacteria inoculation). sampled at top (0-35 cm), middle (35-65 cm), and bottom (65-100 cm). Means (± SE, n = 4) followed by different letter(s) are significantly different ($p < 0.05$) from each other among amendments within the same tailing type.

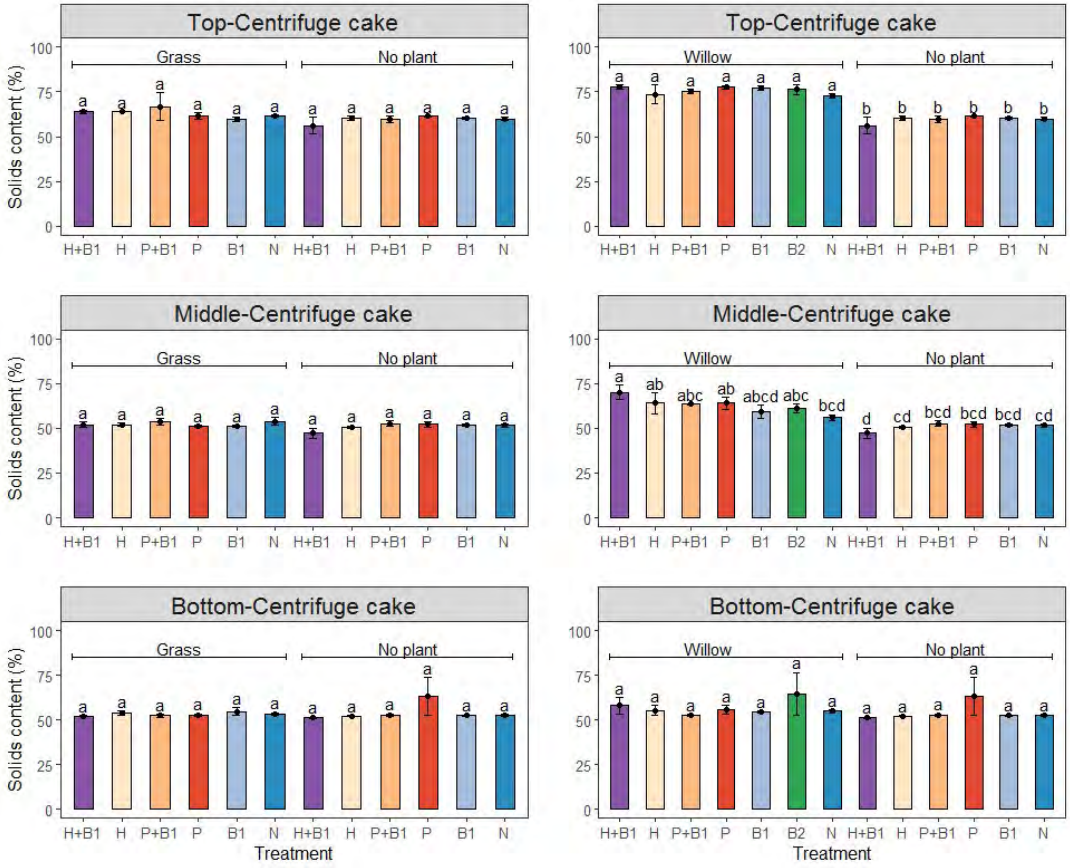


Figure 13. Mean solids content (%) for thickened tailings planted with *Elymus trachycaulus* (grass) and *Salix interior* (willow), and with no plants under different amendments (H+B1= hydrochar + bacteria (type 1); H= hydrochar only; P+B1= peat + bacteria (type 1); P= peat only; B1= bacteria only (type 1); B2= bacteria only (type 2); N= no amendment or bacteria inoculation). Sampled at top (0-35 cm), middle (35-65 cm), and bottom (65-100 cm). Means (± SE, n = 4) followed by different letter(s) are significantly different ($p < 0.05$) from each other among amendments within the same tailing type.

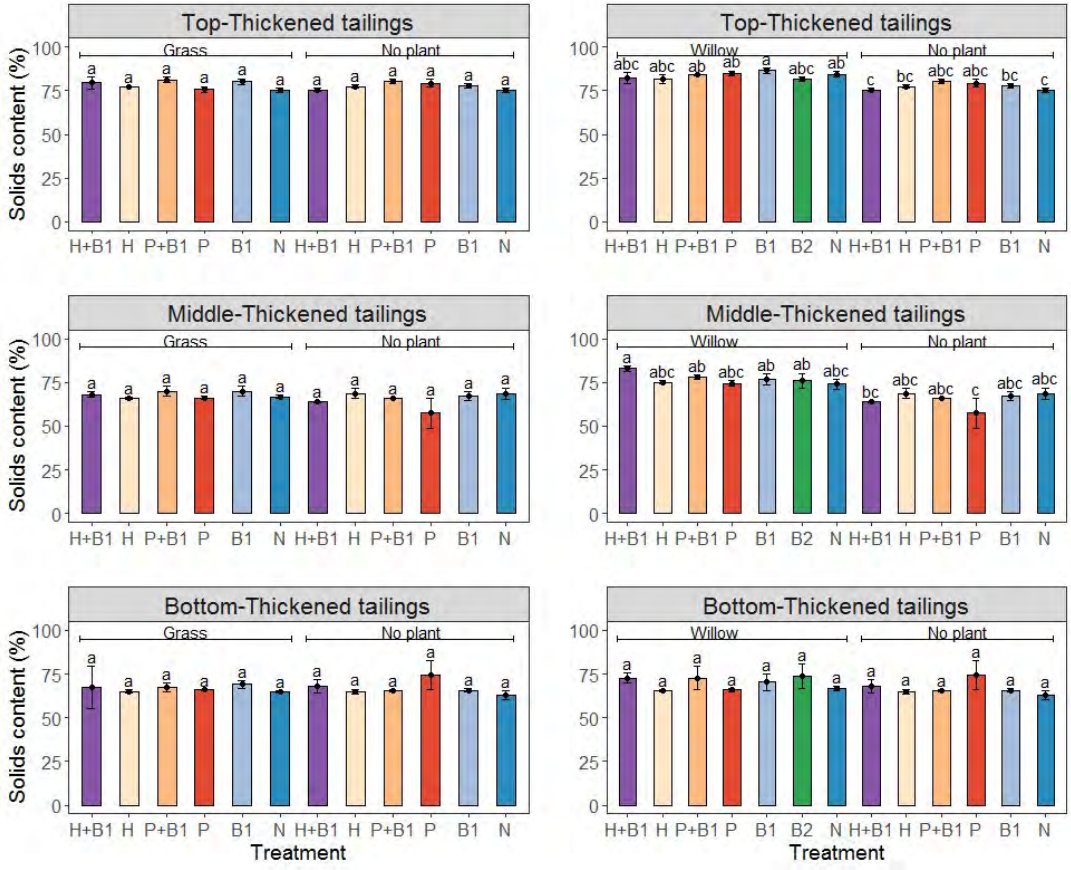


Figure 14. Mean consolidation and percentage of tailings under water for centrifuge cake planted with *Elymus trachycaulus* (grass) and *Salix interior* (willow), and with no plants under different amendments (H+B1= hydrochar + bacteria (type 1); H= hydrochar only; P+B1= peat + bacteria (type 1); P= peat only; B1= bacteria only (type 1); B2= bacteria only (type 2); N= no amendment or bacteria inoculation). Means (± SE, n = 6) followed by different letter(s) are significantly different ($p < 0.05$) from each other among amendments within the same tailing type.

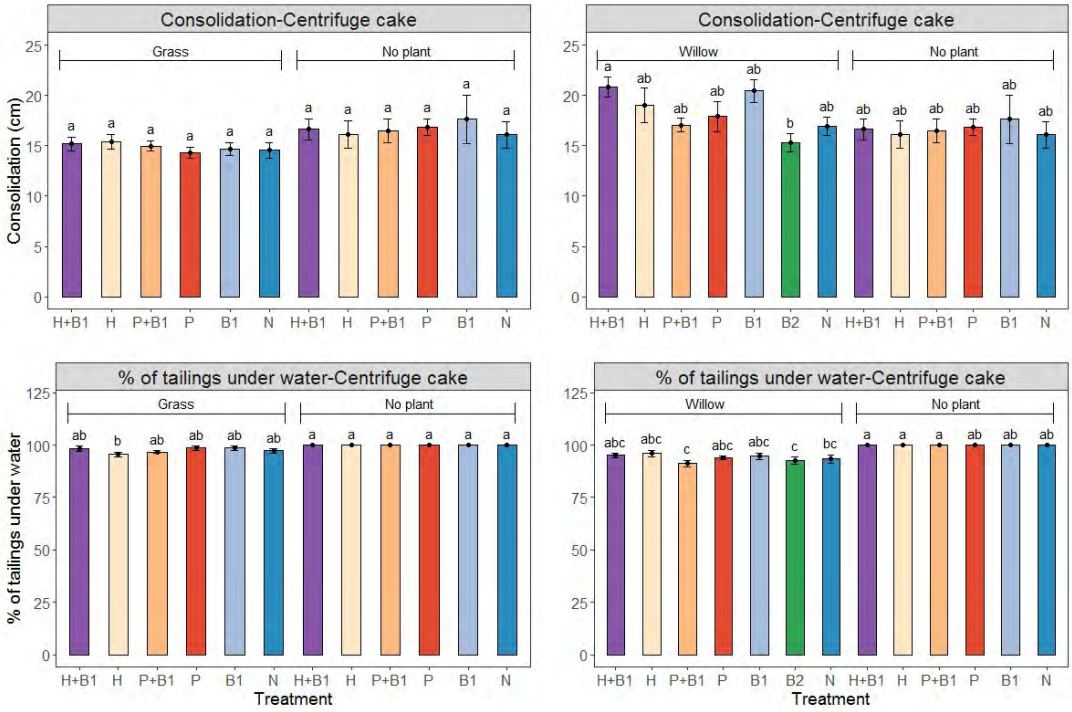


Figure 15. Mean consolidation and percentage of tailings under water for thickened tailings planted with *Elymus trachycaulus* (grass) and *Salix interior* (willow), and with no plants under different amendments (H+B1= hydrochar + bacteria (type 1); H= hydrochar only; P+B1= peat + bacteria (type 1); P= peat only; B1= bacteria only (type 1); B2= bacteria only (type 2); N= no amendment or bacteria inoculation). Means (± SE, n = 6) followed by different letter(s) are significantly different ($p < 0.05$) from each other among amendments within the same tailing type.

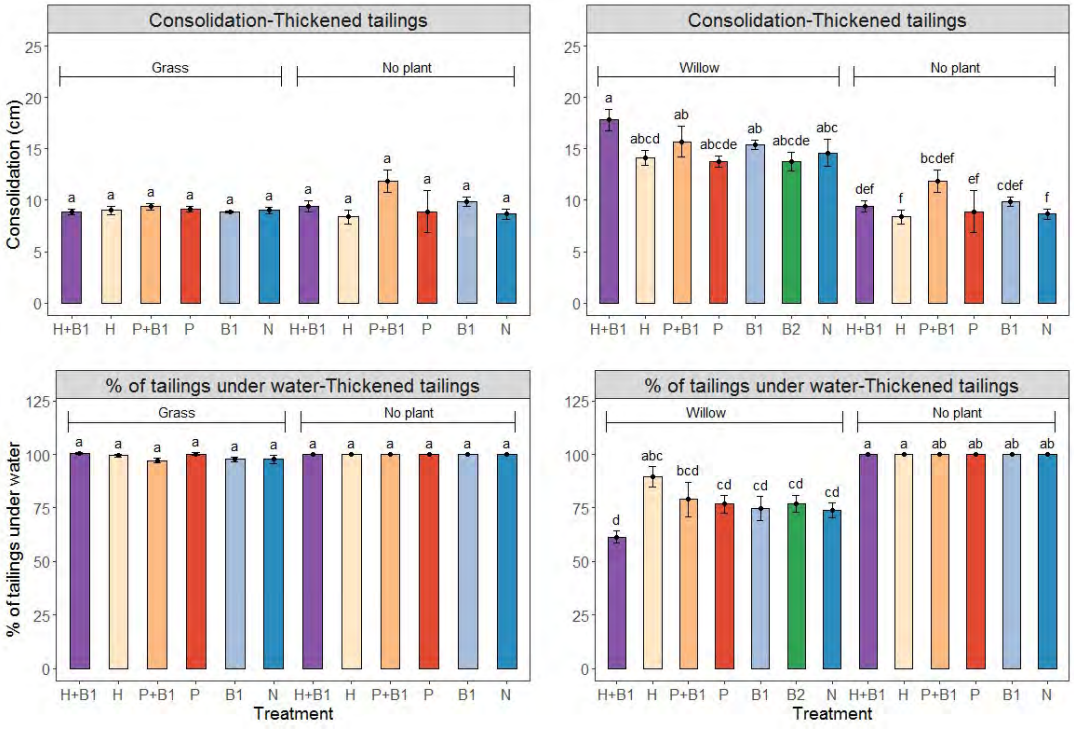


Figure 16. Mean evapotranspiration (L) for centrifuge cake and thickened tailings planted with *Elymus trachycaulus* (grass) and *Salix interior* (willow), and evaporation (with no plants) under different amendments (H+B1= hydrochar + bacteria (type 1); H= hydrochar only; P+B1= peat + bacteria (type 1); P= peat only; B1= bacteria only (type 1); B2= bacteria only (type 2); N= no amendment or bacteria inoculation). Means (± SE, n = 5) followed by different letter(s) are significantly different ($p < 0.05$) from each other among amendments within the same tailing type.

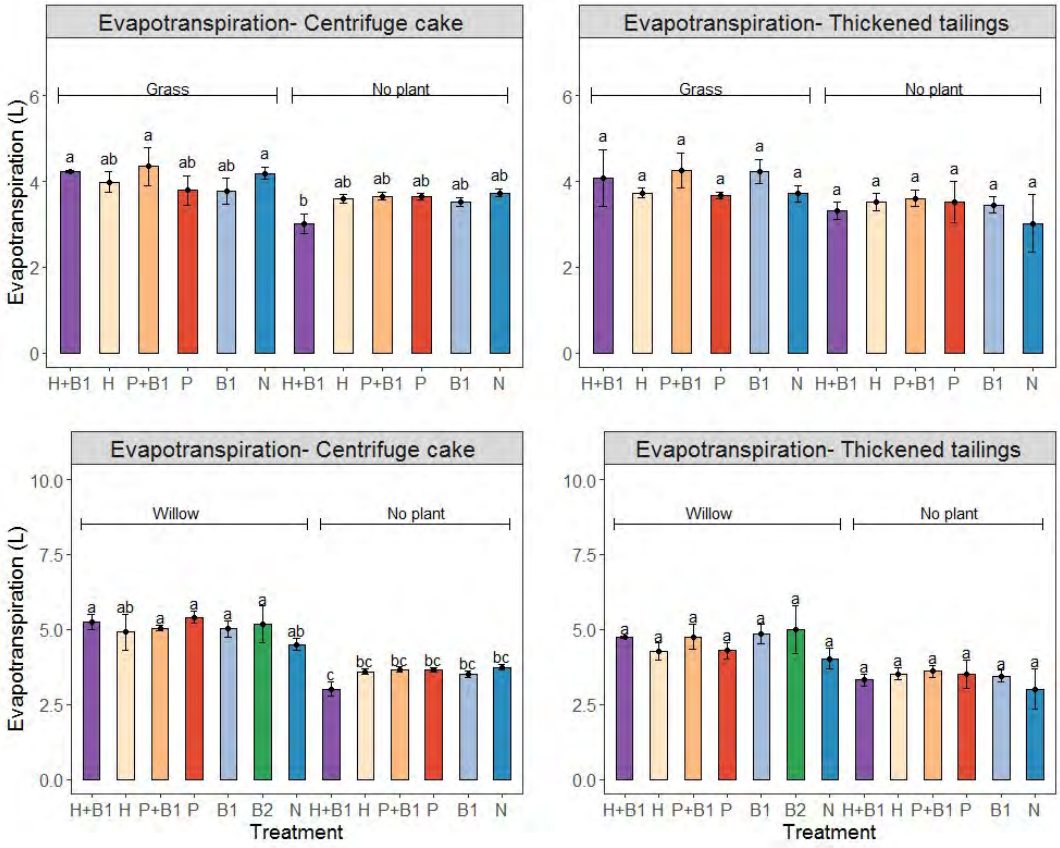


Figure 17. Mean water used (mm) for centrifuge cake and thickened tailings planted with *Elymus trachycaulus* (grass) and *Salix interior* (willow), and with no plants under different amendments (H+B1= hydrochar + bacteria (type 1); H= hydrochar only; P+B1= peat + bacteria (type 1); P= peat only; B1= bacteria only (type 1); B2= bacteria only (type 2); N= no amendment or bacteria inoculation). Means (± SE, n = 5) followed by different letter(s) are significantly different ($p < 0.05$) from each other among amendments within the same tailing type.

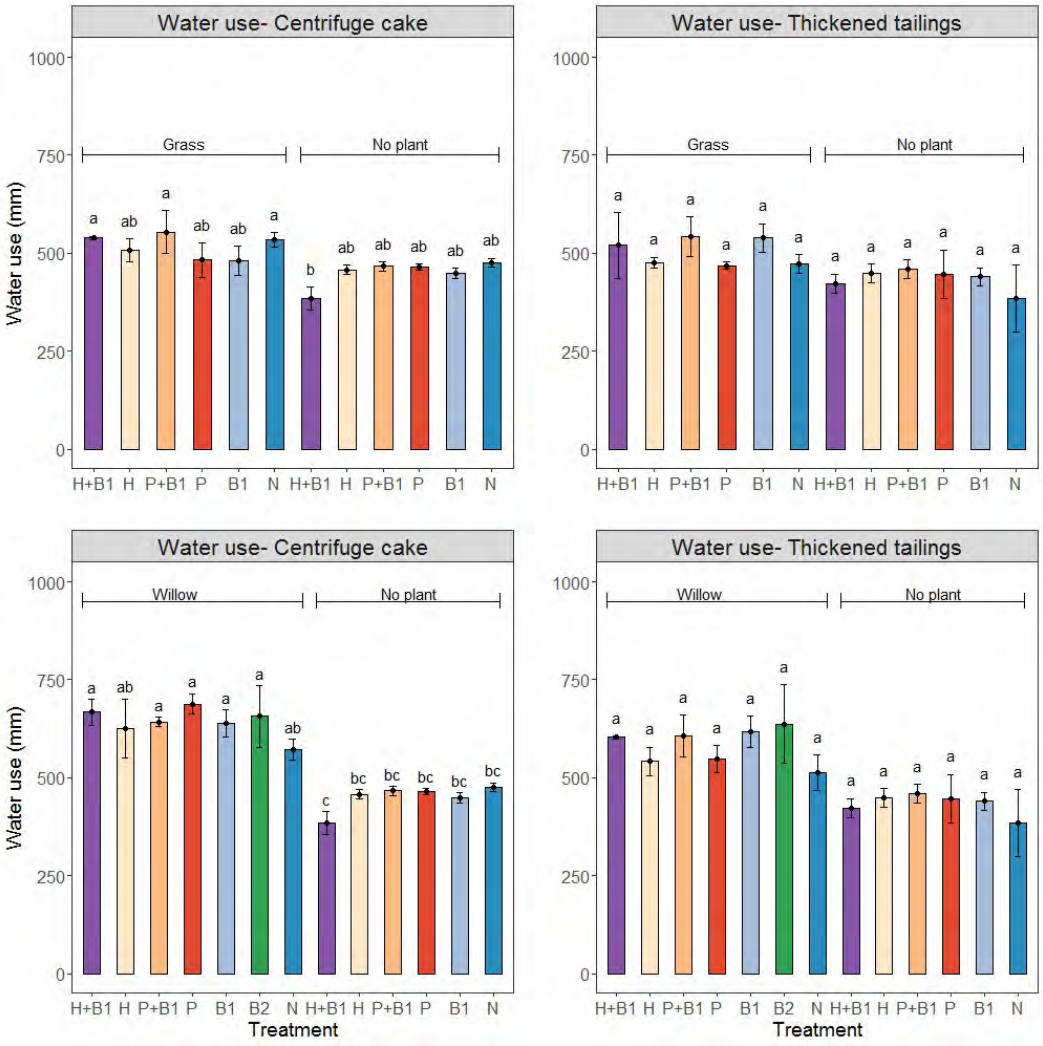


Figure 18. Mean undrained shear strength for centrifuge cake planted with *Elymus trachycaulus* (grass) and *Salix interior* (willow), and with no plants under different amendments (H+B1= hydrochar + bacteria (type 1); H= hydrochar only; P+B1= peat + bacteria (type 1); P= peat only; B1= bacteria only (type 1); N= no amendment or bacteria inoculation). Means (± SE, n = 5).

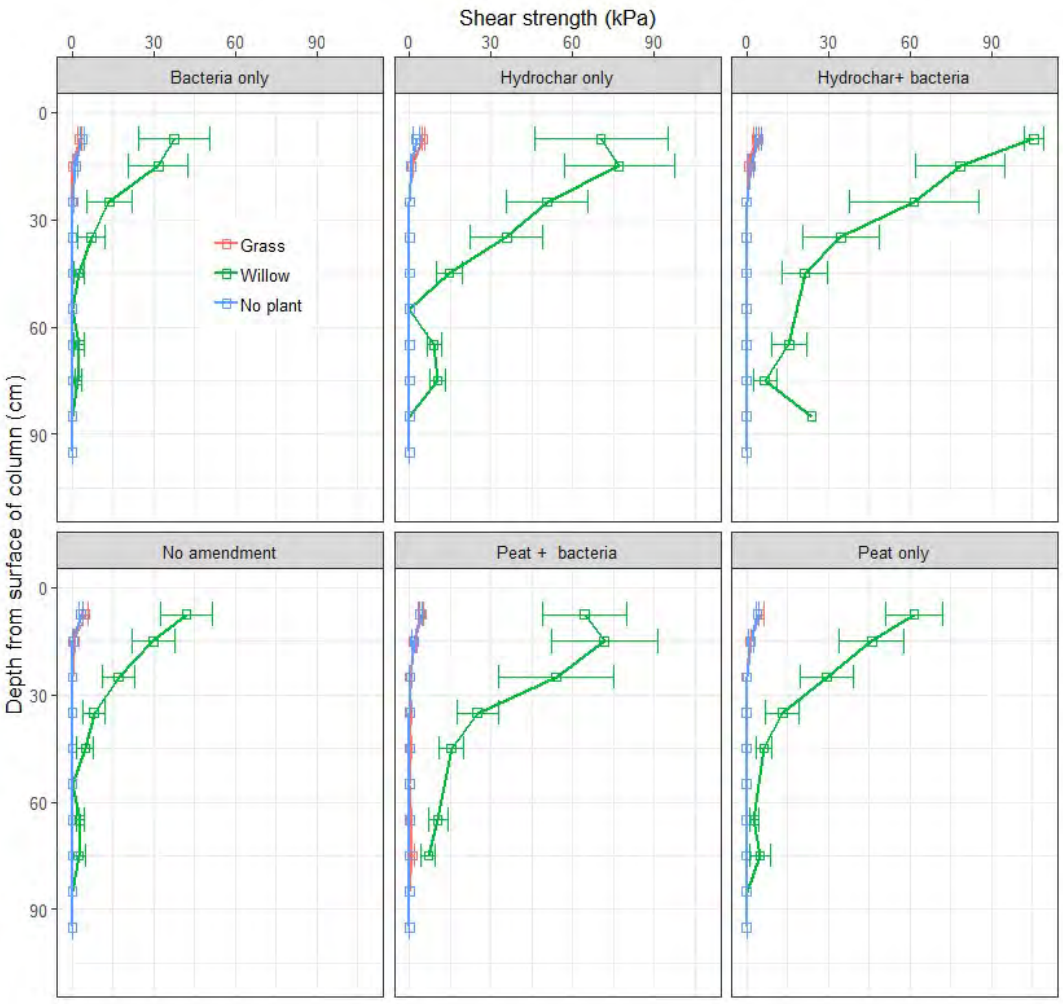


Figure 19. Mean predicted and measured shear strength for centrifuge cake planted with *Salix interior* (willow) under different amendments (H+B1= hydrochar + bacteria (type 1); H= hydrochar only; P+B1= peat + bacteria (type 1); P= peat only; B1= bacteria only (type 1); N= no amendment or bacteria inoculation). Means (± SE, n = 3).

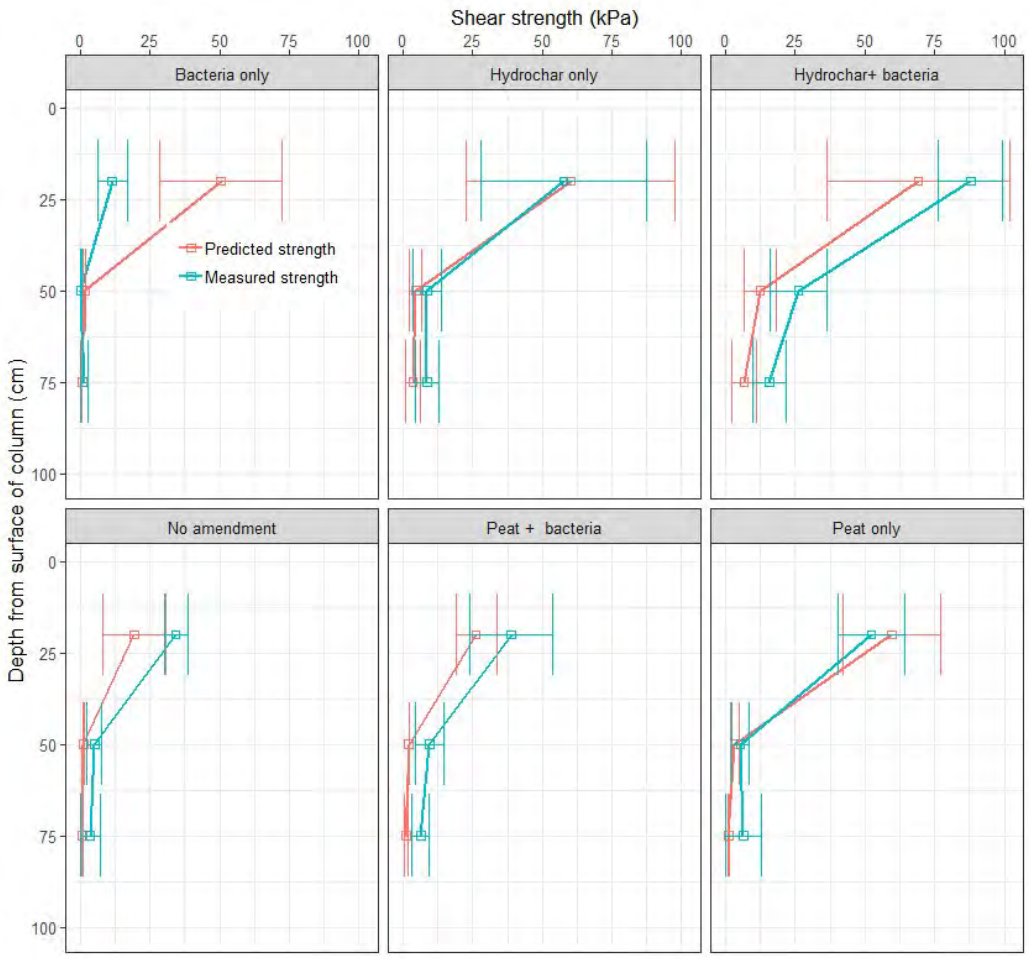


Figure 20. Mean undrained shear strength for thickened tailings planted with *Elymus trachycaulus* (grass) and *Salix interior* (willow), and with no plants under different amendments (H+B1= hydrochar + bacteria (type 1); H= hydrochar only; P+B1= peat + bacteria (type 1); P= peat only; B1= bacteria only (type 1); N= no amendment or bacteria inoculation). Means (± SE, n = 5).

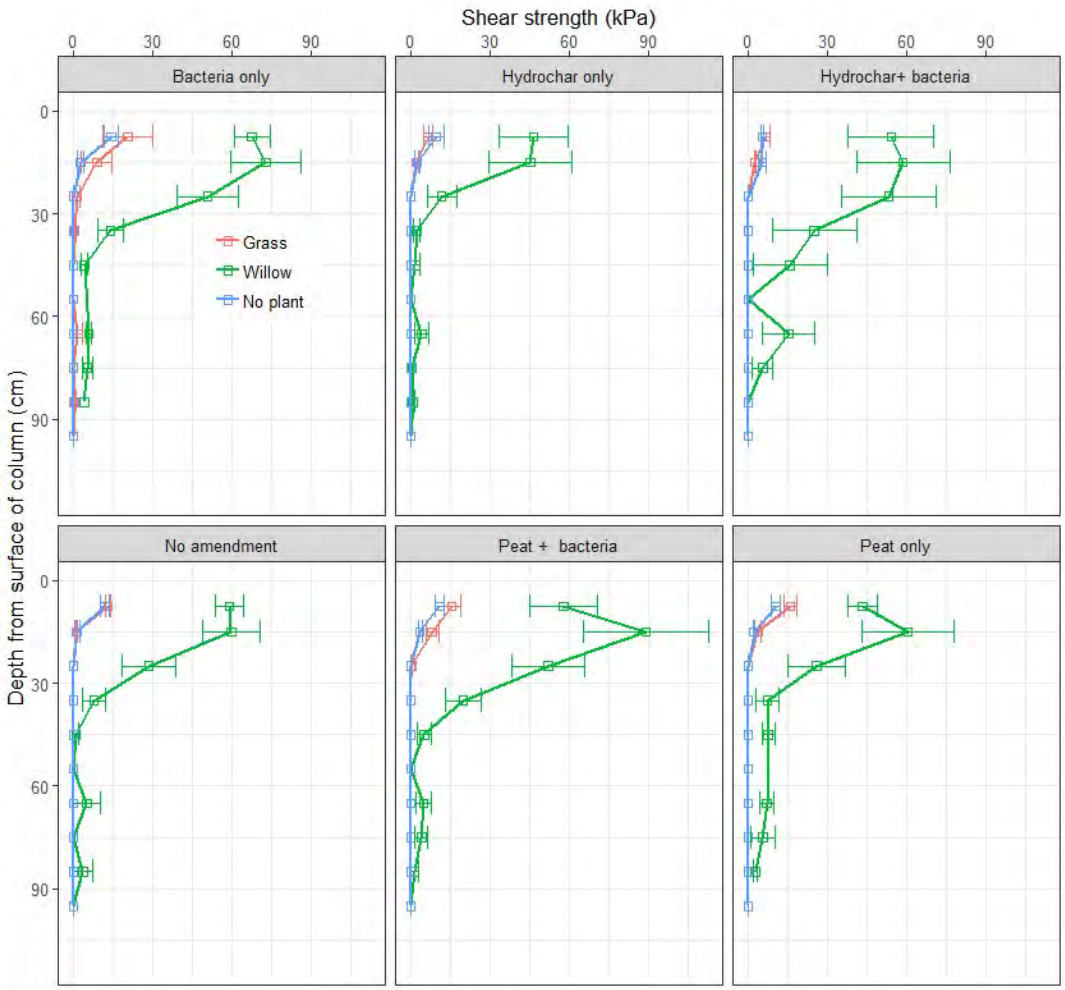


Figure 21. Mean predicted and measured shear strength for thickened tailings planted with *Salix interior* (willow) under different amendments (H+B1= hydrochar + bacteria (type 1); H= hydrochar only; P+B1= peat + bacteria (type 1); P= peat only; B1= bacteria only (type 1); N= no amendment or bacteria inoculation). Means (± SE, n = 3).

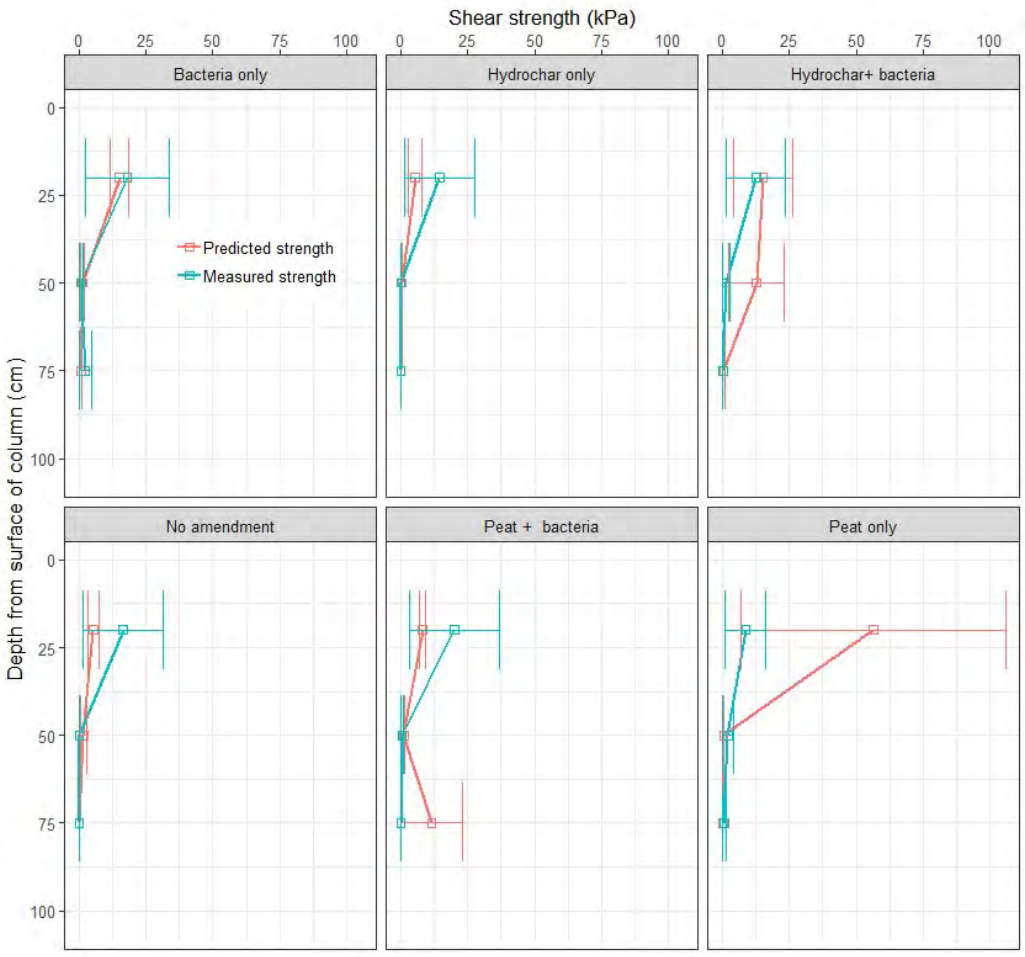


Figure 22. Relationship between tailings consolidation, evapotranspiration, water use, and leaf biomass of *Salix interior* (willow) planted in centrifuge cake. Regression lines for this relationship are shown at p <0.05

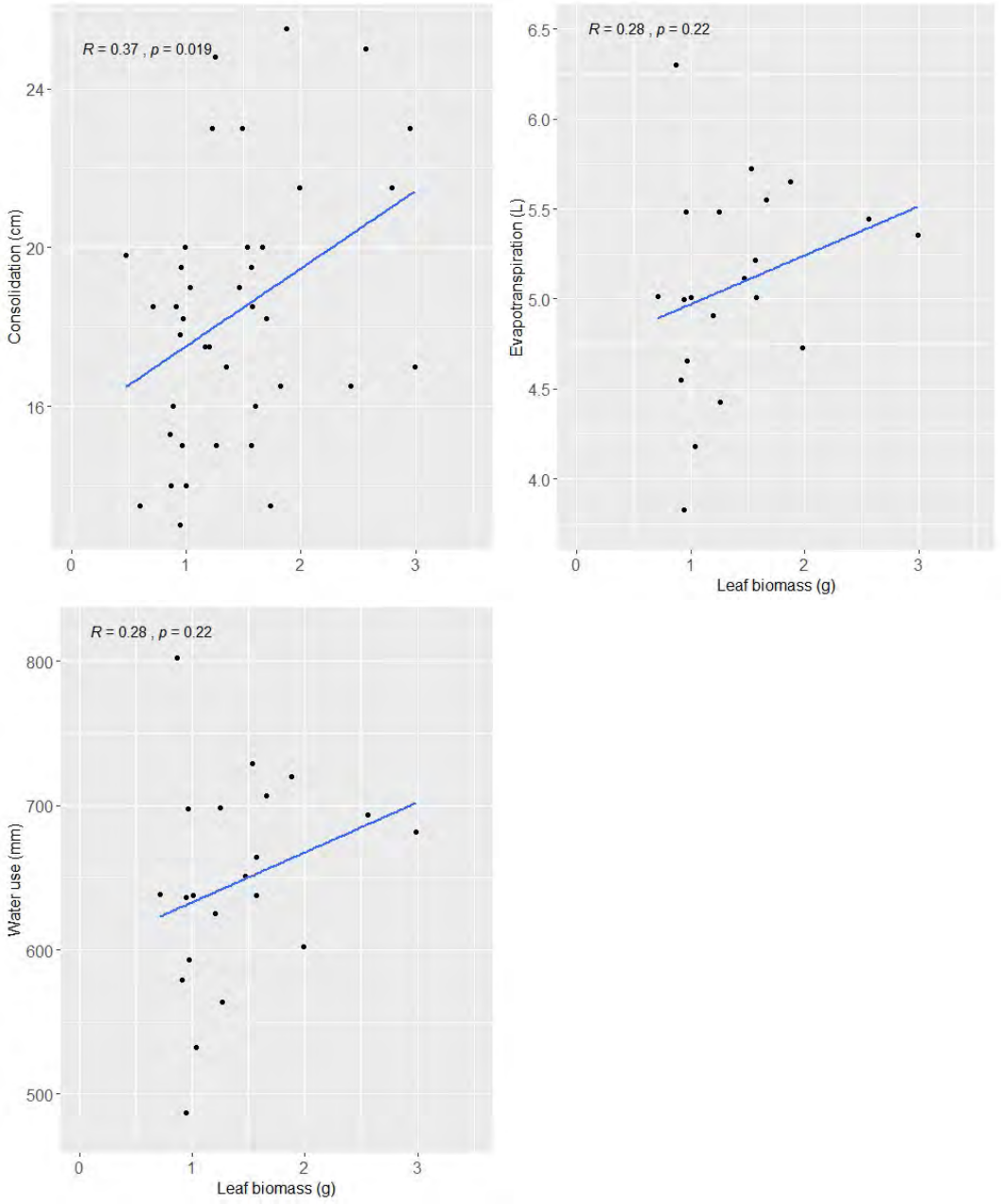


Figure 23. Relationship between solids content [top (0-35 cm), middle (35-65 cm), and bottom (65-100 cm)], measured shear strength [top (0-35 cm), middle (35-65 cm), and bottom (65-100 cm)], and leaf biomass of *Salix interior* (willow) planted in centrifuge cake. Regression lines for this relationship are shown at p <0.05.

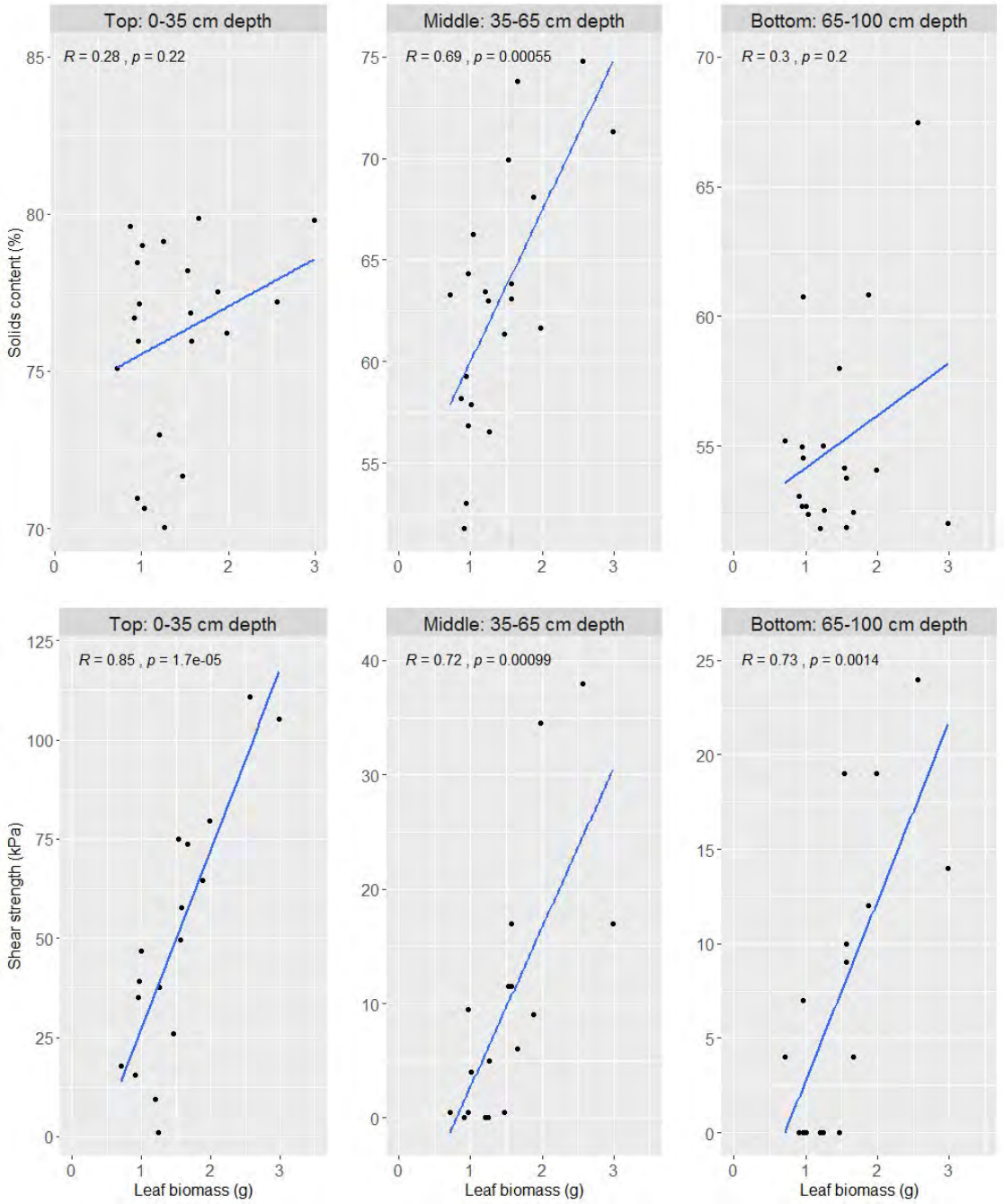
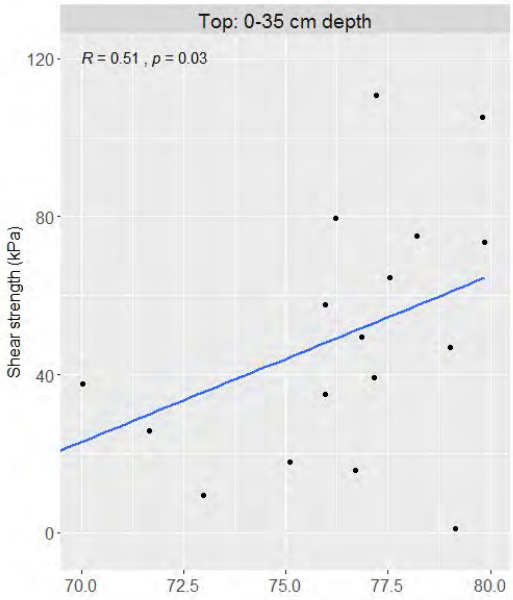
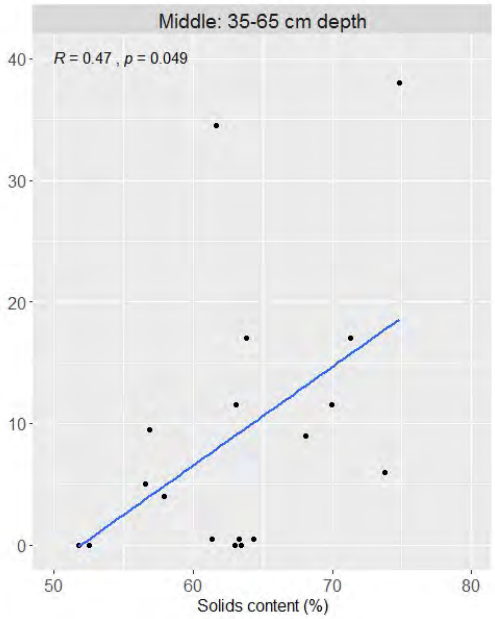


Figure 24. Relationship between solids content and measured shear strength of centrifuge cake at top [0-35 cm], middle [35-65 cm], and bottom [65-100 cm] of column. Regression lines for this relationship are shown at p <0.05


Top: 0-35 cm depth
R = 0.51 , p = 0.03
Shear strength (kPa)
120
80
40
0
70.0
72.5
75.0
77.5
80.0


Middle: 35-65 cm depth
R = 0.47 , p = 0.049
40
30
20
10
0
50
60
70
80
Solids content (%)

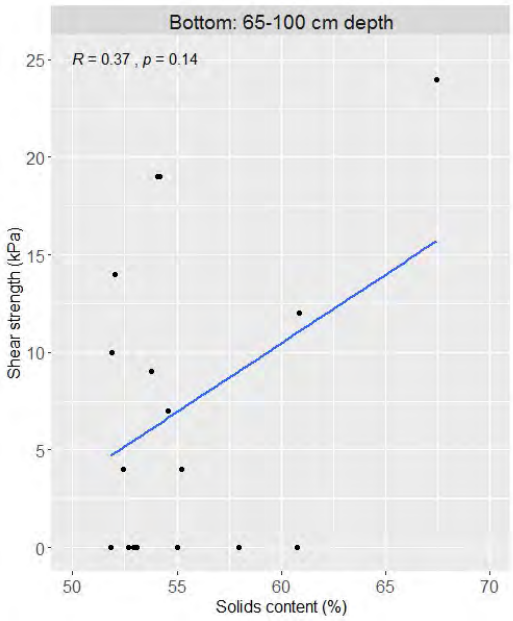

Bottom: 65-100 cm depth
R = 0.37 , p = 0.14
Shear strength (kPa)
25
20
15
10
5
0
50
55
60
65
70
Solids content (%)

Figure 25. Relationship between solids content [top (0-35 cm), middle (35-65 cm), and bottom (65-100 cm)], measured shear strength [top (0-35 cm), middle (35-65 cm), and bottom (65-100 cm)], and leaf biomass of *Salix interior* (willow) planted in thickened tailings. Regression lines for this relationship are shown at p <0.05.

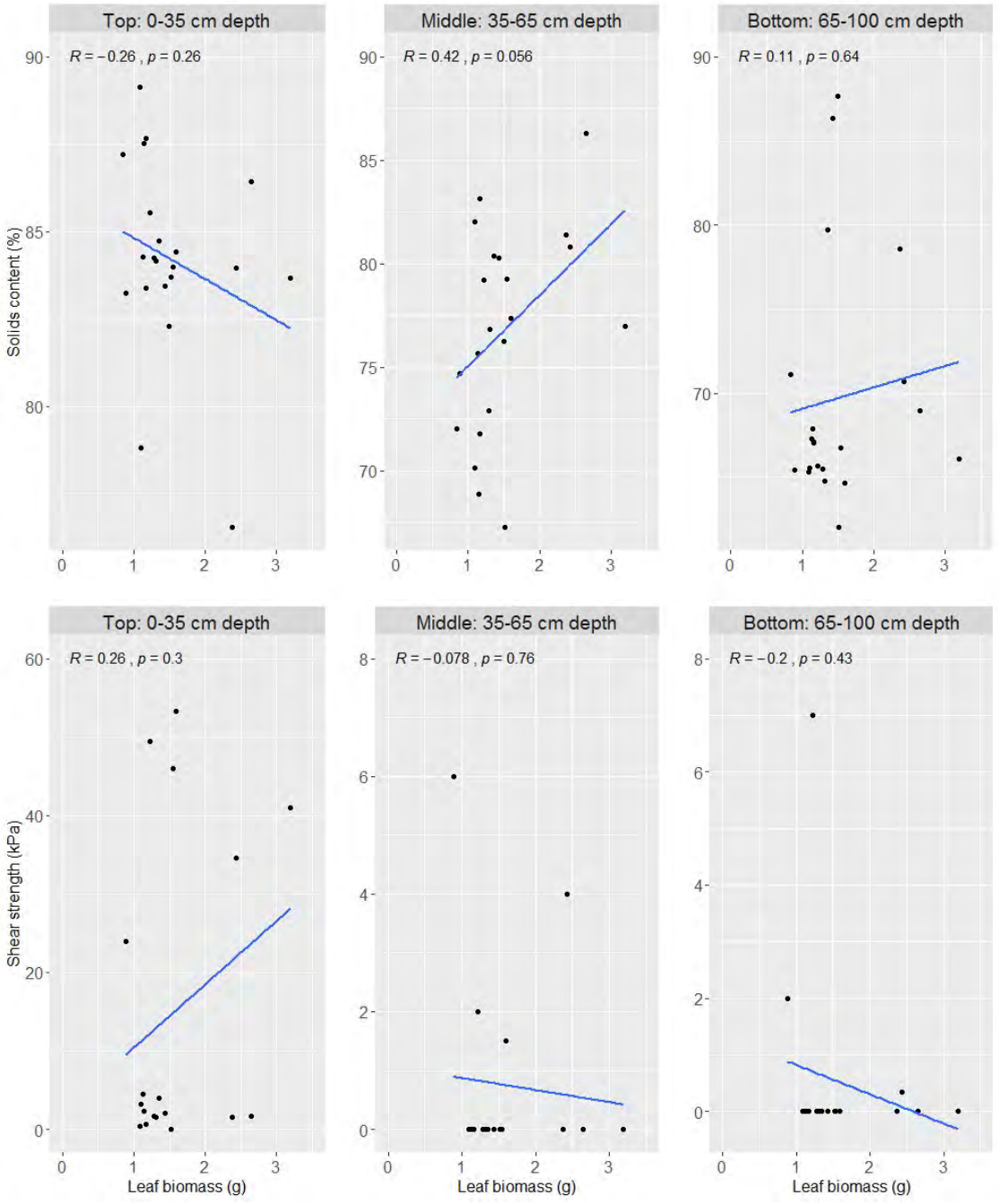
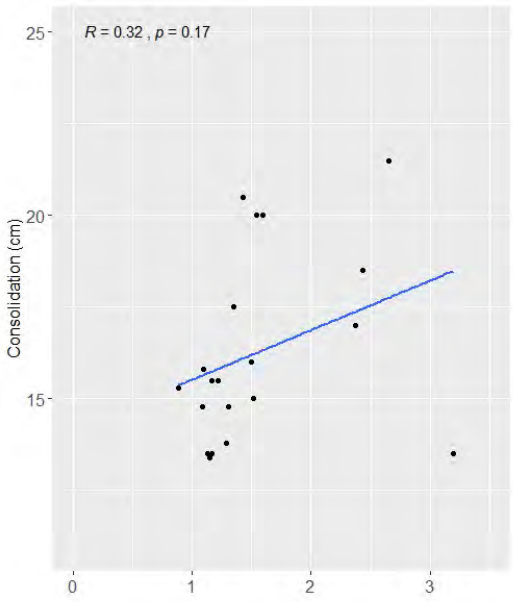
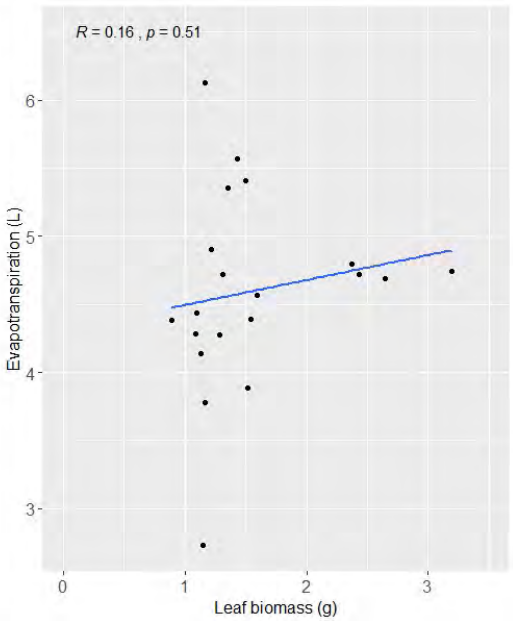


Figure 26. Relationship between tailings consolidation, evapotranspiration, water use, and leaf biomass of *Salix interior* (willow) planted in thickened tailings. Regression lines for this relationship are shown at p <0.05


R = 0.32 , p = 0.17
Consolidation (cm)
25
20
15
0
1
2
3


R = 0.16 , p = 0.51
Evapotranspiration (L)
6
5
4
3
0
1
2
3
Leaf biomass (g)

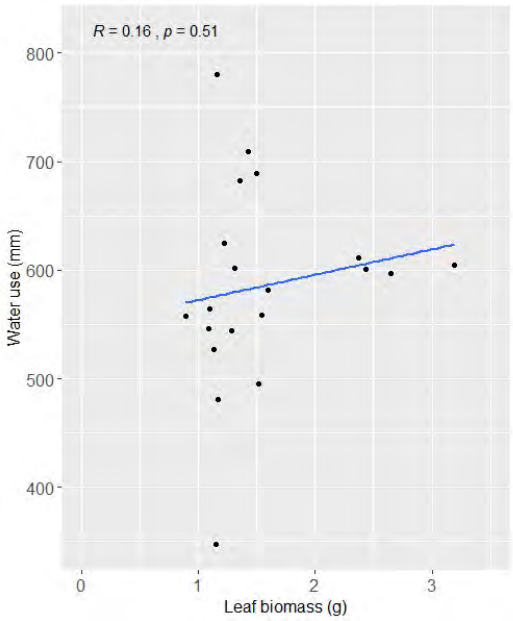

R = 0.16 , p = 0.51
Water use (mm)
800
700
600
500
400
0
1
2
3
Leaf biomass (g)

Figure 27. Relationship between solids content and measured shear strength of thickened tailings at top [0-35 cm], middle [35-65 cm], and bottom [65-100 cm] of column. Regression lines for this relationship are shown at p <0.05

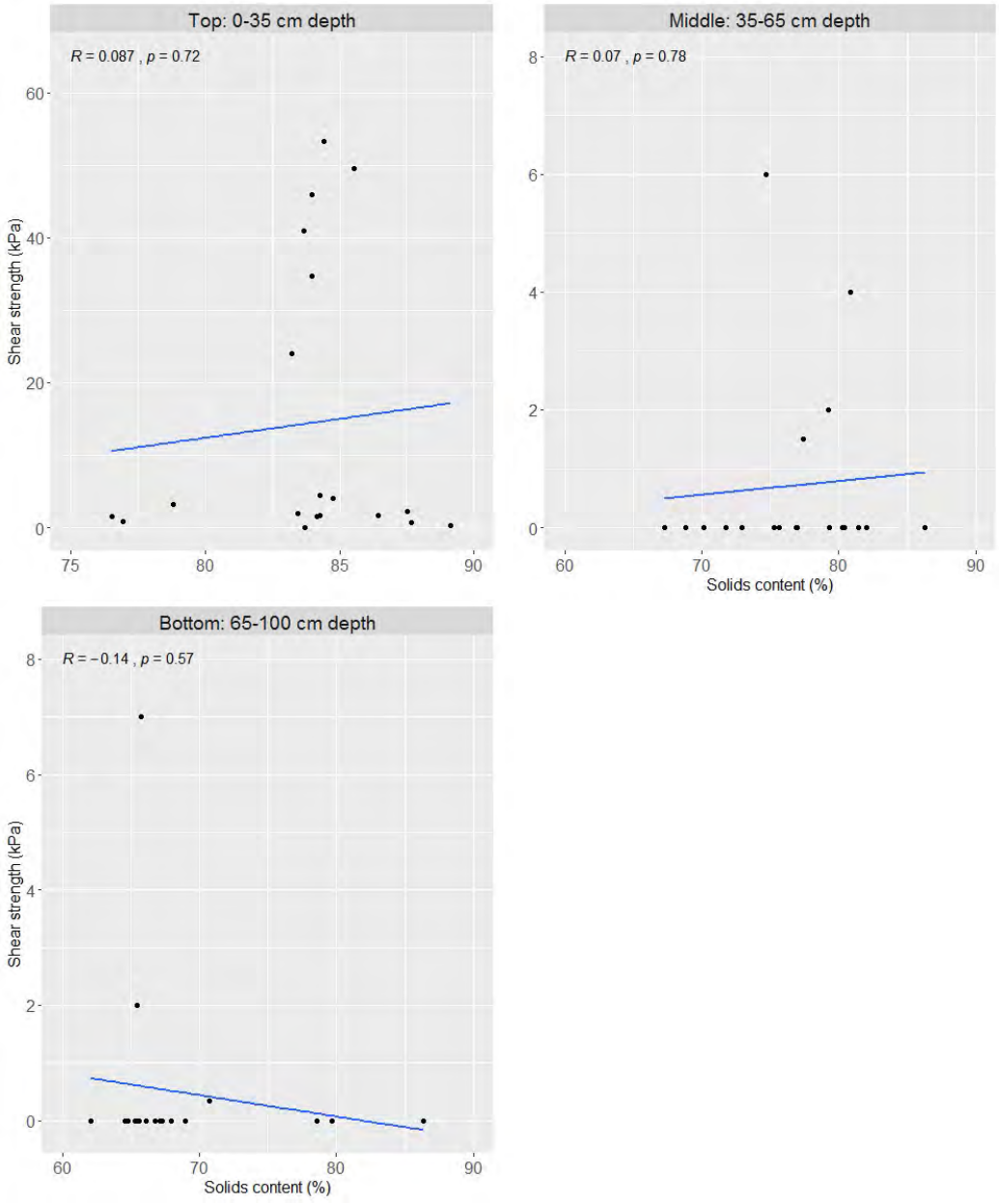


Figure 28. Principal component analysis (PCA) of Bacteria community data at Order level based on 16S rRNA sequencing of composite samples and alignment using the SILVA database. These PCA explain 32.2% (PC1) and 15% (PC2) of the observed variance.

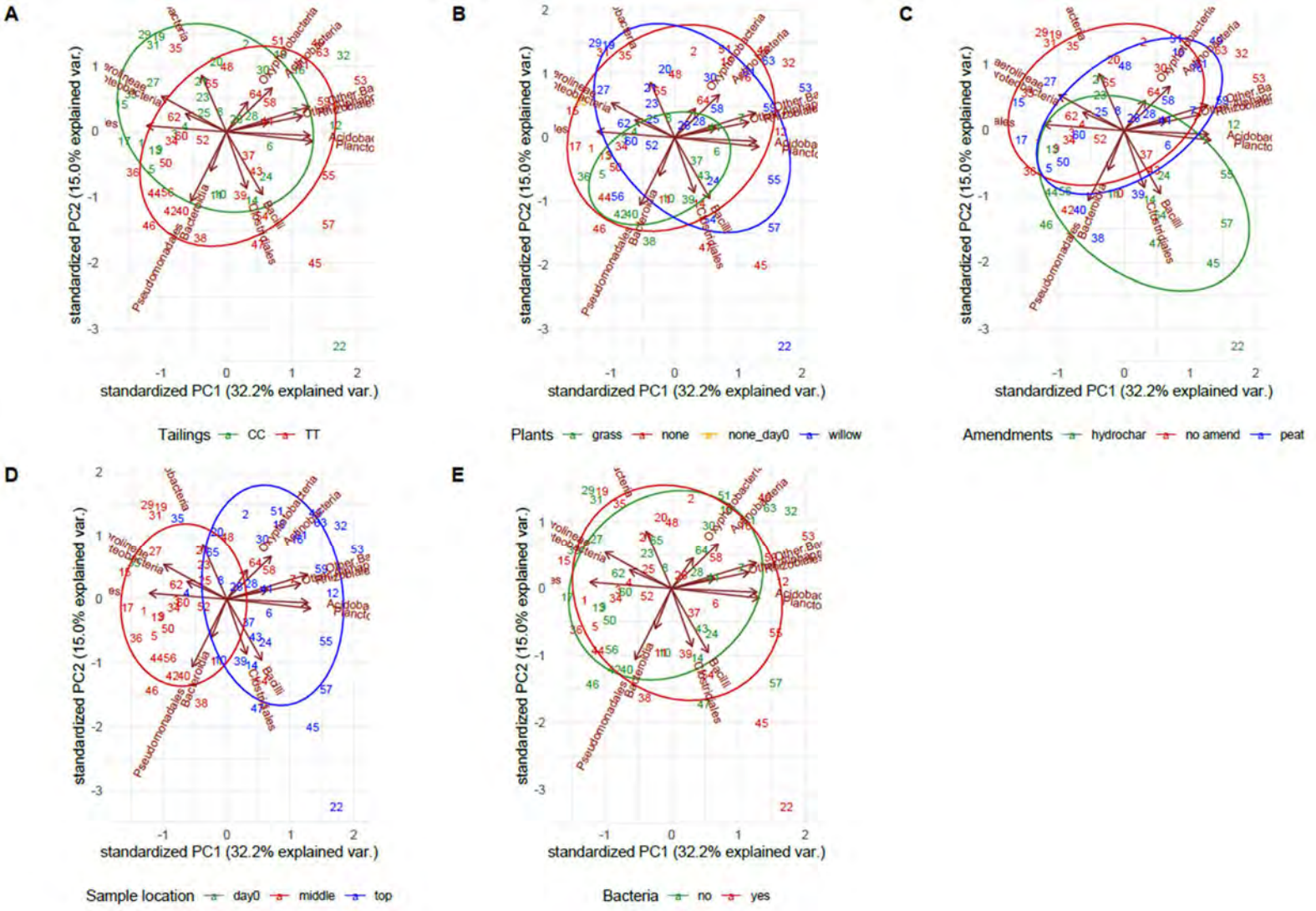
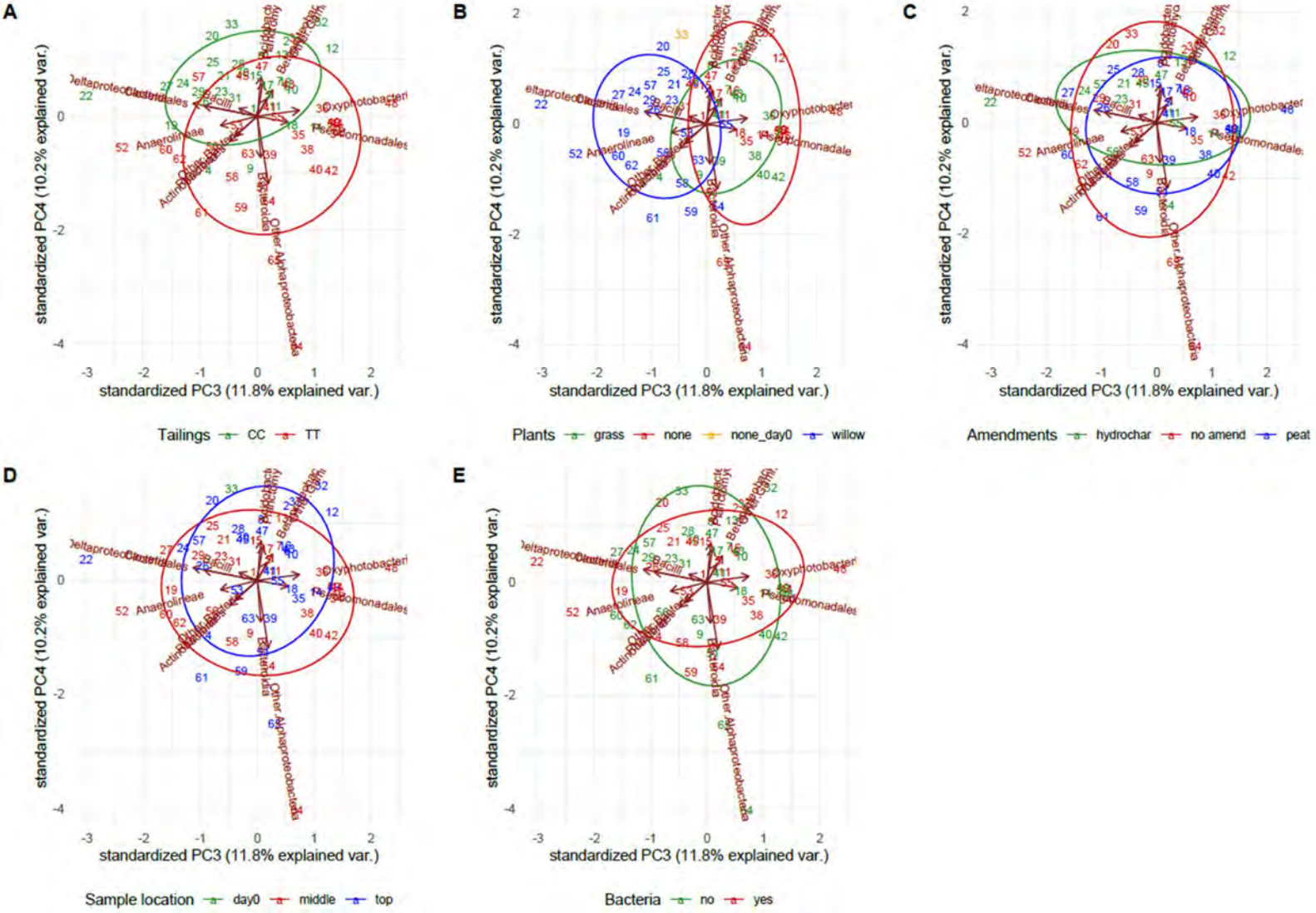


Figure 29. Principal component analysis (PCA) of Bacteria community data at Order level based on 16S rRNA sequencing of composite samples and alignment using the SILVA database. These PCA explain 11.8% (PC3) and 10.2% (PC42) of the observed variance.



9.0 APPENDIX A

Table A1. Treatment combinations for outdoor greenhouse study. Tailings type CC and TT represent centrifuge cake and thickened tailings, standard soils are forest soil and reclaimed soil. Plant species sandbar willow (*Salix interior)* and slender wheatgrass (*Elymus trachycaulus*) were used in this study. Type 1 bacteria indicates bacterial inoculum cultured on substrate developed in Phase 1, type 2 indicates mulched sandbar willow roots from willows previously grown on the respective tailings material then added to willow columns as indicated.

	Tailings Type			Plant Species			Amendment			Bacteria Treatment		
Treatment #	CC	TT	Standard soil	Grass	Willow	No plant	Peat	Hydrochar	None	Type 1	Type 2	No bacteria
1			X1	X					X			X
2			X1		X				X			X
3			X2	X					X			X
4			X2		X				X			X
5	X			X				X		X		
6	X			X				X				X
7	X			X			X			X		
8	X			X			X					X
9	X			X					X	X		
10	X			X					X			
11	X			X					X			X
12	X				X			X		X		
13	X				X			X				X
14	X				X		X			X		
15	X				X		X					X
16	X				X				X	X		
17	X				X				X		X	
18	X				X				X			X
19	X					X		X		X		
20	X					X		X				X
21	X					X	X			X		
22	X					X	X					X
23	X					X			X	X		
24	X					X			X			
25	X					X			X			X
26		X		X				X		X		
27		X		X				X				X
28		X		X			X			X		

	Tailings Type			Plant Species			Amendment			Bacteria Treatment		
Treatment #	CC	TT	Standard soil	Grass	Willow	No plant	Peat	Hydrochar	None	Type 1	Type 2	No bacteria
29		X		X			X					X
30		X		X					X	X		
31		X		X					X			
32		X		X					X			X
33		X			X			X		X		
34		X			X			X				X
35		X			X		X			X		
36		X			X		X					X
37		X			X				X	X		
38		X			X				X		X	
39		X			X				X			X
40		X				X		X		X		
41		X				X		X				X
42		X				X	X			X		
43		X				X	X					X
44		X				X			X	X		
45		X				X			X			
46		X				X			X			X

Table A2. Arithmetic mean of predicted and measured soil strength of centrifuge cake planted with *Elymus trachycaulus* (grass) and *Salix interior* (willow), and with no plants under different amendments (H+B1= hydrochar + bacteria (type 1); H= hydrochar only; P+B1= peat + bacteria (type 1); P= peat only; B1= bacteria only (type 1); N= no amendment or bacteria inoculation). Means (± SE, n = 5)

Plant species	Treatment	Centrifuge cake (CC)					
		Top (0-35 cm)		Middle (35-65 cm)		Bottom (65-100 cm)	
		Predicted (kPa)	Measured (kPa)	Predicted (kPa)	Measured (kPa)	Predicted (kPa)	Measured (kPa)
Grass	Hydrochar+bacteria (type 1)	2.30±0.39	1.67±0.27	0.478±0.10	0.00	0.47±0.03	0.00
	Hydrochar only	2.30±0.02	2.20±0.53	0.461±0.06	0.00	0.58±0.10	0.00
	Peat +bacteria (type 1)	1.40±0.77	2.80±0.72	0.574±0.13	0.58±0.58	0.48±0.06	1.67±1.67
	Peat only	1.76±0.46	2.10±1.02	0.415±0.03	0.00	0.50±0.04	0.00
	Bacteria only (type 1)	1.26±0.21	0.87±0.59	0.4020.03	0.00	0.68±0.19	0.00
	No amendment	1.54±0.16	2.40±0.61	0.891±0.44	0.00	0.53±0.02	0.00
Willow	Hydrochar+bacteria (type 1)	69.19±32.67	88.00±11.53	12.41±35.57	26.17±10.13	1.71±1.18	15.67±6.01
	Hydrochar only	25.20±23.01	57.60±29.71	4.544±2.35	8.67±4.91	0.80±0.32	8.67±4.37
	Peat +bacteria (type 1)	26.23±7.36	38.87±14.91	2.104±0.07	9.50±5.01	0.48±0.04	6.33±3.18
	Peat only	59.56±17.42	52.27±11.87	3.285±1.66	5.33±3.25	0.83±0.29	6.33±6.33
	Bacteria only (type 1)	28.87±5.66	16.70±0.90	1.245±0.66	0.25±0.20	0.60±0.07	2.00±1.63
	No amendment	19.25±11.35	34.20±4.23	1.054±0.24	5.00±2.60	0.69±0.14	3.50±2.86
No plant	Hydrochar+bacteria (type 1)	1.02±0.38	1.70±0.47	0.285±0.08	0.00	0.42±0.02	0.00
	Hydrochar only	1.33±0.23	1.27±0.70	0.389±0.03	0.00	0.46±0.03	0.00
	Peat +bacteria (type 1)	1.30±0.33	2.30±0.67	0.499±0.09	0.00	0.50±0.04	0.00
	Peat only	1.54±0.09	2.40±0.59	0.486±0.08	0.00	0.48±0.04	0.00
	Bacteria only (type 1)	1.36±0.14	31.53±30.44	0.455±0.03	5.17±5.17	0.48±0.02	2.83±2.83
	No amendment	1.33±0.23	1.35±0.46	0.403±0.03	0.00	0.47±0.02	0.00

Table A3. Arithmetic mean of predicted and measured soil strength of thickened tailings planted with *Elymus trachycaulus* (grass) and *Salix interior* (willow), and with no plants under different amendments (H+B1= hydrochar + bacteria (type 1); H= hydrochar only; P+B1= peat + bacteria (type 1); P= peat only; B1= bacteria only (type 1); N= no amendment or bacteria inoculation). Means (± SE, n = 5)

Plant species	**Treatment**	**Thickened tailings (TT)**					
		Top (0-35 cm)		Middle (35-65 cm)		Bottom (65-100 cm)	
		Predicted (kPa)	Measured (kPa)	Predicted (kPa)	Measured (kPa)	Predicted (kPa)	Measured (kPa)
Grass	Hydrochar+bacteria (type 1)	14.16±13.51	19.75±17.88	0.17±0.04	0.11±0.11	0.05±0.03	0.00
	Hydrochar only	0.83±0.05	11.00±8.96	0.12±0.01	1.33±1.33	0.10±0.02	0.00
	Peat +bacteria (type 1)	4.22±2.64	23.75±21.42	0.28±0.14	2.17±2.17	0.17±0.07	1.33±1.33
	Peat only	0.70±0.19	16.89±8.48	0.12±0.02	4.00±3.28	0.11±0.00	1.00±0.58
	Bacteria only (type 1)	2.92±1.77	23.83±12.06	0.27±0.14	3.17±1.64	0.22±0.09	1.33±1.33
	No amendment	0.74±0.09	9.69±8.37	0.14±0.03	0.00	0.10±0.01	0.00
Willow	Hydrochar+bacteria (type 1)	15.22±11.22	12.61±11.03	12.72±10.23	1.33±1.33	0.54±0.33	0.11±0.11
	Hydrochar only	5.41±2.40	14.49±13.26	0.57±0.13	0.00	0.11±0.01	0.00
	Peat +bacteria (type 1)	8.14±1.11	19.94±16.71	1.13±0.40	0.50±0.50	11.57±11.45	0.00
	Peat only	56.35±49.45	8.72±7.64	0.52±0.14	2.00±2.00	0.11±0.01	0.00
	Bacteria only (type 1)	15.08±3.57	17.94±15.81	1.19±0.51	0.67±0.67	0.61±0.50	2.33±2.33
	No amendment	35.53±0.33	12.85±11.07	1.28±0.76	0.00	0.12±0.02	0.00
No plant	Hydrochar+bacteria (type 1)	0.60±0.11	0.00	0.09±0.01	0.00	0.27±0.18	0.00
	Hydrochar only	0.95±0.19	0.17±0.17	0.22±0.10	0.00	0.11±0.02	0.00
	Peat +bacteria (type 1)	2.35±0.92	0.67±0.44	0.12±0.00	0.00	0.11±0.01	0.00
	Peat only	2.45±1.46	0.33±0.33	0.08±0.04	0.00	0.11±0.01	0.00
	Bacteria only (type 1)	1.03±0.25	0.78±0.78	0.16±0.06	0.00	0.11±0.02	0.00
	No amendment	0.64±0.17	1.25±0.93	0.35±0.24	0.00	0.09±0.03	0.00

Table A4. Summary output for linear mixed effects models, replicate blocks were treated as a random effect for tailings type (centrifuge cake and thickened tailings), amendments, and a combination of each of tailing type and amendment [shown in table as centrifuge cake (CC) or thickened tailings (TT)].

Planted species	**Source of Variation**	**Tailings (centrifuge cake and thickened tailings)**			**Amendment**		
		df	*F*	*P*	*df*	*F*	*P*
Grass	Leaf biomass	1	3.37	0.0717	5	5.65	0.0003
	Maximum height	1	26.11	<0.0001	5	19.44	<0.0001
	Leaf area index	1	0.70	0.4074	5	4.35	0.0019
	Root mass	1	0.42	0.5229	5	1.29	0.2945
	Root: leaf	1	0.20	0.6575	5	1.95	0.12
Willow	Leaf biomass	1	0.59	0.4449	6	21.45	<0.0001
	Maximum height	1	20.23	<0.0001	6	0.96	0.4598
	Leaf area index	1	12.08	0.0009	6	1.31	0.2667
	Root mass	1	0.89	0.3515	6	2.47	0.0434
	Root: leaf	1	1.00	0.3253	6	2.06	0.0877
	Total biomass	1	4.43	0.0392	6	2.82	0.0171
		Centrifuge cake (CC)			**Thickened tailings (TT)**		
		df	*F*	*p*	*df*	*F*	*P*
Grass	Top solid content	11	1.03	0.454	11	1.41	0.2354
	Middle solid content	11	1.22	0.3287	11	1.5	0.2
	Bottom solid content	11	1.07	0.4224	11	0.46	0.9103
	Evapotranspiration	11	7.75	<0.001	11	2.88	0.08
	Water use	11	7.75	<0.001	11	2.88	0.08
	Consolidation	11	1.35	0.2321	11	1.42	0.1972
	% of tailings submerged	11	2.22	0.0313	11	1.49	0.1713
Willow	Top solid content	12	17.35	<0.0001	12	5.11	0.0003
	Middle solid content	12	7.37	<0.0001	12	4.75	0.0005
	Bottom solid content	12	0.93	0.5328	12	0.99	0.4855
	Evapotranspiration	12	7.75	<0.001	12	2.88	0.08
	Water use	12	7.75	<0.001	12	2.88	0.08
	Consolidation	12	2.33	0.0187	12	10.91	0.0003
	% of tailings submerged	12	8.10	<0.0001	12	24.18	<0.0001

Table A5. Basic chemical analyses of treated tailings prior to phase 2 study. Anions nitrate, nitrite, bromide, and phosphate were below detection, sulfur was below detection in CHNS analyses. Means (± 95% confidence interval, n = 2).

Anions (mg/kg)			
	Fluoride	**Chloride**	**Sulfate**
Thickened tailings	1.9±-	17.1±0.51	456.3±15.96
Centrifuge cake	2.0±-	159.0±22.02	422.7±73.29
CHNS (Carbone, Hydrogen, Nitrogen, Sulfur; %wt)			
	Nitrogen	**Carbon**	**Hydrogen**
Thickened Tailings	0.025±0.003	0.98±0.048	0.32±0.021
Centrifuge Cake	0.041±0.004	3.68±0.15	0.68±0.03

Figure A1. Consistency of solids content during fill of columns. TT indicates the thickened tailings samples and CC indicates the centrifuge cake samples.

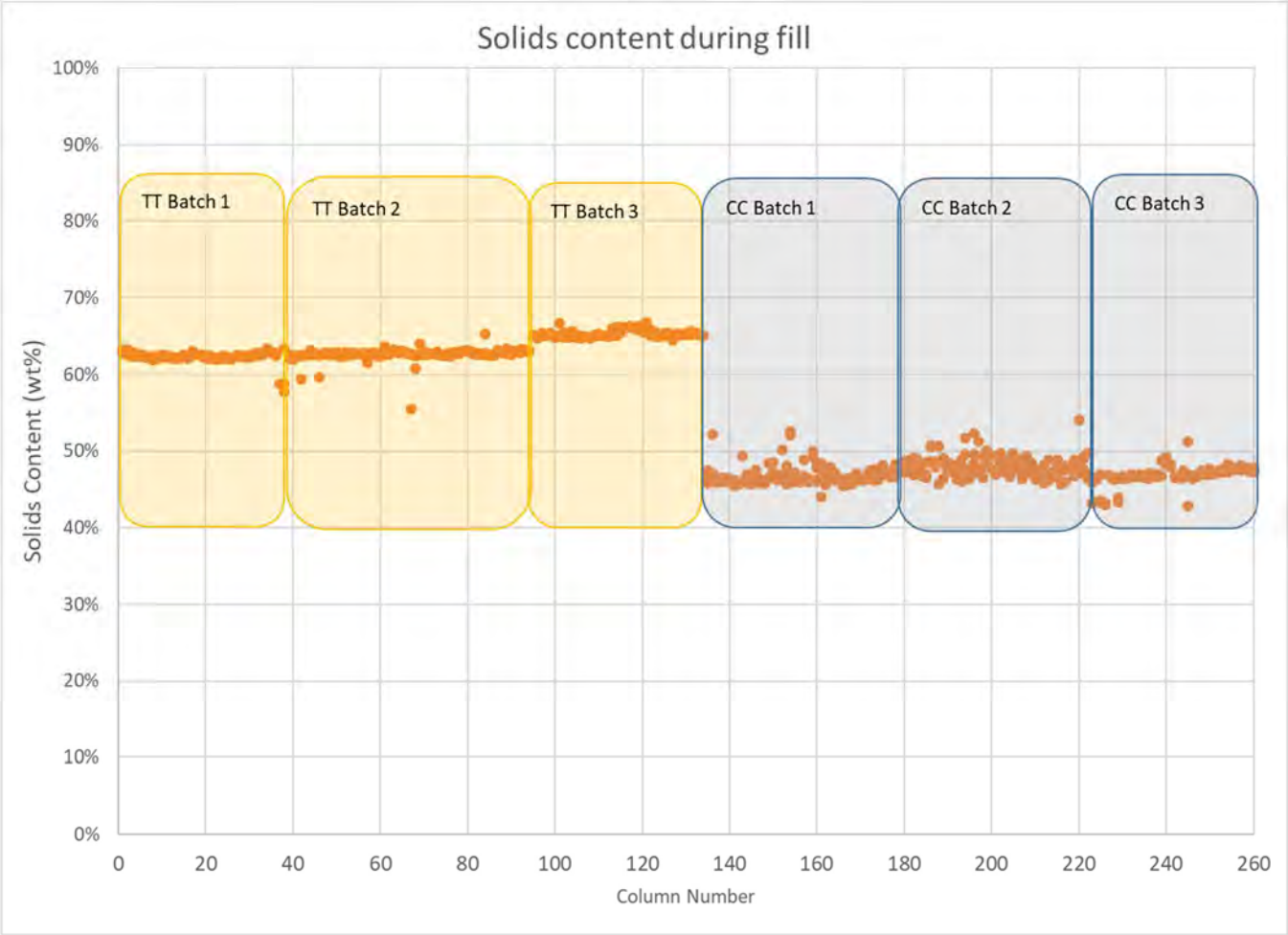


Figure A2. Particle size distribution for the three batches of thickened tailings and centrifuge cake. The thickened tailings was slightly coarser than the centrifuge cake, having a sand to fines ratio (SFR) of approximately 0.3 compared to an SFR of 0.04 for the centrifuge cake.

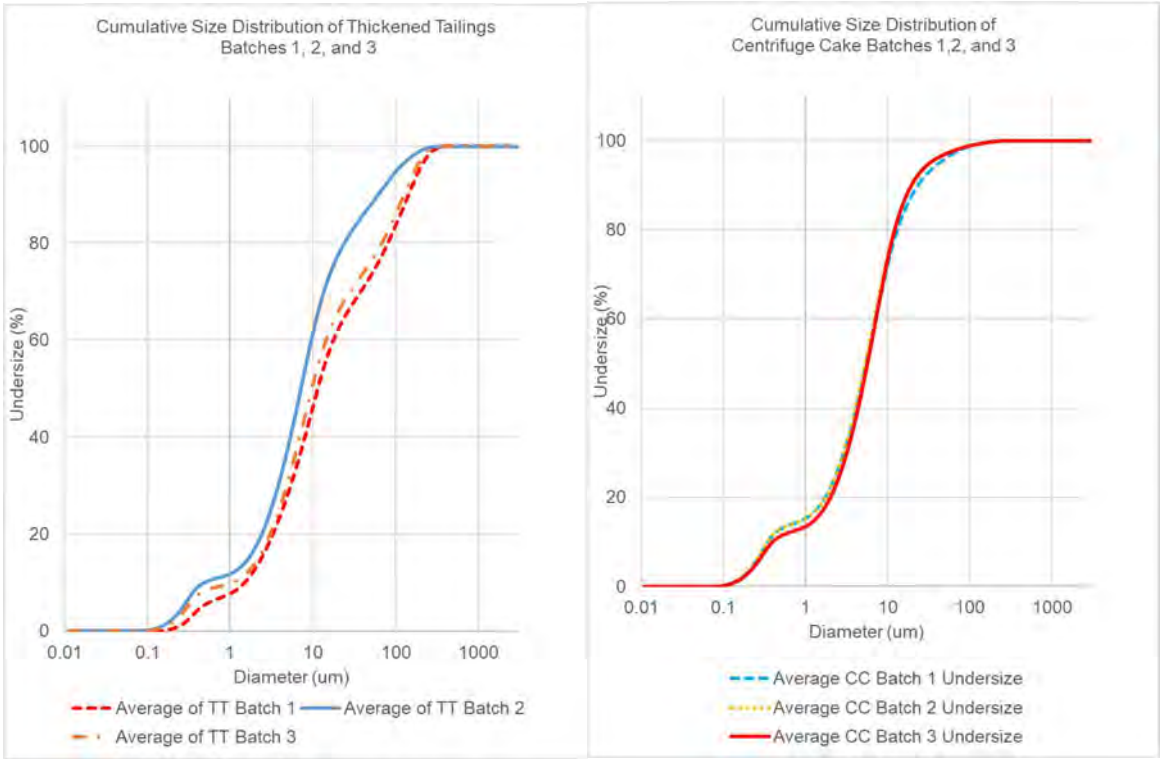


Figure A3. Difference between initial and final solids content for centrifuge cake planted with *Elymus trachycaulus* (grass) and *Salix interior* (willow), and with no plants under different amendments (H+B1= hydrochar + bacteria (type 1); H= hydrochar only; P+B1= peat + bacteria (type 1); P= peat only; B1= bacteria only (type 1); B2= bacteria only (type 2); N= no amendment or bacteria inoculation). Means (± SE, n = 4) followed by different letter(s) are significantly different ($p < 0.05$) from each other among amendments within the same tailing type.

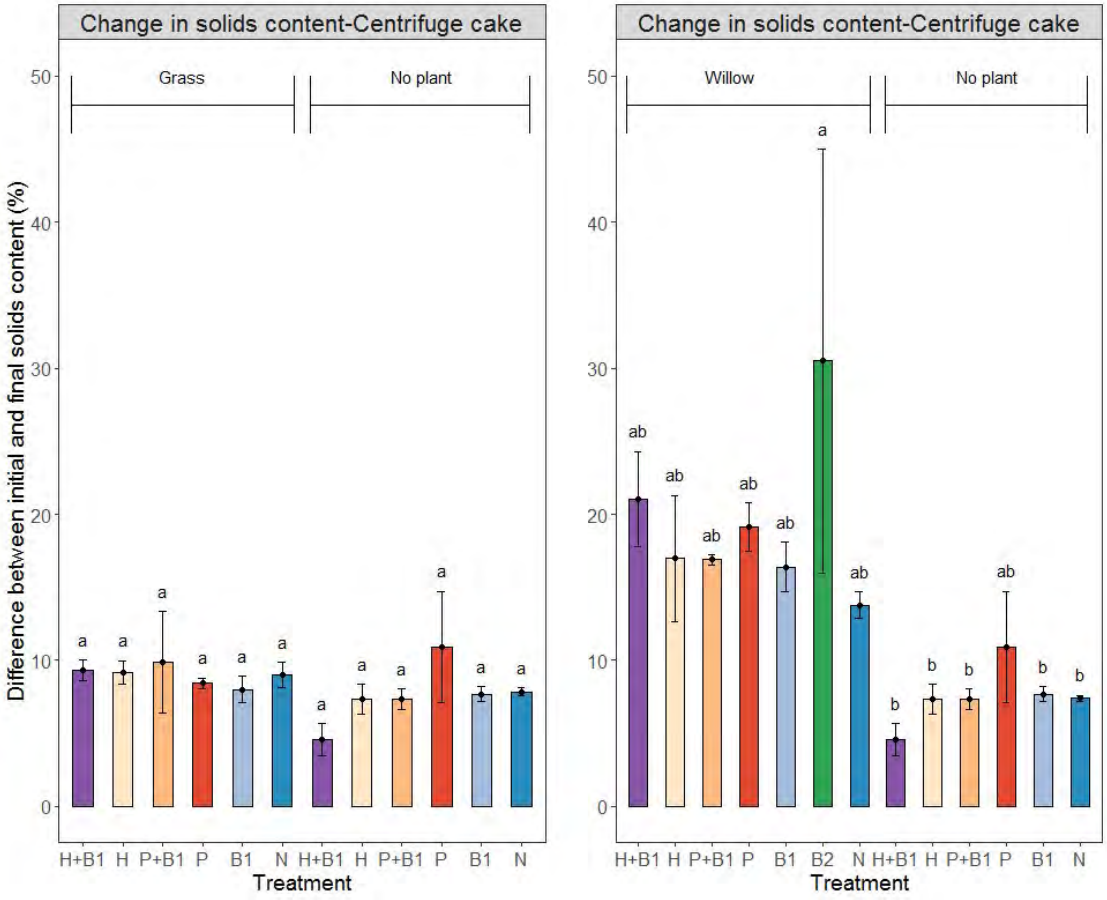


Figure A4. Difference between initial and final solids content for thickened tailings planted with *Elymus trachycaulus* (grass) and *Salix interior* (willow), and with no plants under different amendments (H+B1= hydrochar + bacteria (type 1); H= hydrochar only; P+B1= peat + bacteria (type 1); P= peat only; B1= bacteria only (type 1); B2= bacteria only (type 2); N= no amendment or bacteria inoculation). Means (± SE, n = 4) followed by different letter(s) are significantly different ($p < 0.05$) from each other among amendments within the same tailing type.

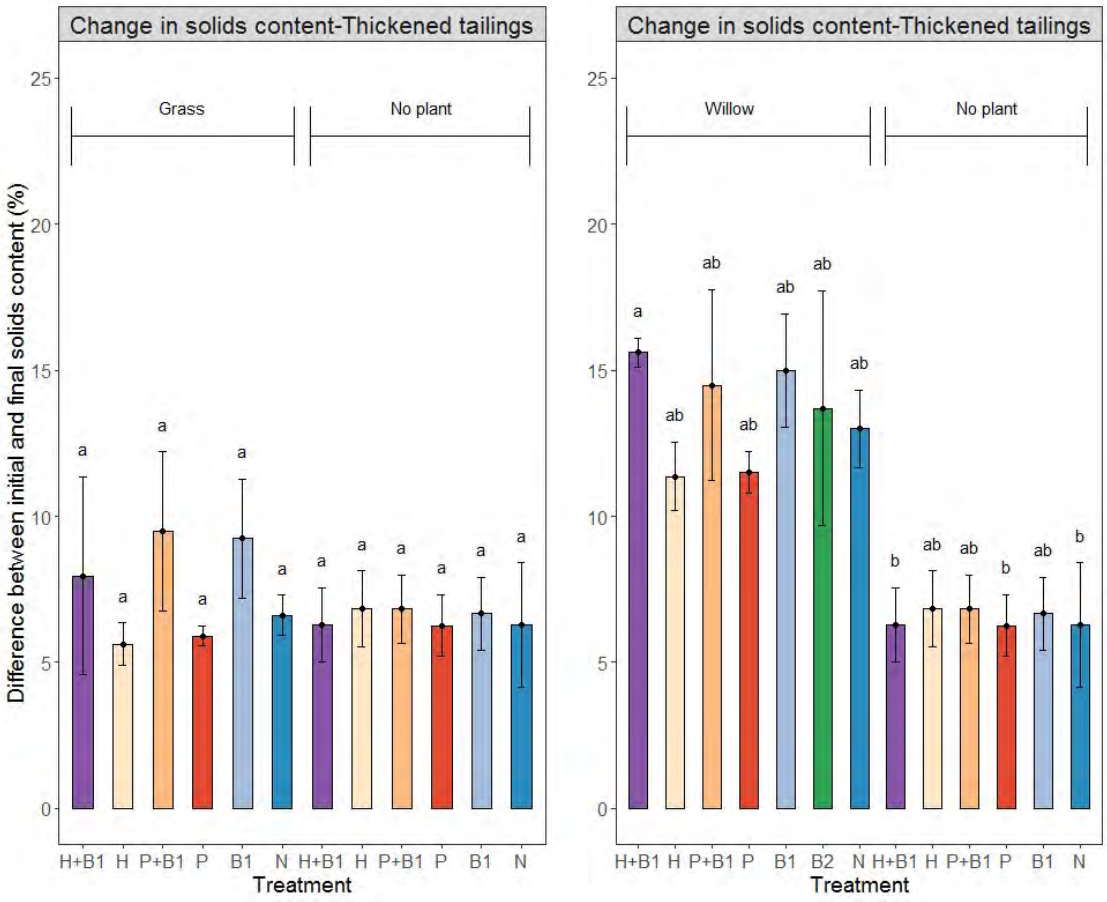


Figure A5. Community composition indicating percentage of Bacteria based on 16S rRNA sequencing of composite samples and alignment using the SILVA database. **CC** represent centrifuge cake, **W** and **G** indicate willow or grass, **t** and **m** refer to the top and middle of the column, and **H**, **B**, and **P** indicates hydrochar, bacteria type 1 inoculum from Phase 1, and peat amendments, respectively. D0_CC indicates centrifuge cake at day 0. Reads present at less than 2% of the population were grouped as Other Bacteria.

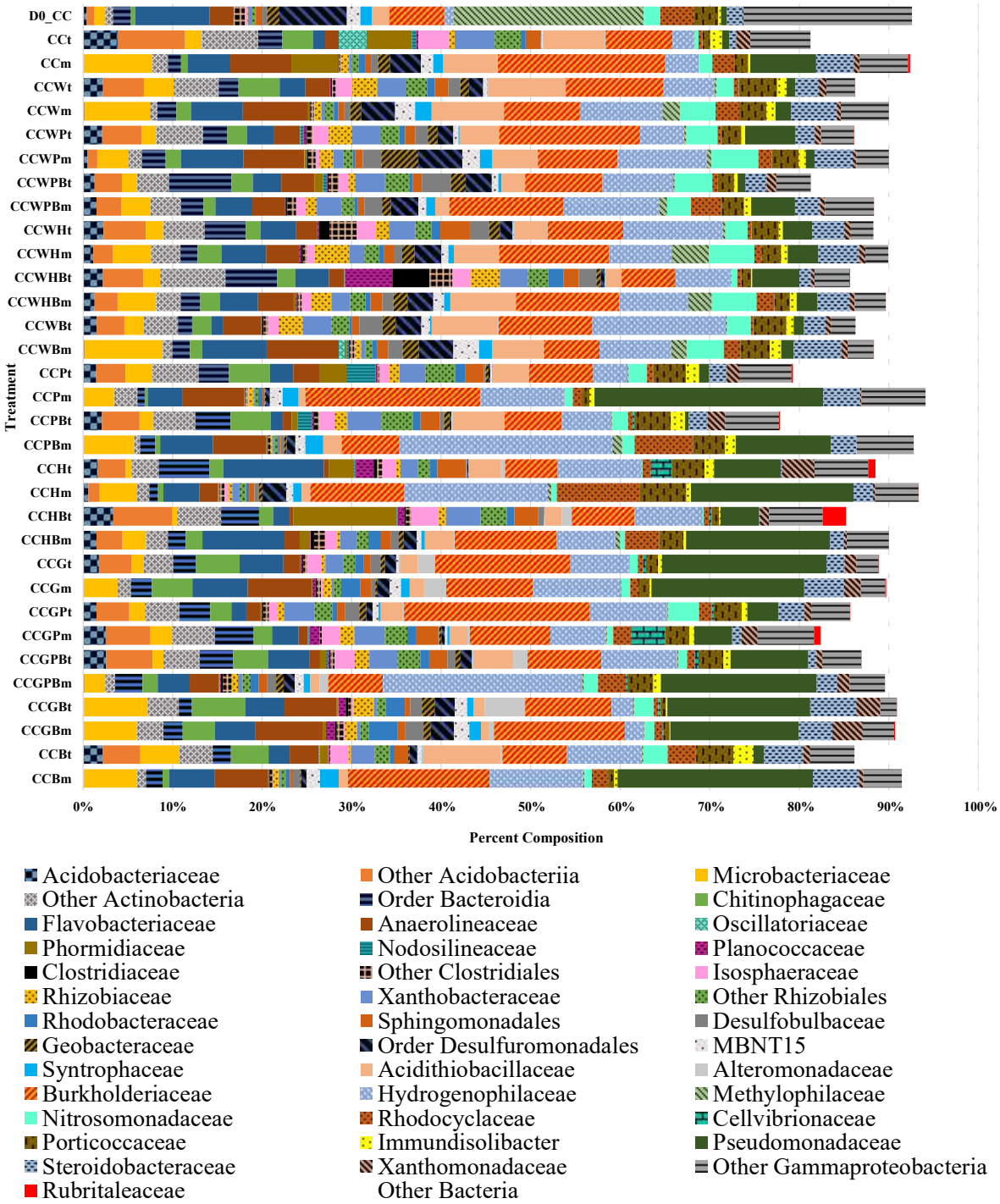


Figure A6. Community composition indicating percentage of Bacteria based on 16S rRNA sequencing of composite samples and alignment using the SILVA database. **TT** represent thickened tailings, **W** and **G** indicate willow or grass, **t** and **m** refer to the top and middle of the column, and **H**, **B**, and **P** indicates hydrochar, bacteria type 1 inoculum from Phase 1, and peat amendments, respectively. Reads present at less than 2% of the population were grouped as Other Bacteria.

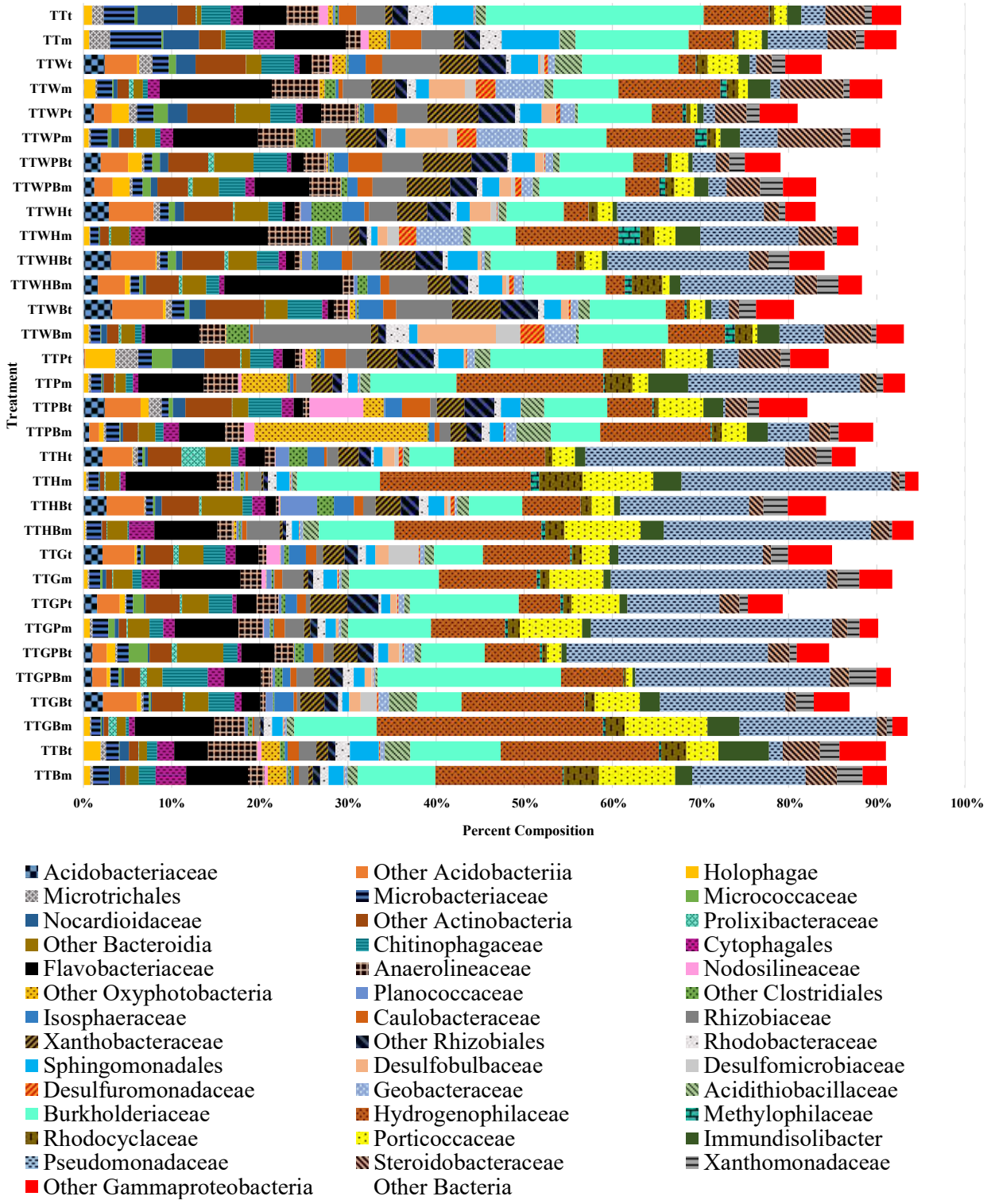
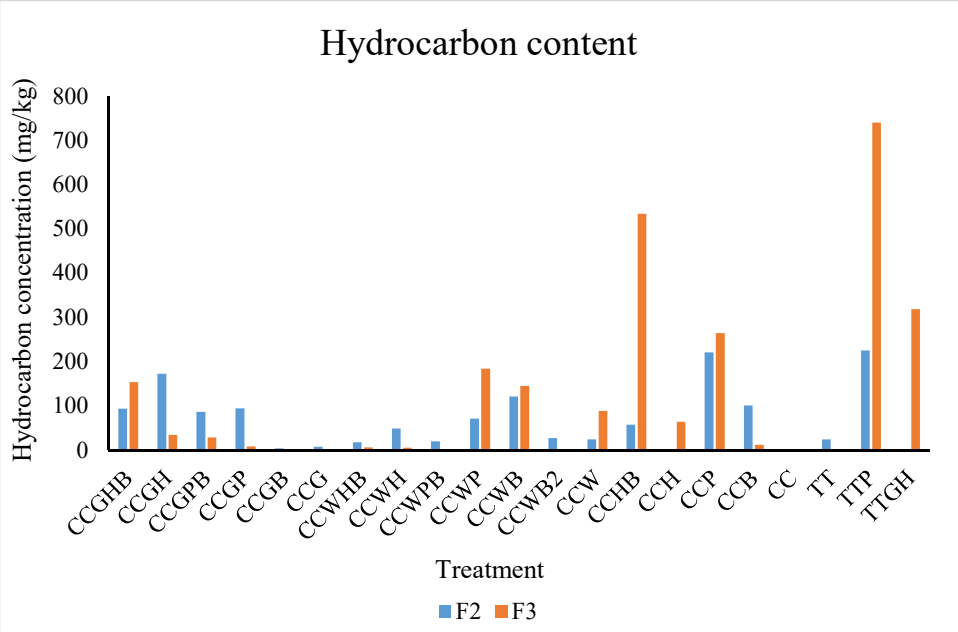


Figure A7. Hydrocarbon concentrations of F2 and F3 fractions in Phase 2 columns. **CC** and **TT** represent centrifuge cake and thickened tailings, respectively. **W** and **G** indicate willow or grass, and **H**, **B**, and **P** indicates hydrochar, bacteria type 1 inoculum from Phase 1, and peat amendments, respectively. **B2** indicates treatment with mulched willow roots. Samples where hydrocarbons were below detection are not shown. Hydrocarbon extractions were performed on single samples from each treatment.



10.0 APPENDIX B

Hydrochar rate trial.

Context for study

In the bugs and veggies trial, fixed concentration of 200 mg L^{-1} hydrochar was applied to each column where each column contains approximately 7.8 L of tailings. We observed mortality and reduced growth in many of the columns containing this hydrochar, hence there was a need to test different application rates of hydrochar in tailings to determine optimum application rates.

The overall objective of this trial is to test increasing application rates of hydrochar incorporated into oil sands mine tailings to determine optimum application rates for plant establishment. It will further evaluate the use of hydrochar over a larger range of application rates in order to better understand toxicity thresholds and to identify optimum concentration of hydrochar required.

Experimental design and set up

The experimental design was set-up where plants were grown in 1 L clear-plastic containers, in the greenhouse from July – August 2018. This study utilized the following treatments:

1. **Species:** native grass (*Elymus trachycaulus*) and shrub-*Salix interior* (willow). These species are same species used in the bugs and veggies study.
2. **Amendment:** hydrochar (also known as the carbonaceous product of wet pyrolysis, produced from meat and bone meal feedstock). Hydrochar was applied at control (0%), 0.05%, 0.1%, 0.2%, 0.5%, and 1% of total material weight.

Each species was established in centrifuge cake (CC) from Albian mine. Four replicates of each treatment combination were evaluated resulting in a total of 48 plastic containers used in the experiment. Prior to filling containers, tailings was homogenized to create a consistent initial solids content. Clear 1 L containers were filled with centrifuge cake (CC) and following filling, cuttings of *Salix* were hand planted, and *Elymus trachycaulus* was hand seeded into the plastic containers. 0.24 grams of Urea (46-0-0) fertilizer was sprinkled on the substrate surface of vegetation columns as these tailings were deficient in nitrogen.

Plant measurements

At the completion of study, plants were harvested with hand clippers in order to remove the aboveground biomass from the CC surface and oven dried at 70˚C for 48 hours or until constant weight. Aboveground plant biomass was determined to the nearest 0.1 g.

Statistical analysis

Effects of different hydrochar application on the aboveground biomass of either (i) *Elymus trachycaulus*, or (ii) *Salix interior* were analyzed using the statistical program R 3.4.1 (R Core Team 2018). Analysis of variance was performed by fitting linear-mixed effect models, with the aov() function. Replicates were treated as a random effect. Tukey adjusted multiple mean comparison test was used to identify and separate significant treatment effects at $P < 0.05$. The posthoc analysis and the calculation of least squares means was completed using the *lsmeans* package (Lenth, 2018).

Results and Discussion

The biomass of *Elymus trachycaulus* in control treatment was similar to the biomass in tailings amended with 0.05 or 0.1% w/w of hydrochar (**Figure B1**). We observed a significant decline in shoot biomass of *Elymus trachycaulus* when hydrochar was applied at 0.2 to 0.5% w/w of tailings (α=0.00003). At 1% w/w hydrochar/tailings, there was complete plant mortality (this was not represented in the plot (α=0.00065).

Similarly, *Salix* biomass declined with increasing concentration of hydrochar (**Figure B1**). Up to 0.2% application rate of hydrochar did not affect willow biomass, however, at 1%w/w, there was a significant decline in willow biomass.

These results suggest that *Elymus trachycaulus* when hand seeded in tailings amended with up to 0.1% w/w of hydrochar can successfully establish in tailings, whereas *Salix* (cutting) can successfully grow in up to 0.2% w/w of hydrochar amended tailings.

Figure B1. Mean aboveground biomass of *Elymus trachycaulus* (grass) and *Salix interior* (willow), planted in centrifuge cake amended with different application rates of hydrochar (0, 0.05, 0.1, 0.2, 0.5, and 1% w/w hydrochar/tailings). Means (± SE, n = 4) followed by different letter(s) are significantly different ($p < 0.05$) from each other among different application rates.

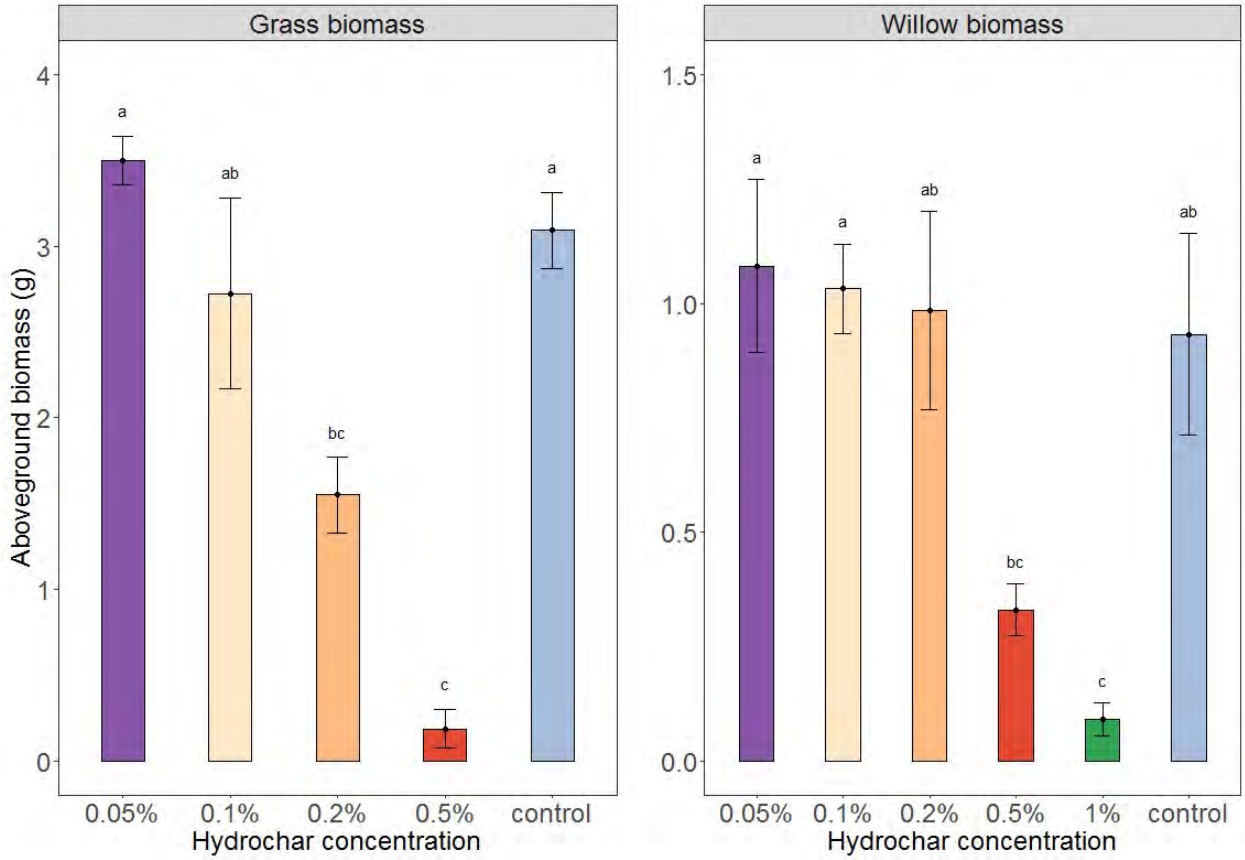
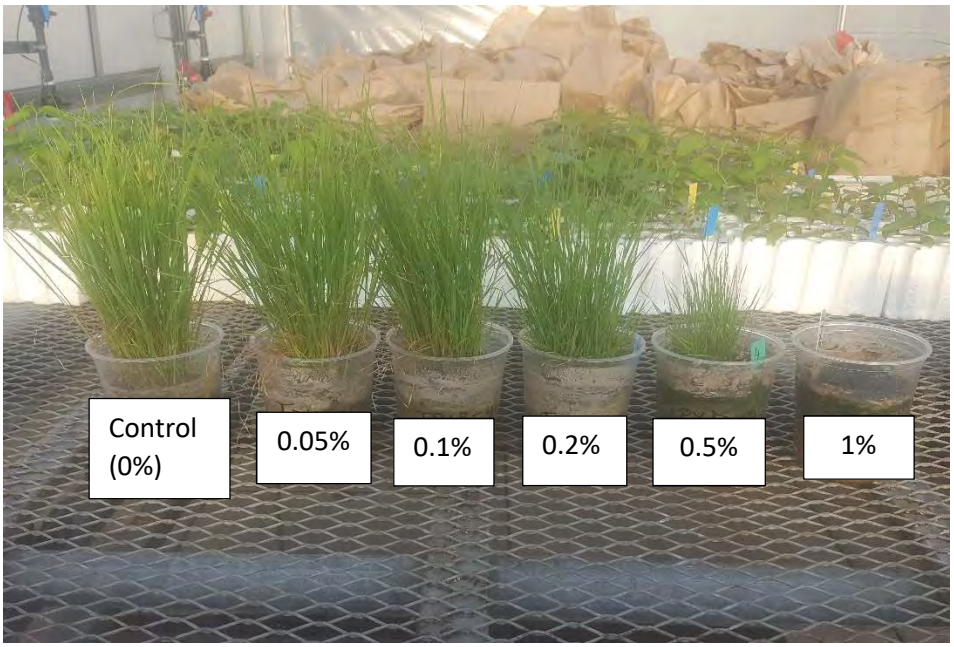
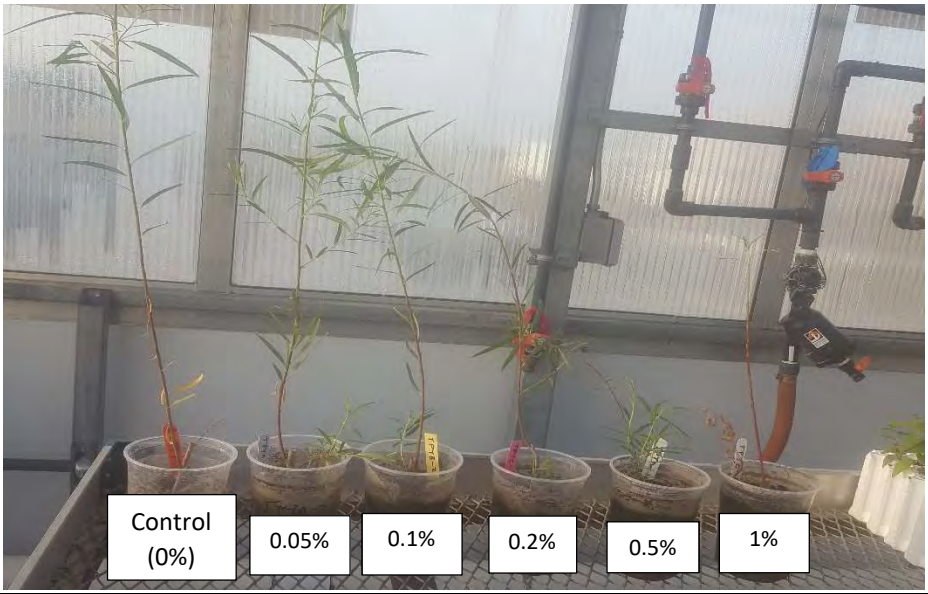


Figure B2. Photos from August 28, 2018 of **(a)** *Elymus trachycaulus* (grass) vegetation and **(b)** *Salix interior* (willow) grown in centrifuge cake.

(a)



(b)



11.0 APPENDIX C

Peat rate trial.

Context for study

Peat was identified by the industry stewards as a readily available organic amendment that could be utilized to aid in plant establishment on soft tailings deposits. Prior to incorporating this treatment in the main column study, we conducted a short pilot study in order to confirm potential rates of application that could show benefit to plants.

The rationale behind the rates selected was to pseudo-emulate two land application rates of 10 T ha^{-1} and 50 T ha^{-1} of peat. As these rates were applied on an area basis in a column (10 cm diameter), it was further assumed that there would be more downward root growth (rather than lateral growth) so these rates were therefore doubled to account for this difference in root growth pattern.

Experimental design and set up

The experimental design was set-up where plants were grown in 1 L clear-plastic containers filled with centrifuge treated tailings (Albian mine), in the greenhouse from March – April 2018. This study utilized the following treatments:

1. Species: native grass (*Elymus trachycaulus*), this was one of two species used in the main column study.
2. Amendment: Peat was applied at a rate of 0g (control), 16 g, and 80 g per container.
3. Bacteria: Root rescue™ (an endo/ecto blend of fungi species [*Glomus, Gigaspora, Rhizopogon, Pisolithus, Laccaria, and Suillus*]) was applied at a rate of 0.6 g per container.

Three replicates of each treatment combination were evaluated resulting in a total of 18 containers used in the experiment. Prior to filling containers, the tailings were homogenized to create a consistent initial solids content. Clear 1 L containers were filled with centrifuge cake (CC) and following filling, *Elymus trachycaulus* was hand seeded into the plastic containers. 0.43 g of fertilizer (containing the following elements [kg ha^{-1}]: N=29.3; phosphoric acid=58.5; potash=13.5; sulfur=2.6; magnesium=1.4; calcium=1.8; iron=2.3; zinc=0.5 and organic matter =33.8) and 0.29 g of urea were sprinkled on the substrate surface of the containers after seedling emergence (~5 days after sowing). Five plants per container were grown (all other germinant were thinned out) for 33 days. Containers were watered as required to ensure sufficient moisture for plant growth.

Plant measurements

At the completion of the study, the aboveground materials were harvested with hand clippers from the CC surface and oven dried at 70˚C for 48 hours or until constant weight. Aboveground plant biomass was determined to the nearest 0.1 g.

Statistical analysis

Effects of different peat application on the aboveground biomass of *Elymus trachycaulus* were analyzed using the statistical program R 3.4.1 (R Core Team 2018). Analysis of variance was performed by fitting linear-mixed effect models, with the aov() function. Replicates were treated as a random effect. Tukey adjusted multiple mean comparison test was used to identify and separate significant treatment effects at $P < 0.05$. The posthoc analysis and the calculation of least squares means was completed using the lsmeans package (Lenth, 2018).

Results and Discussion

Different application rate of peat did not affect ($\alpha=0.120$) the biomass of *Elymus trachycaulus* (**Figure C1**). The application of fertilizer significantly enhanced ($\alpha<0.0001$) the biomass of *Elymus trachycaulus* by over 100% in each peat application rate, including the control treatments. Application of bacteria did not affect the biomass of *Elymus trachycaulus* in all the tested peat application rates ($\alpha=0.1062$)

These results suggest that *Elymus trachycaulus* when hand seeded in tailings amended with up to 80 g of peat can successfully establish in tailings. This study also clearly showed the importance of inorganic fertilization of these tailings as previous characterization has demonstrated they are N deficient.

Figure C1. Mean aboveground biomass of *Elymus trachycaulus* (grass) planted in centrifuge cake amended with different application rates of peat (0, 16, and 80g), with and without the application of fertilizer. Means (± SE, n = 4) followed by different letter(s) are significantly different ($p < 0.05$) from each other among different application rates.

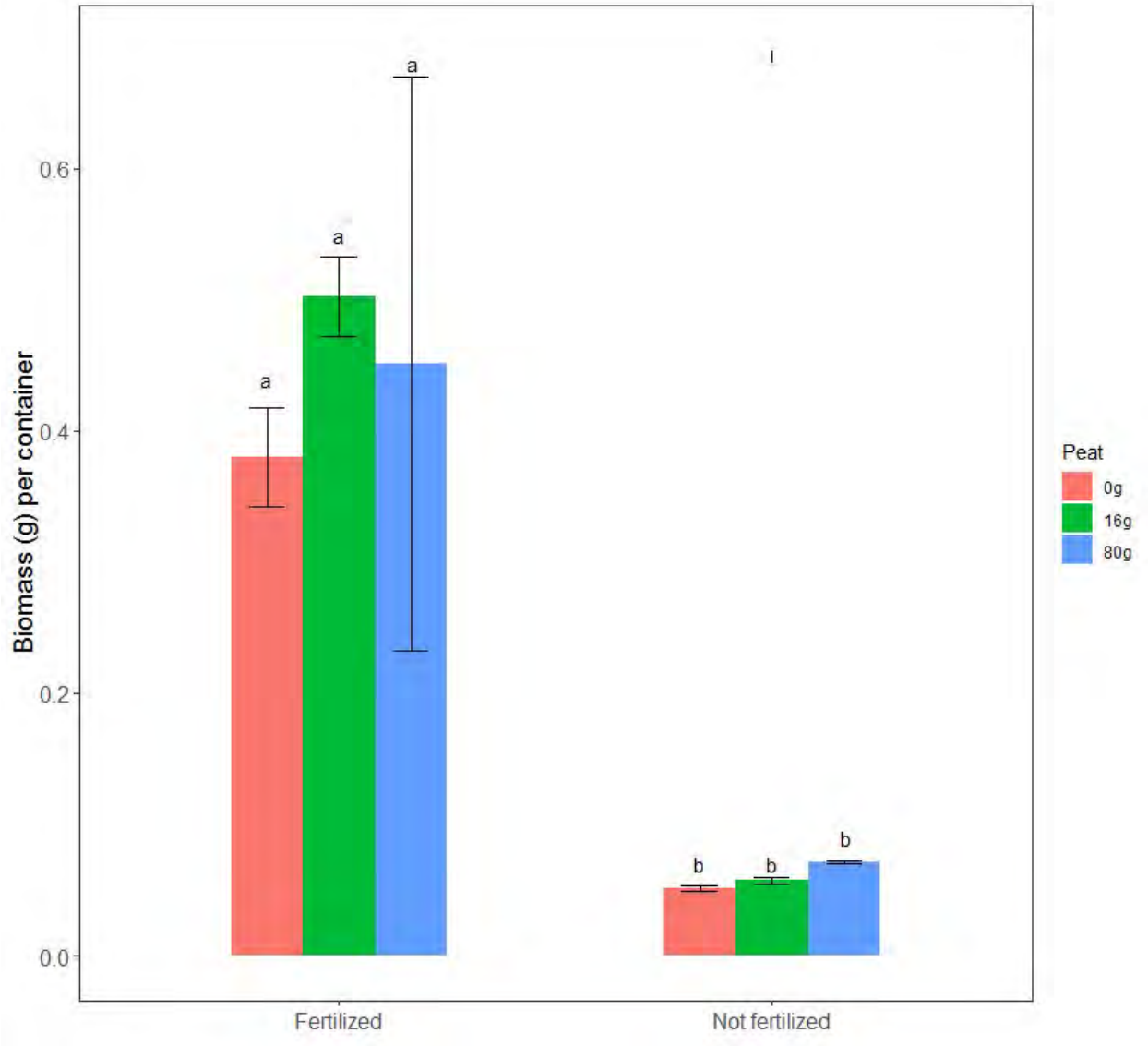


Figure C2. Photos from April 16, 2018 of *Elymus trachycaulus* (grass) vegetation planted in tailings amended with **(a)** 80 g of peat and fertilizer [left], and 16 g of peat, fertilizer and bacteria [right] and, **(b)** 80 g of peat and fertilizer [left] and 80 g of peat without fertilizer [right].

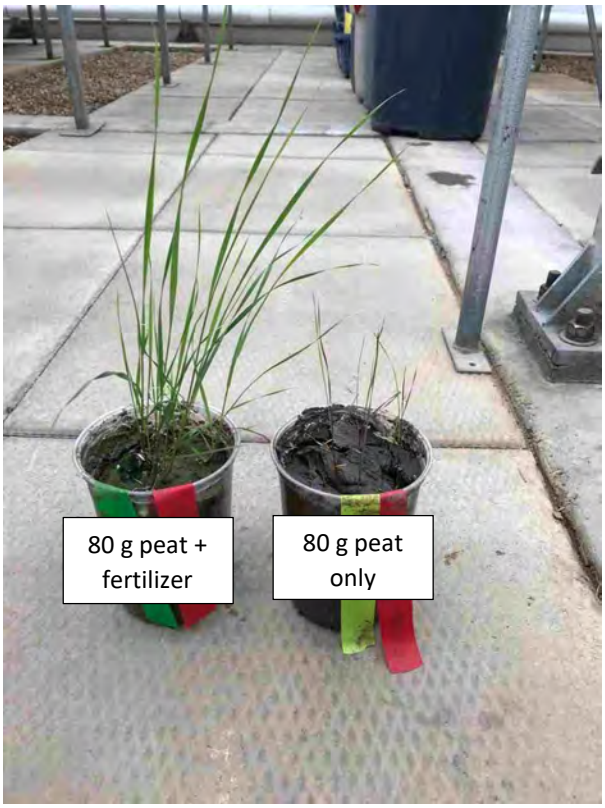
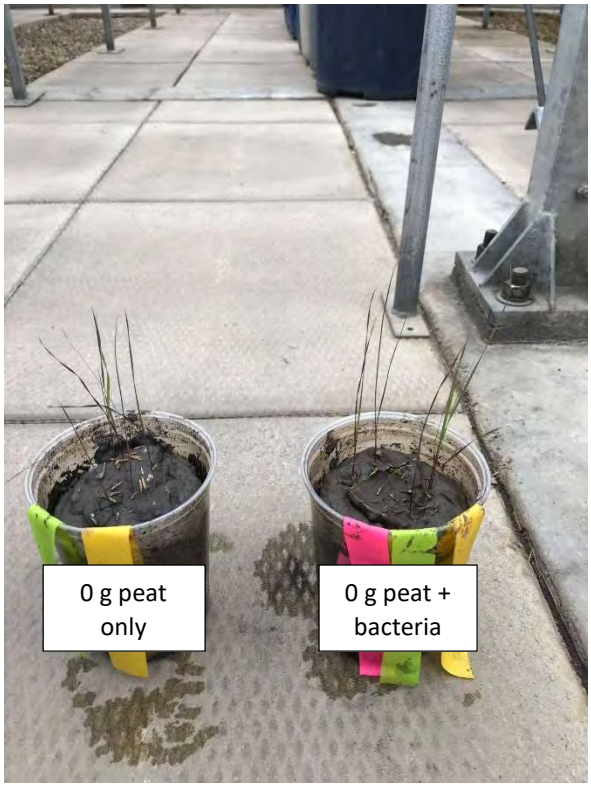
(a)	**(b)**
	

Figure C3. Photos from April 16, 2018 of *Elymus trachycaulus* (grass) vegetation planted in tailings amended with **(a)** 0 g of peat without fertilizer [left], and 0 g of peat, with bacteria [right].

(a)



12.0 APPENDIX D
Photograph panel.

Figure D1. Photos of *Salix interior* vegetation columns grown in thickened tailings amended with hydrochar and bacteria [type 1] (a-f) from September 6, 2018

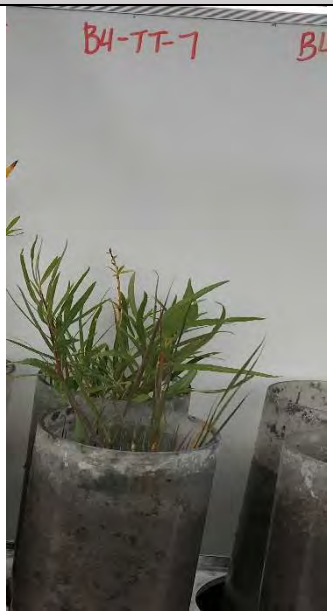
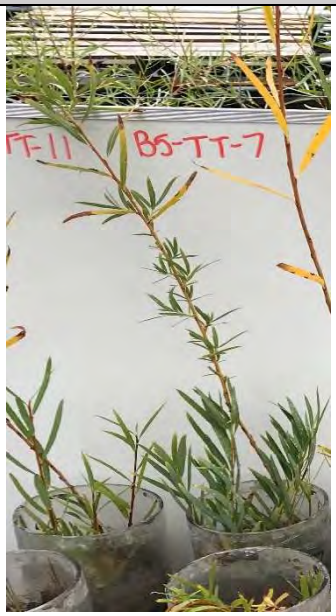
(a)	(b)	(c)
		
(d)	**(e)**	**(f)**
		

Figure D2. Photos of *Salix interior* vegetation columns grown in thickened tailings amended with hydrochar only (a-f) from September 6, 2018

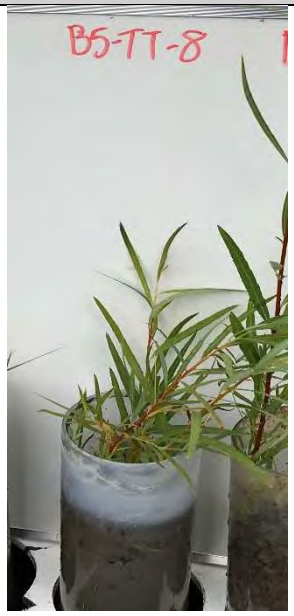
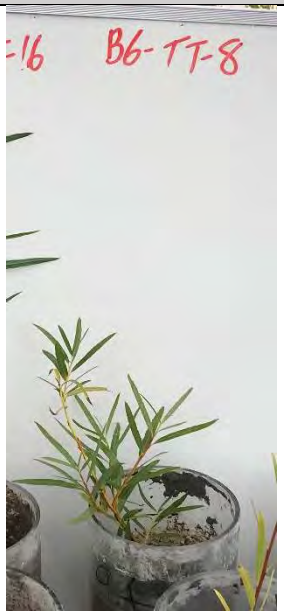
(a)	(b)	(c)
		
(d)	**(e)**	**(f)**
		

Figure D3. Photos of *Salix interior* vegetation columns grown in thickened tailings amended with peat and bacteria [type 1] (a-f) from September 6, 2018

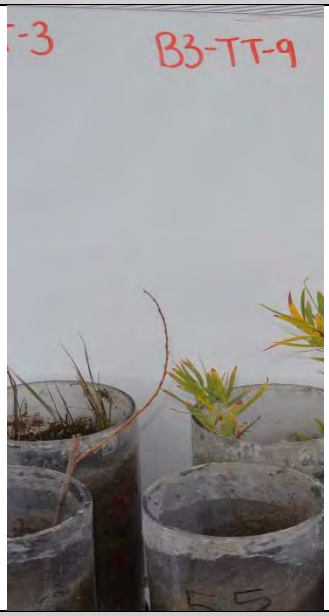
(a)	(b)	(c)
		
(d)	**(e)**	**(f)**
		

Figure D4. Photos of *Salix interior* vegetation columns grown in thickened tailings amended with peat only (a-f) from September 6, 2018

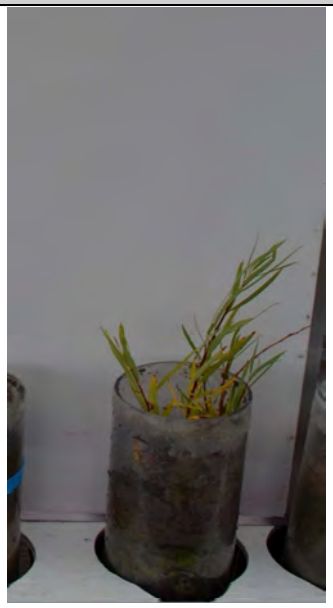
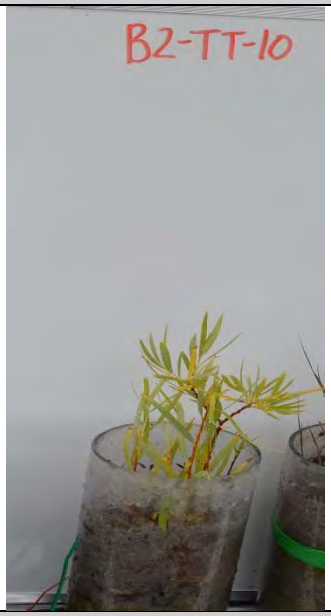
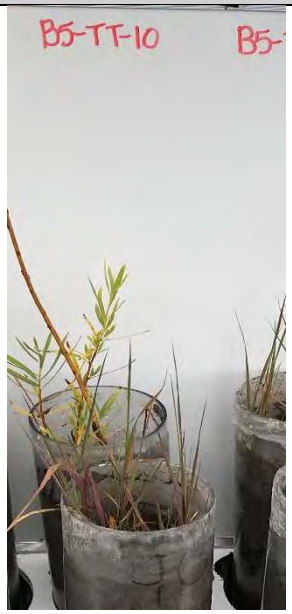
(a)	(b)	(c)
		
(d)	**(e)**	**(f)**
		

Figure D5. Photos of *Salix interior* vegetation columns grown in thickened tailings amended with bacteria only [type 1] (a-f) from September 6, 2018

(a)	(b)	(c)
		
(d)	**(e)**	**(f)**
		

Figure D6. Photos of *Salix interior* vegetation columns grown in thickened tailings amended with bacteria only [type 2] (a-f) from September 6, 2018

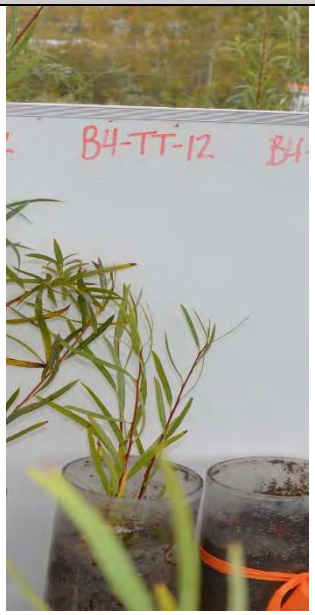
(a)	(b)	(c)
		
(d)	**(e)**	**(f)**
		

Figure D7. Photos of *Salix interior* vegetation columns grown in thickened tailings without amendment (a-f) from September 6, 2018

(a)	(b)	(c)
		
(d)	**(e)**	**(f)**
		

Figure D8. Photos of *Elymus trachycaulus* vegetation columns grown in thickened tailings amended with hydrochar and bacteria [type 1] (a-f) from September 6, 2018

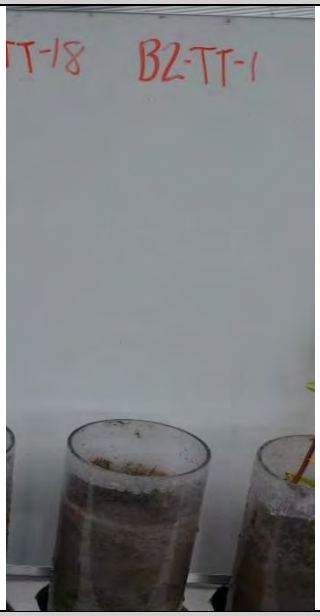
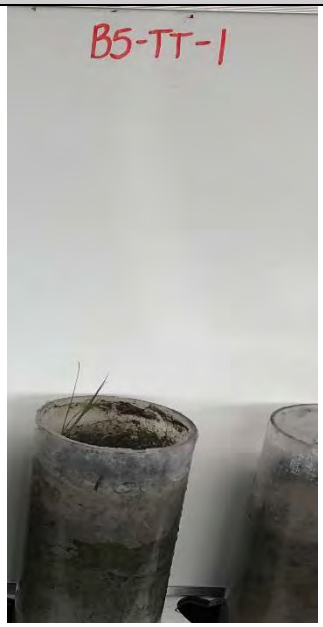
(a)	(b)	(c)
		
(d)	**(e)**	**(f)**
		

Figure D9. Photos of *Elymus trachycaulus* vegetation columns grown in thickened tailings amended with hydrochar only (a-f) from September 6, 2018

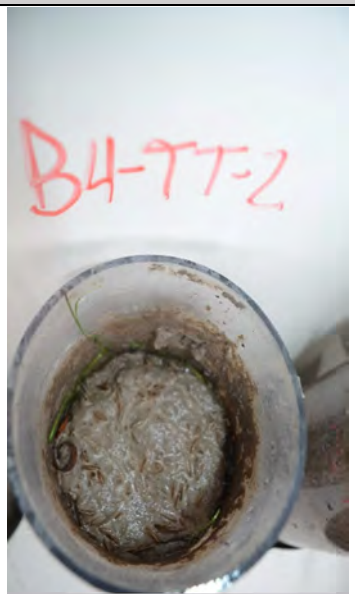
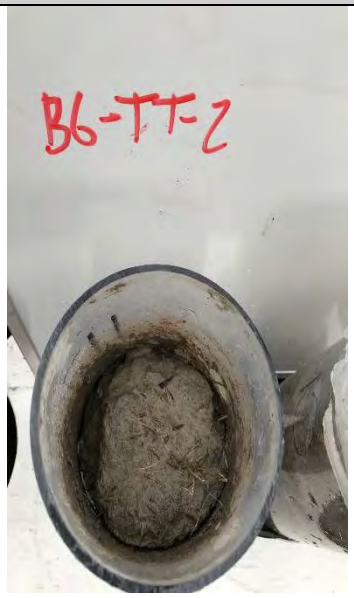
(a)	(b)	(c)
		
(d)	**(e)**	**(f)**
		

Figure D10. Photos of *Elymus trachycaulus* vegetation columns grown in thickened tailings amended with peat and bacteria [type 1] (a-f) from September 6, 2018

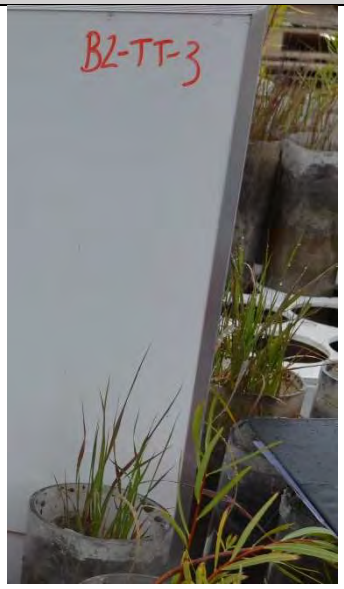
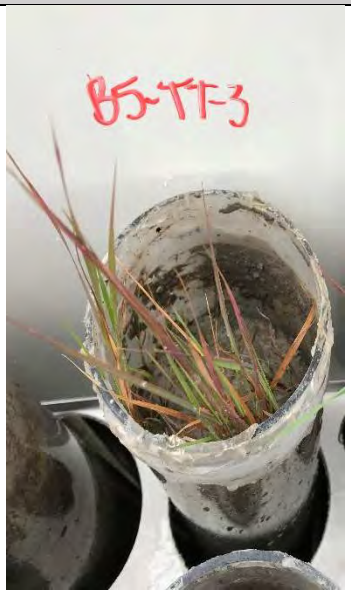
(a)	(b)	(c)
		
(d)	**(e)**	**(f)**
		

Figure D11. Photos of *Elymus trachycaulus* vegetation columns grown in thickened tailings amended with peat only (a-f) from September 6, 2018

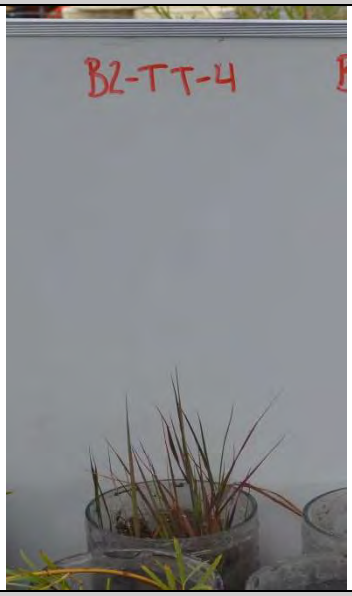
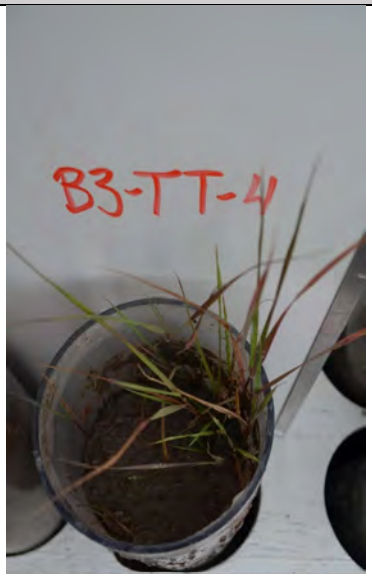
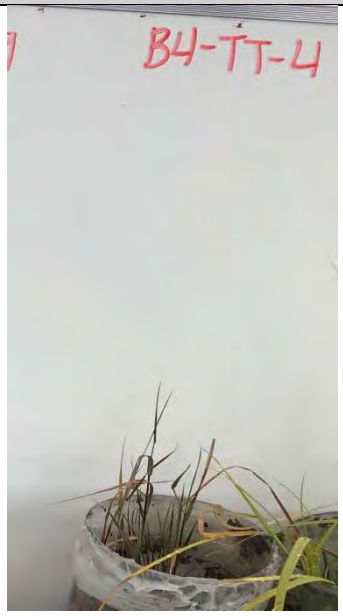
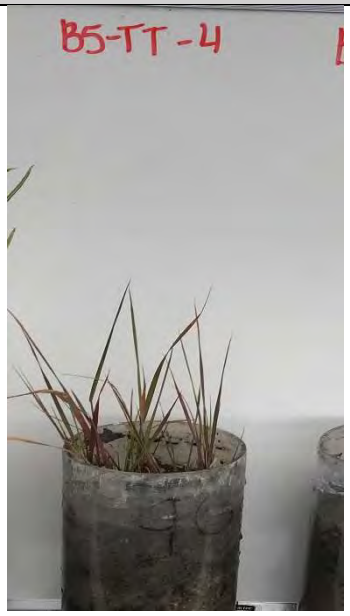
(a)	(b)	(c)
		
(d)	**(e)**	**(f)**
		

Figure D12. Photos of *Elymus trachycaulus* vegetation columns grown in thickened tailings amended with bacteria [type 1] only (a-f) from September 6, 2018

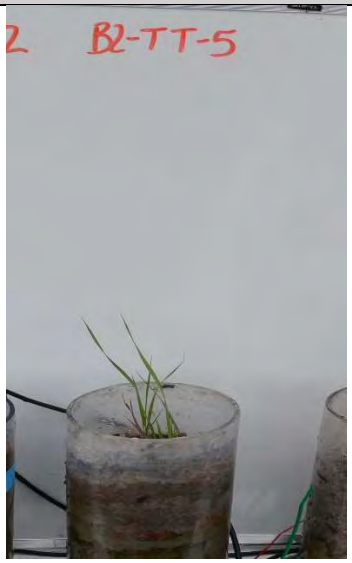
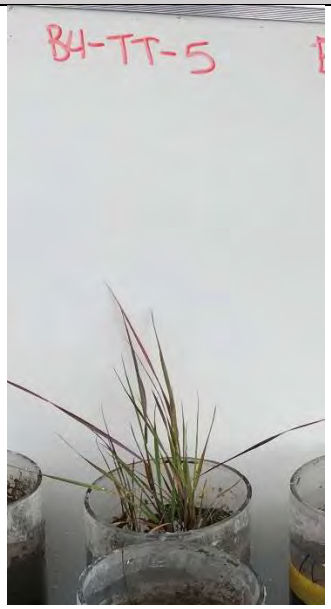
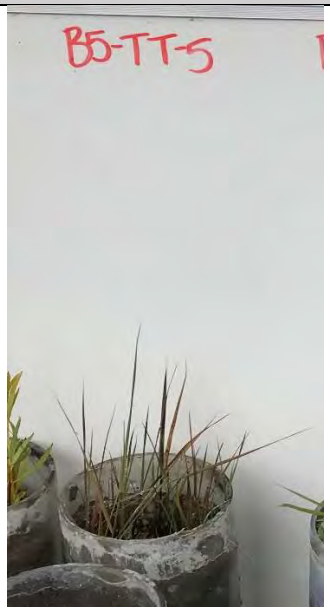
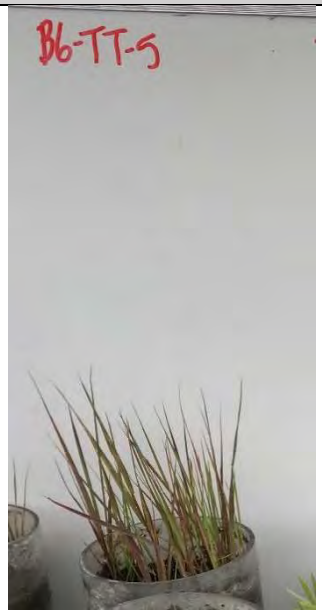
(a)	(b)	(c)
		
(d)	**(e)**	**(f)**
		

Figure D13. Photos of *Elymus trachycaulus* vegetation columns grown in thickened tailings without amendment (a-f) from September 6, 2018

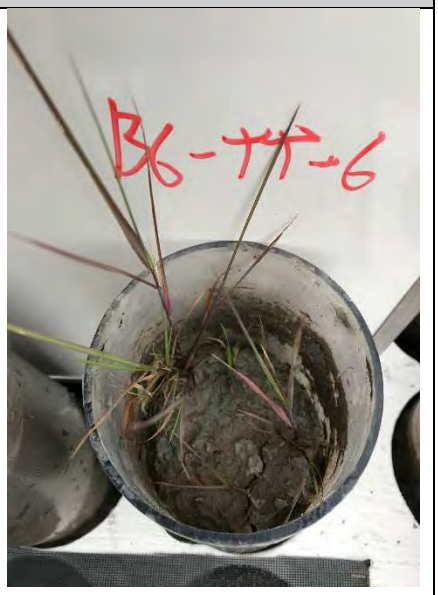
(a)	(b)	(c)
		
(d)	**(e)**	**(f)**
		

Figure D14. Photos of *Salix interior* vegetation columns grown in centrifuge cake amended with hydrochar and bacteria [type 1] (a-f) from September 6, 2018

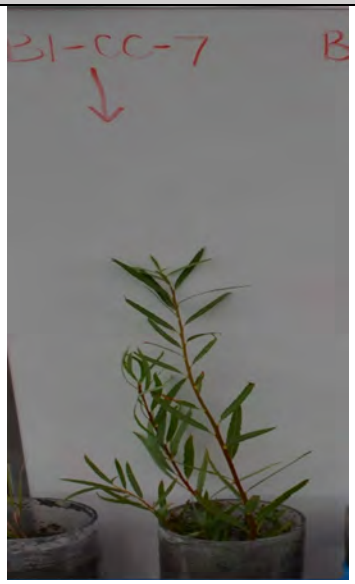
(a)	(b)	(c)
		
(d)	**(e)**	**(f)**
		

Figure D15. Photos of *Salix interior* vegetation columns grown in centrifuge cake amended with hydrochar only (a-f) from September 6, 2018

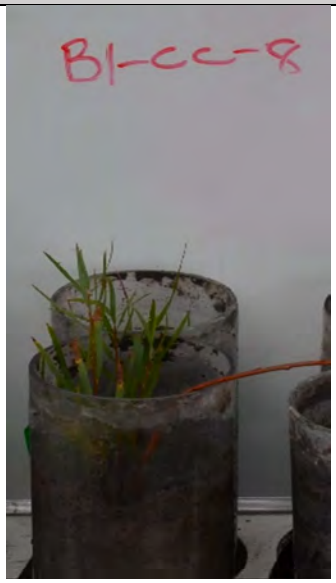
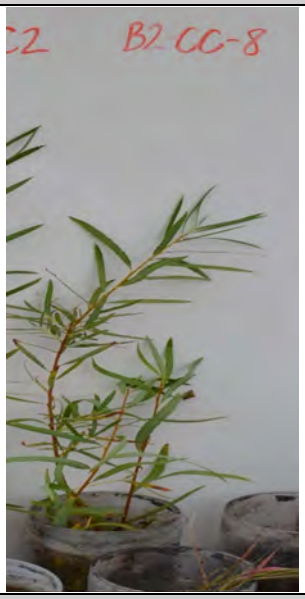
(a)	(b)	(c)
		
(d)	**(e)**	**(f)**
		

Figure D16. Photos of *Salix interior* vegetation columns grown in centrifuge cake amended with peat and bacteria [type 1] (a-f) from September 6, 2018

(a)	(b)	(c)
		
(d)	**(e)**	**(f)**
		

Figure D17. Photos of *Salix interior* vegetation columns grown in centrifuge cake amended with peat only (a-f) from September 6, 2018

(a)	(b)	(c)
		
(d)	**(e)**	**(f)**
		

Figure D18. Photos of *Salix interior* vegetation columns grown in centrifuge cake amended with bacteria only [type 1] (a-f) from September 6, 2018

(a)	(b)	(c)
		
(d)	**(e)**	**(f)**
		

Figure D19. Photos of *Salix interior* vegetation columns grown in centrifuge cake amended with bacteria only [type 2] (a-f) from September 6, 2018

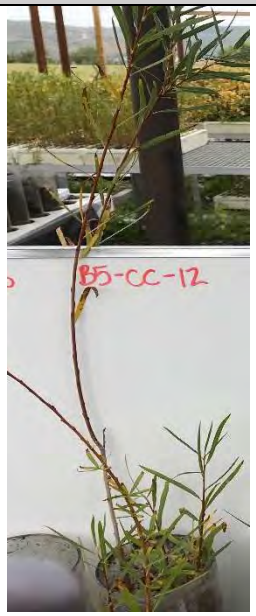
(a)	(b)	(c)
		
(d)	**(e)**	**(f)**
		

Figure D20. Photos of *Salix interior* vegetation columns grown in centrifuge cake without amendment (a-f) from September 6, 2018

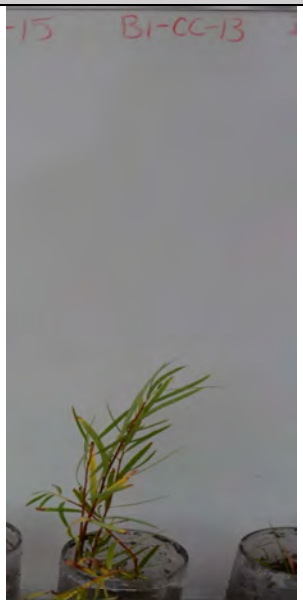
(a)	(b)	(c)
		
(d)	**(e)**	**(f)**
		

Figure D21. Photos of *Elymus trachycaulus* vegetation columns grown in centrifuge cake amended with hydrochar and bacteria [type 1] (a-f) from September 6, 2018

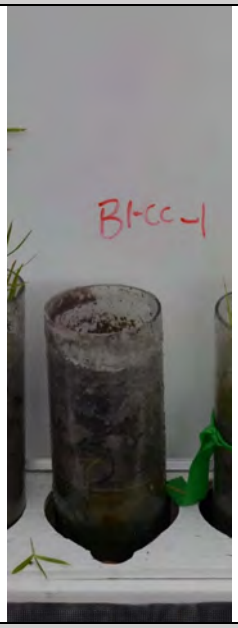
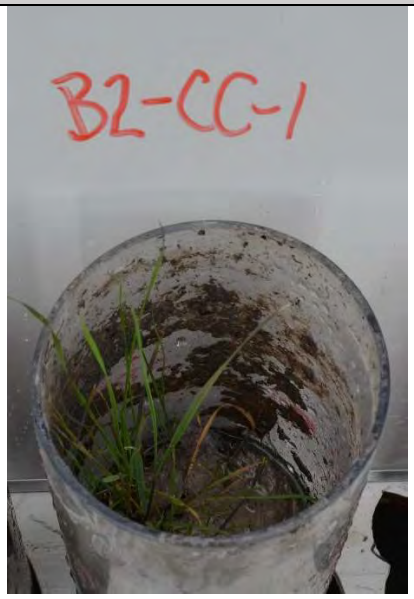
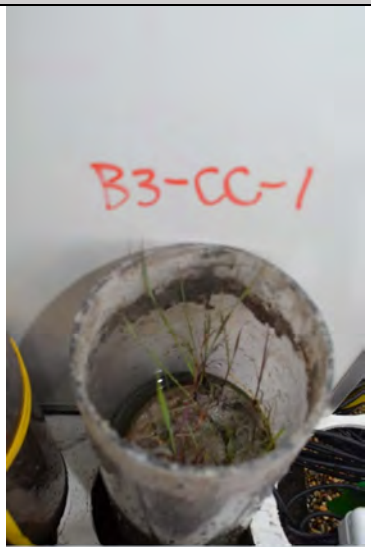
(a)	(b)	(c)
		
(d)	**(e)**	**(f)**
		

Figure D22. Photos of *Elymus trachycaulus* vegetation columns grown in centrifuge cake amended with hydrochar only (a-f) from September 6, 2018

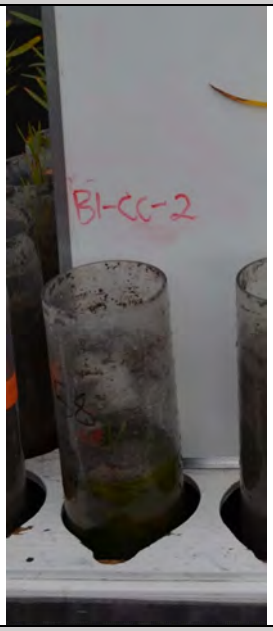
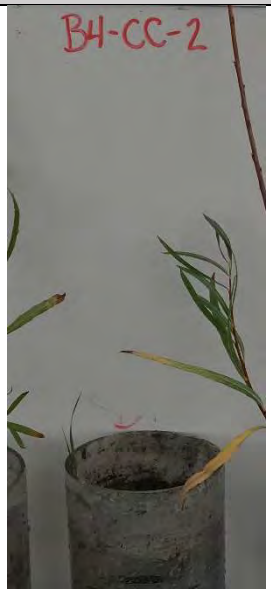
(a)	(b)	(c)
		
(d)	**(e)**	**(f)**
		

Figure D23. Photos of *Elymus trachycaulus* vegetation columns grown in centrifuge cake amended with peat and bacteria [type 1] (a-f) from September 6, 2018

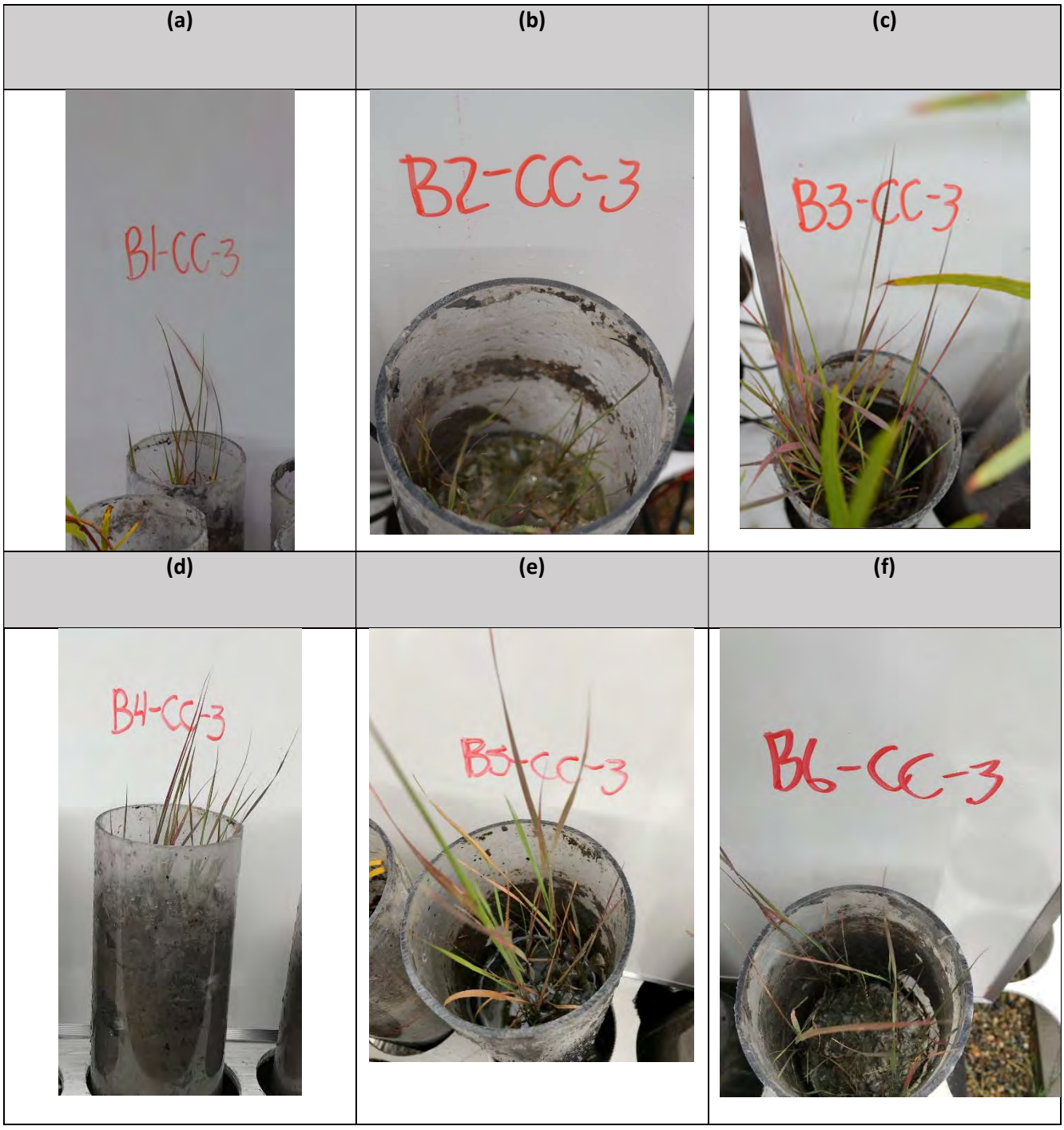


Figure D24. Photos of *Elymus trachycaulus* vegetation columns grown in centrifuge cake amended with peat only (a-f) from September 6, 2018

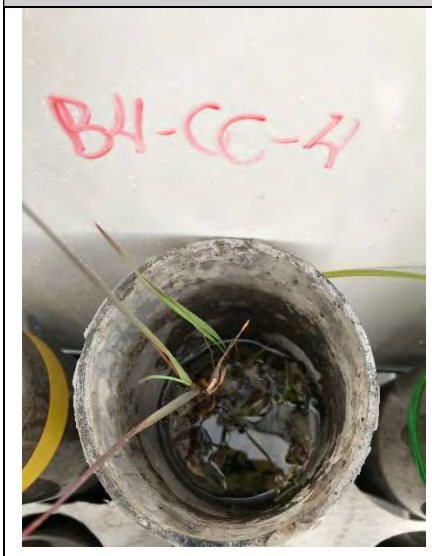
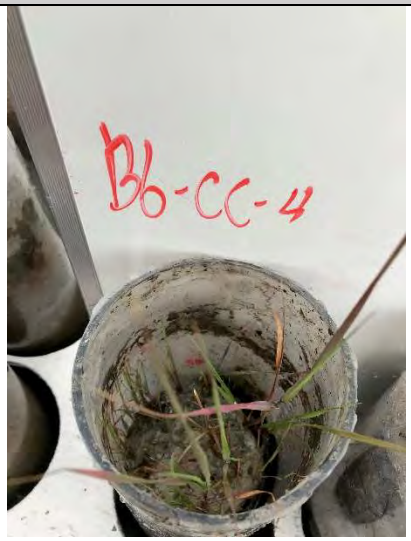
(a)	(b)	(c)
		
(d)	**(e)**	**(f)**
		

Figure D25. Photos of *Elymus trachycaulus* vegetation columns grown in centrifuge cake amended with bacteria [type 1] only (a-f) from September 6, 2018

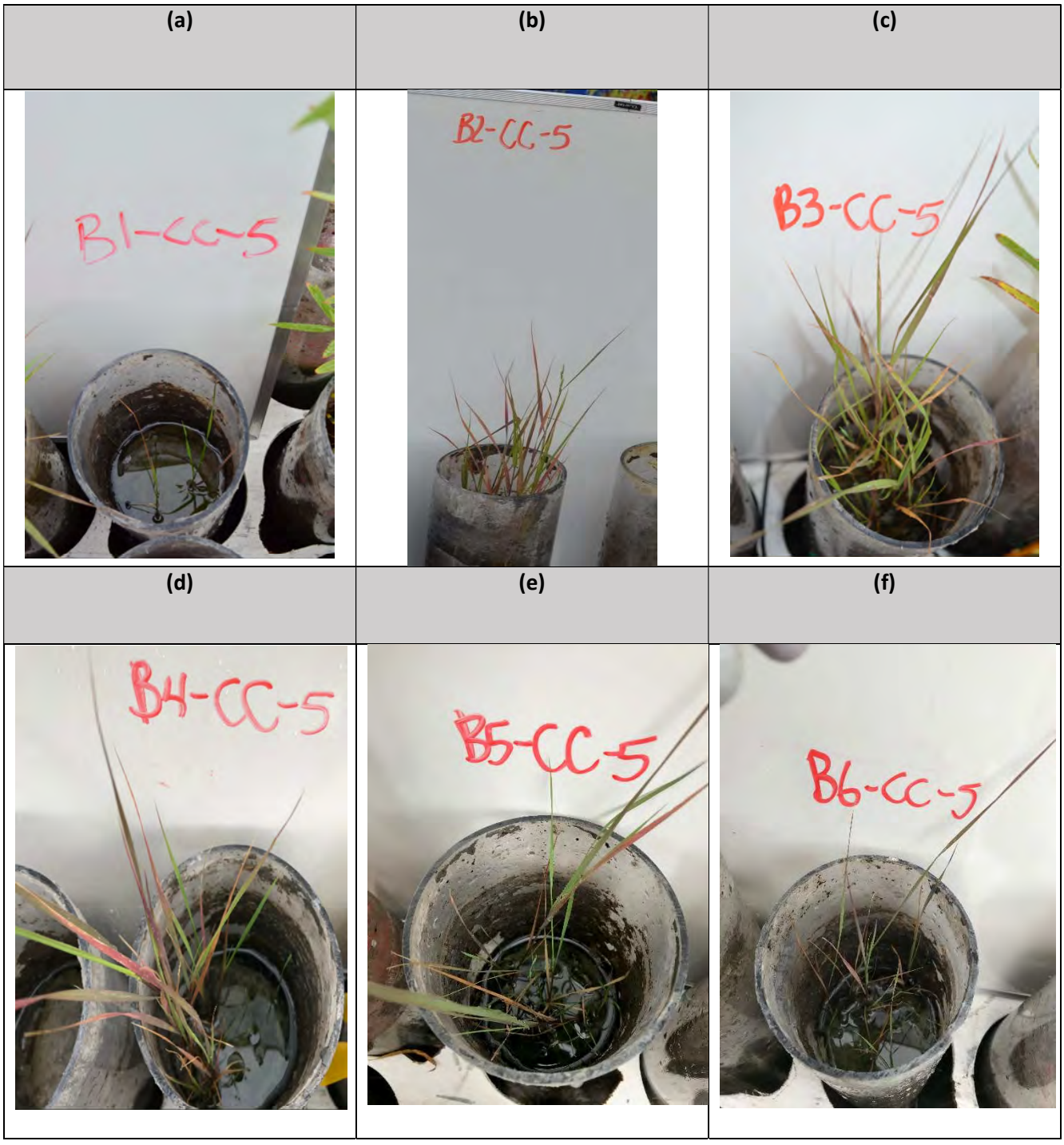


Figure D26. Photos of *Elymus trachycaulus* vegetation columns grown in centrifuge cake without amendment (a-f) from September 6, 2018

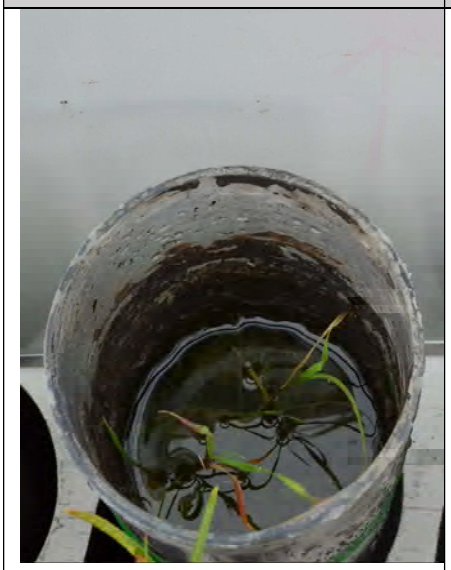
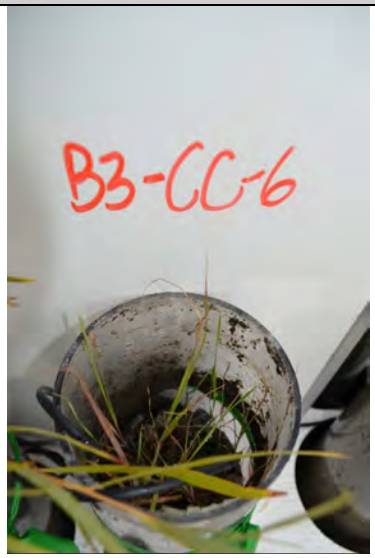
(a)	(b)	(c)
		
(d)	**(e)**	**(f)**
		

Figure D27. Photos of column establishment in June, 2018 depicting centrifuge cake in basin, filled columns, and members of the setup team preparing to homogenize tailings with mixer and drill