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Plant Genotype Influences Physicochemical Properties of Substrate as well as Bacterial and Fungal Assemblages in the Rhizosphere of Balsam Poplar

1 **Karelle Rheault[1], Denis Lachance[1], Marie-Josée Morency[1], Évelyne Thiffault[2], Marie**
2 **Guittonny[3], Nathalie Isabel[1], Christine Martineau[1] and Armand Séguin[1*]**

3 [1] Natural Resources Canada, Canadian Forest Service, Laurentian Forestry Centre, Quebec City, QC,
4 Canada

5 [2] Research Centre on Renewable Materials, Department of Wood and Forest Sciences, Université
6 Laval, Quebec City, QC, Canada

7 [3] Research Institute of Mining and Environment (RIME), Université du Québec en Abitibi-
8 Témiscamingue, Rouyn-Noranda, QC, Canada

9 *** Correspondence:**
10 Armand Séguin
11 armand.seguin@canada.ca

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13 **genetics, Mine waste revegetation, Plant-microbe interactions.**

14 **Abstract**

15 Abandoned unrestored mines are an important environmental issue since they typically remain
16 unvegetated for decades, exposing vast amounts of mine waste to erosion. Several factors limit the
17 revegetation of these sites, including extreme abiotic conditions and unfavorable biotic conditions.
18 However, some pioneer tree species having high level of genetic diversity, such as balsam poplar
19 *(Populus balsamifera)*, are able to naturally colonize these sites and initiate plant succession. This
20 suggests that some tree genotypes are likely more suited for acclimation to the conditions of mine
21 wastes. In this study, two contrasting mine waste storage facilities (waste rock versus tailings) from
22 the Abitibi region of Quebec (Canada), on which poplars have grown naturally, were selected. First,
23 we assessed *in situ* the impact of vegetation presence on each type of mine wastes. The presence of
24 balsam poplars improved soil health locally by improving physicochemical properties (e.g. higher
25 nutrient content and pH) of the mine wastes and causing an important shift in their bacterial and
26 fungal community compositions, going from lithotrophic communities that dominate mine waste
27 environments to heterotrophic communities involved in nutrient cycling. Next, in a greenhouse
28 experiment, ten genotypes of *P. balsamifera* collected on both mine sites and from a natural forest
29 nearby were grown in these mine wastes. Tree growth was monitored during two growing seasons,
30 after which the effect of genotype-by-environment interactions was assessed by measuring the
31 physicochemical properties of the substrates and the changes in microbial communities, using a
32 metabarcoding approach. Although substrate type was identified as the main driver of rhizosphere
33 microbiome diversity and community structure, a significant effect of tree genotype was also
34 detected, particularly for bacterial communities. Plant genotype also influenced aboveground tree
35 growth and the physicochemical properties of the substrates. These results highlight the influence of

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36 balsam poplar genotype on the soil environment and the potential importance of tree genotype
37 selection in the context of mine waste revegetation.

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40 tables.

41 # 1 Introduction

42 Abandoned and unrestored mine sites represent an important environmental issue since they typically
43 remain unvegetated for decades. They often constitute a major eyesore to adjacent communities and
44 pose further risk to surrounding ecosystems as vast amounts of soil, waste rock and tailings are
45 exposed to aeolian and water erosion (Mendez and Maier, 2008). Soil microorganisms are in part
46 responsible for the negative impacts of mine sites, mainly through the formation of acid mine
47 drainage, mostly at pH lower than five (Bussière *et al.*, 2005). In addition, the nature and
48 composition of mine wastes make them challenging substrates for plant growth: they are nutrient
49 poor, have either a very low or very high pH and have poor physical structure and deficient water
50 holding capacity (Kumaresan *et al.*, 2017).

51 Revegetation could help initiate the restoration of these ecosystems. Established plants, together with
52 their associated microbiome, have the ability to reduce acid mine drainage and modify the
53 physicochemical properties of their environment, improving soil quality and fertility (Hubbard *et al.*,
54 2018). Furthermore, plants form a biological cap that reduces soil erosion while increasing water
55 retention and organic matter content in coarse mine wastes, which contributes to soil stability
56 (Tordoff *et al.*, 2000).

57 Parameters used to assess the success of revegetation are based on plant, soil and microbial criteria.
58 Plant criteria include plant survival and biomass, leaf and shoot metal concentrations, establishment
59 of other native colonizers and ability to self-propagate (Tordoff *et al.*, 2000). Soil criteria include
60 improvement in soil structure such as increased soil aggregation and reduced erosion; and
61 improvement in soil physicochemical properties such as less acidic pH, increased organic matter
62 content and increased metal bioavailability and mobility (Mendez and Maier, 2008; Lauber *et al.*,
63 2009). Although currently not widely used, microbial criteria include a decrease in autotrophic
64 bacteria followed by an increase in heterotrophic bacteria and fungi (Rosario *et al.*, 2007) and an
65 increase in bacterial diversity and richness (Garbeva *et al.*, 2004).

66 The efficiency of revegetation is largely dependent on the establishment of a large root network and
67 beneficial root-soil microbe interactions (Callender *et al.*, 2016). Poplars with their high level of
68 genetic diversity could thus be ideal candidates for revegetation purposes. Indeed, poplars are pioneer
69 trees: they rapidly grow in a vast range of environmental conditions and they easily propagate by root
70 suckering and crown breakage (Dickmann and Kuzovkina, 2014). They also have a large and deep
71 root system (Braatne *et al.*, 1996). As a perennial species, they can tolerate harsh environments and
72 promote the establishment of primary and successive plant species through the addition of soil
73 nutrients from their abundant litter production, increasing ecosystem health and function (Pardon *et*
74 *al.*, 2017). They can establish root associations with both arbuscular mycorrhizal and
75 ectomycorrhizal fungi (Gehring *et al.*, 2006), as well as other endophytic and rhizospheric organisms,
76 thereby increasing access to nutrients, relieving abiotic stresses such as hydric stress, suppressing
77 plant pathogens, altering phenology and promoting plant growth (Bulgarelli *et al.*, 2013). Finally,
78 *Populus* is considered as a model genus for the study of woody perennials and therefore massive

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79 genomic resources are available (e.g. fully sequenced genome, Tuskan *et al.*, 2006) which provides
80 unique opportunity to support selection of most suited genotypes to address ecosystem restoration
81 issues (Fini *et al.*, 2017).

82 Plant species produce specific root exudates that will attract specific microorganisms, resulting in
83 dissimilar selective pressures on microbial communities (Philippot *et al.*, 2013). In addition,
84 differences between plant genotypes can have a significant impact on the microbiomes of their
85 rhizosphere (Badri *et al.*, 2009; Lebeis *et al.*, 2015; Veach *et al.*, 2019). This suggests that plant
86 genotype could significantly affect the recruitment of specific microorganisms, thus leading to further
87 differences in plant growth and ecosystem functioning. Many studies have attempted to characterize
88 the root microbiome of *Populus*. It has been shown that soil type is the main driver of microbial
89 community assembly, since physicochemical properties, such as granulometry, pH and nutrient
90 content, influence microbial composition and functional group prevalence (Gottel *et al.*, 2011;
91 Danielsen *et al.*, 2012; Shakya *et al.*, 2013; Cregger *et al.*, 2018). However, genetic variations of the
92 host plant are also associated with differential microbial colonization (Bonito *et al.*, 2014).
93 Differentiating between the effects of soil properties and those of the host plant genotype has not yet
94 been sufficiently addressed (Bonito *et al.*, 2019).

95 The aim of this study was to assess the suitability of *P. balsamifera* for the revegetation of abandoned
96 mine sites and to determine the impact of genotype-by-environment interactions on the improvement
97 of soil health and the rhizosphere microbiome. To do so, two contrasting mine sites from Abitibi,
98 Quebec, where poplars have naturally grown, were selected. First, in a field study, the impact of
99 balsam poplar presence on the waste rock of the first site and on tailings of the second site, was
100 assessed, regarding their physicochemical properties and the composition of their microbiome using
101 a metabarcoding approach. Then, in a greenhouse experiment, ten genotypes of *P. balsamifera*
102 collected from both mine sites and from a natural forest nearby were grown in mine wastes (waste
103 rock and tailings) and evaluated. Tree growth was monitored over two growing seasons, after which
104 the effect of genotype-by-environment interactions on microbial community dynamics were
105 investigated using the same genomic tools as for the field study. The main objectives were (1) to
106 assess the effect of vegetation presence on two contrasting mine wastes by measuring
107 physicochemical properties and characterizing bacterial and fungal communities of the mine wastes
108 *in situ*; (2) to assess the effect of balsam poplar genotype on the physicochemical properties of their
109 substrate and the diversity and composition of their rhizosphere microbiome; and (3) to assess the
110 effect of genotype-by-environment interactions on the rhizosphere microbiome in contrasting
111 substrates.

112 # 2 Material and Methods

113 ## 2.1 Field site description and sampling methods

114 Two mine sites, 50 km apart, located in Abitibi (western Quebec, Canada) were chosen for the
115 contrasting characteristics of their mine wastes and for the fact that they both have balsam poplars
116 naturally growing on the periphery, among other plant species such as willows (*Salix*), trembling
117 aspen (*P. tremuloides)*, alder (*Alnus*) and birch (*Betula*). The Westwood site, formerly the Doyon
118 site, is characterized by its acid generating and coarse waste rock piles; the La Corne Mine site is
119 dominated by neutral and fine-grained tailing piles. Both mine wastes are nutrient poor. The
120 Westwood site is a former gold mine, recently put back into operation and owned by IAMGOLD
121 Corporation. The La Corne Mine site is a former molybdenum and bismuth mine out of operation
122 since 1972 and owned by Romios. See Figures S1 and S2 for pictures of the sites and Figure S3 for a

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123 summary of the field and the greenhouse experiments. Mine wastes, vegetated soil samples and tree
124 cuttings were sampled in November 2016.

125 2.1.1 Soil sampling on site

126 Approximately 150 L of mine waste was collected from the tailing stockpiles at the La Corne Mine
127 site as well as from waste rock stockpiles from the former Doyon mine site at the Westwood site.
128 Samples were collected from the top 20 cm using a shovel and were stored in 25 L plastic boxes at
129 4°C until used. Five subsamples of 15 g from both mine wastes were also taken for DNA extraction
130 and physicochemical analyses. In addition, from each site, five samples of 15 g of bulk soil were
131 collected from areas colonized by balsam poplars to assess the impact of vegetation on mine
132 substrates in a natural setting. Upon arrival to the laboratory, subsamples were taken from each 15 g
133 samples, placed in 1.5 mL tubes and stored at -20°C until DNA extraction. The remainder of each
134 sample was air-dried for physicochemical analyses.

135 2.1.2 Cuttings sampling and genotyping

136 Eight mature balsam poplars per mine site and two from a natural forest near the La Corne Mine site
137 were selected, from which branches were harvested to produce cuttings. Branches were dormant
138 when collected. Harvested branches were kept on ice during transport and stored frozen at -5°C
139 immediately upon arrival to the laboratory, until used. In order to reduce the risk of harvesting the
140 same genotype (clone), trees were sampled at a minimum distance of approximately 200 m. Their
141 unique genotypes were then verified using a 40 SNP-array designed to reveal *P. balsamifera*
142 intraspecific variations (Table S1), as described by Meirmans *et al.* (2017). For this, DNA was
143 extracted from bud tissue using a Nucleospin 96 Plant II kit (Macherey-Nagel, Bethlehem, PA, USA)
144 following the manufacturer's protocol for centrifugation processing with the following modification:
145 buffer PL2 was used at the cell lysis step and was heated for 2 h at 65°C instead of 30 min. All
146 samples were sent to the Genome Quebec Innovation Centre at McGill University to be genotyped.

147 2.2 Cuttings selection and growth

148 From November 2016 to January 2017, cuttings were first started using a hydroponic system (see
149 below) to better allow for root development, then transferred into pots and grown until the end of
150 April 2017. Trees were at least 30 cm tall at the start of the experiment. Genotypes for which a
151 minimum of nine replicates (three replicates per substrate type) remained at the end of the tree
152 production process were kept for the experiment, leaving four genotypes per mine site and two
153 genotypes from the natural forest.

154 Ten cuttings, each containing three buds, were prepared from the tree branches collected from each
155 tree in the field. The base of each was cut diagonally, dipped in a rooting powder (STIM-ROOT No3,
156 Plant Product Co. LTD) and inserted into a rooting medium (ROOTCUBES® 1½' square, Smithers-
157 OASIS) in the hydroponic system. Buds containing flowers were removed. The cuttings were
158 watered automatically twice a day, at 08:00 and 20:00, for 4 minutes (just enough time for the
159 container to be filled and drained slowly). Day/night greenhouse temperatures were set at 22/18°C
160 with 16 hours supplemented lighting (less than 250 W/m^2) between 08:00 and 24:00. Every four
161 weeks of growth in hydroponic, a rooting fertilizer (8 mL / 40 L; Roots&Rhizo, Fred T. Lizer) was
162 added to the irrigation system to help root development.

163 After two months of growth in the hydroponic system, cuttings were transferred to pots. The potting
164 mix consisted of four parts peat (Agro Mix G6, Fafard), two parts vermiculite (Perlite Canada Inc.)
165 and one part Turface (calcined clay particles, Turface MVP, Turface Athletics®). The potting mix

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166 was watered and autoclaved to kill insects potentially present in the peat. Nine grams of slow release
167 fertilizer 18.6.8 (Nutricote Total, Type:100, Chisso-Asahi Fertilizer Co. LTD) was then added per
168 liter of potting mix. Cuttings were planted in 250 mL square pots. Temperature and light conditions
169 were the same as for the hydroponic growth. For the first two months, the trees were watered
170 automatically by a drip irrigation system twice a day for 3 minutes at 08:00 and 20:00. For the last
171 month, the watering program was changed to three times per day for 5, 3 and 5 minutes at 08:00,
172 16:00 and 24:00. Trees were given extra manual watering as required.

173 2.3 Greenhouse experiment

174 2.3.1 Soil sieving and mixing

175 Mine wastes were sieved through a 10 mm sieve before use. A peat mix composed of four parts peat,
176 two parts vermiculite and one part Turface was also prepared. Using a cement mixer, equal volumes
177 of mine wastes and the peat mix were combined. Thus, three treatments were obtained: (1) the mix
178 containing waste rock from the Westwood site and the peat mix (waste rock; WR); (2) the mix
179 containing tailings from the La Corne Mine site and the peat mix (tailing; TA); and (3) a control
180 substrate composed of peat, vermiculite, Turface and slow release fertilizer as described in section
181 2.2 (control; CO).

182 2.3.2 Experimental design

183 At the end of April, three trees from each genotype were repotted in 4 L pots with each mixture
184 treatment and randomly distributed in a greenhouse. The trees were again watered automatically
185 using the drip irrigation system three times per day for 5 minutes at 08:00, 16:00 and 24:00.
186 Temperature and light settings were as described previously.

187 The trees were transferred outside in August after they formed buds and started to lose leaves so as to
188 harden off naturally. Plants were watered manually as required. The trees were returned to the
189 greenhouse in January set to a day/night temperature of 10/5°C with 10 hours of supplemented
190 lighting from 08:00 to 16:00 hours. The trees were immediately cut back to between 30 cm and 50
191 cm high so as to keep 10 buds per tree, including both lateral and terminal buds. Temperature and
192 lighting were gradually raised by 5°C and two hours respectively at two weeks interval until reaching
193 maximum day/night temperatures of 22/18°C and 14 hours lighting (between 06:00 and 20:00).
194 Irrigation started one week after the first buds started to flush, again using the drip irrigation system.
195 Trees were initially watered for 3 minutes at 08:00 once every three days. After ten days, this was
196 increased to once every two days, then to once a day a week later, and finally to twice a day, for 5
197 minutes, at 08:00 and 20:00 11 days after that. The trees were grown for about three months, until
198 they naturally set bud.

199 2.3.3 Tree growth and health measurements

200 The following parameters were measured to assess tree growth and health during the two growing
201 seasons: variation in height (growth); chlorophyll content of leaves; shoot diameter; and dry biomass
202 of shoots and leaves. Height was measured from the soil surface to the terminal bud at the beginning
203 and end of the first season. Height was not measured during the second season because trees were cut
204 back to 40-50 cm at the start of the season. Growth was expressed as a percentage of the difference in
205 heights between the beginning of the experiment and the end of the first season of growth:

206 $$\%\mathbf{Growth} = \frac{\mathbf{Height}_{\mathbf{day\ 91}} - \mathbf{Height}_{\mathbf{day\ 4}}}{\mathbf{Height}_{\mathbf{day\ 91}}} \times \mathbf{100}$$

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207 As an estimation of leaf nitrogen content, the leaf chlorophyll content, or "greenness", was measured
208 with a spectrophotometer, following the manufacturer's instructions (SPAD 502 Plus Chlorophyll
209 Meter, Spectrum Technologies, Inc.). Chlorophyll measurements were made around the middle of
210 each growing season, starting on the fifth leaf from the base of the plant and on every third leaf
211 thereafter, avoiding diseased or immature leaves that were not representative of the whole tree. Shoot
212 diameter was measured 20 cm above the initial cutting at the end of the experiment. Dry biomass was
213 the total mass of the shoot and leaves from the second growth season after drying at 50°C for 7 days.
214 Appearance of leaves was noted once a week during the two growing seasons.

215 **2.3.4 Rhizosphere and bulk sampling**

216 Trees were removed from their pots and the shallow roots removed. Fine roots less than 2 mm in
217 diameter were then sampled, avoiding the taproot in the center of the pots. Bulk soil was obtained by
218 collecting the soil that detached from those roots with gentle shaking. Rhizosphere soil, the soil still
219 attached to the roots after shaking, was then collected by placing the roots into 50 mL Falcon tubes
220 containing 25 mL of sterile PBS (8 g NaCl, 0.2 g KCl, 1.44 g Na_2HPO_4, 0.24 g KH_2PO_4, pH 7.4, 1 L
221 distilled water). After briefly shaking the tubes, the roots were removed, the tubes centrifuged at
222 4700 RPM for 10 minutes at 4°C, and the supernatant discarded. The pellet or rhizosphere soil, was
223 then collected and placed on sterile filter papers to absorb excess moisture, then stored in 1.5 mL
224 tubes at -20°C until DNA extraction.

225 **2.4 Processing of samples**

226 **2.4.1 Physicochemical analyses of bulk soil**

227 Samples of bulk soil, either collected directly on the mine sites or from the greenhouse experiment,
228 were air-dried, sieved at 2 mm, and kept in plastic bags until further processing. One gram of soil
229 was ground to a fine powder of 0.5 mm prior to physicochemical analyses for carbon, nitrogen and
230 sulfur.

231 Carbon (C), nitrogen (N) and sulfur (S) were quantified using the TruMac® CNS analyzer (LECO
232 Corporation, MI, USA) following the manufacturer's protocol. Water pH and buffer pH were
233 measured using the methods described by the Canadian Society of Soil Science (Gregorich and
234 Carter, 2007) using the Thermo Scientific™ Orion™ 2-Star Benchtop pH meter. Extractable
235 phosphorus (P) and exchangeable cations (potassium (K), calcium (Ca), magnesium (Mg),
236 manganese (Mn), iron (Fe), aluminum (Al), and sodium (Na)) were extracted with a Mehlich III
237 extraction buffer (Gregorich and Carter, 2007) and analyzed by inductively coupled plasma (ICP)
238 using an optical emission spectrometer (OES) (Optima 7300 DV, Perkin Elmer, Waltham, MA).

239 **2.4.2 DNA isolation and library preparation**

240 Bulk soil samples from the field experiment and rhizosphere soil samples from the greenhouse
241 experiment were kept for microbiome analyses. Up to 250 mg of these samples were transferred to
242 PowerBead tubes for DNA extraction using the DNeasy PowerSoil DNA Isolation Kit (Qiagen,
243 Valencia, CA, USA), in accordance with the manufacturer's instructions, except that DNA was
244 eluted in 50 µL instead of 100 µL. DNA was quantified using a Qubit dsDNA HS Assay Kit and
245 Qubit Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA). Library preparation for Illumina
246 sequencing was performed according to the manufacturer's instructions for user-defined primers

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247 (Illumina, 2013)[1], with the following modifications. Each sample was amplified in triplicate to ensure
248 reproducibility (Schmidt *et al.*, 2013; Kennedy *et al.*, 2014). Bacterial communities were amplified
249 using primers 515F-Y (5'-GTGYCAGCMGCCGCGGTAA-3') and 926R (5'-
250 CCGYCAATTYMTTTRAGTTT-3') targeting the V4-V5 regions of the 16S rRNA gene of bacteria
251 and archaea (Parada *et al.*, 2016; Rivers, 2016). The ITS2 region of the fungal ribosomal DNA was
252 amplified using the primer set ITS9F (5'-GAACGCAGCRAAIIGYGA-3') and ITS4R (5'-
253 TCCTCCGCTTATTGATATGC-3') (White *et al.*, 1990; Rivers, 2016). Primers contained the
254 required Illumina adaptors at the 5′ end of the primer sequences (5′-
255 TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-3′ for the forward primer and 5′-
256 GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-3′ for the reverse primer). PCR reactions
257 were set up by first mixing 37.5 µl of HotStarTaq Plus Master Mix (QIAGEN Inc., Germantown,
258 MD, USA), 27 µL RNase-free water, 1.5 µL of each 10 µM primer and 7.5 µL of gDNA at 5 ng/µL.
259 The final volume of 75 µL was then equally distributed in three 96-well plates placed in distinct
260 thermocyclers. Thermal cycling conditions were as follows: initial denaturation at 95°C for 5
261 minutes; 40 cycles (for ITS2 amplification; and 35 cycles for 16S amplification) at 94°C for 30 s,
262 50°C for 30 s, 72°C for 1 minute; and a final elongation at 72°C for 10 minutes. PCR products were
263 pooled and purified using 81 µL of magnetic beads solution (Agencourt AMPure XP), then unique
264 codes were added to each sample using the Nextera XT Index Kit, in accordance with the above-
265 mentioned Illumina's protocol. Indexed amplicons were purified with magnetic beads, quantified
266 using a Qubit dsDNA BR Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA) and combined
267 at equimolar concentration. Paired-end sequencing (2 × 250 bp) of the pools was carried out on an
268 Illumina MiSeq at the Illumina Sequencing Platform, Nucleic Acids Solutions, Aquatic and Crop
269 Resource Development, National Research Council Canada-Saskatoon. To compensate for low base
270 diversity when sequencing amplicon libraries, PhiX Control v3 Library was denatured and diluted to
271 12.5 pM before being added to the denatured and diluted amplicon library at 15% v/v. The amplicon
272 libraries were sequenced at a concentration of 6.5 pM for most of the sequencing runs. The Illumina
273 data generated in this study was deposited in the NCBI Sequence Read Archive and is available
274 under the project number PRJNA615167.

275 2.5 Bioinformatic analyses

276 2.5.1 Sequences assignation

277 All bioinformatics analyses were performed in QIIME (version 1.9.1) (Caporaso *et al.*, 2010).
278 Briefly, sequence reads were merged with their overlapping paired-end (fastq_mergepairs), trimmed
279 to remove primers (fastx_truncate), and filtered for quality (fastq_filter) using USEARCH (Edgar,
280 2010). Unique identifiers were inserted into the header of the remaining high-quality sequences, and
281 sequences from the different samples were pooled together (add_qiime_labels) prior to further
282 analyses.

283 UPARSE (Edgar, 2013) was then used to dereplicate the sequences (derep_fulllength), discard
284 singletons (sortbysize), group high quality reads into operational taxonomic units (OTUs) using a
285 97% identity threshold (cluster_otus) (Schloss *et al.*, 2009), and identify chimeras (uchime_ref). The
286 taxonomic assignment of OTUs was done using the QIIME "assign_taxonomy" command with
287 Mothur as the assignment method and Greengenes Database (McDonald *et al.*, 2012) files as the
288 reference for bacteria and the UNITE database (Abarenkov *et al.*, 2010) files for fungi. The

[1] https://support.illumina.com/content/dam/illumina-support/documents/documentation/chemistry_documentation/16s/16s-metagenomic-library-prep-guide-15044223-b.pdf

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289 “make_OTU_table’’ command was then used to generate the OTU table in the “biom” format, which
290 was then used by QIIME in the next steps. OTUs from nonbacterial (or nonfungal) taxa were
291 excluded using the “filter_taxa_from_otu_table’’ command. For the 16S rRNA analysis, sequences
292 corresponding to chloroplasts, mitochondria and to the kingdom *Plantae* were removed; for the ITS2
293 analysis, sequences assigned to the kingdom *Protozoa*, *Protista*, *Chromista* and *Plantae* were
294 removed.

295 OTUs with relative abundances below 0.005% were excluded as previously described (Bokulich *et*
296 *al.*, 2013) prior to diversity analyses using the “filter_otus_from_otu_table” command. Each sample
297 was rarefied to the lowest number of reads observed among libraries from each data set with
298 QIIME’s “single_rarefaction” command, so the rarefied samples all contained the same number of
299 sequences. Finally, the OTU rarefied list file was used in QIIME’s “core_diversity_analyses” and
300 “alpha_diversity” commands to generate alpha diversity measures (chao1 and Shannon indices),
301 calculate the beta-diversity between samples (Bray-Curtis dissimilarity), and generate community
302 composition profiles at different taxonomic levels.

303 Fungal functional groups were predicted using a homemade Python script based on a predetermined
304 list of fungi genus associated with their respective function (Tedersoo *et al.*, 2014). First, the script
305 optimizes the list to detect and remove non-unique entries (these data are compared to each other to
306 determine which data contains the most information). The corrected data is then compared to an
307 excel file, where the genus is the search key: the script analyzes each line of the file to extract the
308 genus of each OTU; this genus is then compared to a reference library to identify the associated
309 biological function. OTUs unidentified to the genus level are assigned unidentified function.

310 **2.5.2 Statistical analyses**

311 Statistical analyses were conducted in R version 3.5.3 (Rproject.org) and figures were produced
312 using the package “ggplot2” package. Statistical significance was determined at $p < 0.05$ throughout
313 the analyses. Parametric assumptions were verified before analysis: data normality was checked
314 graphically with normal quantile–quantile plots and computationally with the Shapiro–Wilk test of
315 normality using the “shapiro.test” function. Homoscedasticity was verified using both the Bartlett test
316 (“bartlett.test” function) and the Fligner-Killeen test (“fligner.test” function). Data were transformed
317 using square root (“sqrt” function) or Tukey’s Ladder of Powers (“transformTukey” function,
318 “rcompanion” package) when necessary to meet parametric ANOVA assumptions. A generalized
319 least squares model (“nlme” package) with a stepwise selection and Akaike’s Information Criterion
320 (AIC) minimization approach was performed with vegetation presence (vegetated or unvegetated
321 soil) and waste type (waste rock or tailings) as explanatory variables for the field experiment and tree
322 genotype and substrate type (WR, TA or CO) as explanatory variables for the greenhouse
323 experiment. Genotype origin (La Corne Mine site, Westwood site or natural forest) was also included
324 in these models (“corComSymm” correlation) to account for its overall large influence. Substrate
325 types were weighted (“varIdent”, weights) to reduce variance due to the fact that they were highly
326 different in their physicochemical properties.

327 Two-way ANOVAs (“anova” function) were used to discern how waste type, vegetation presence,
328 substrate type, genotype, and their interactions influenced taxa relative abundances, physicochemical
329 properties of substrates, and alpha diversity indices. When a factor was revealed as a statistically
330 significant predictor, a Tukey HSD post-hoc pairwise comparison test (“predictmeans” function,
331 adjusted to “tukey”, “predictmeans” package) was performed between all treatments. For the
332 greenhouse experiment, if the interaction between substrate type and genotype was deemed

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333 statistically significant, additional analyses were performed for each substrate type separately to
334 better assess the effect of genotype.

335 Spearman linear correlation analyses were performed using the “corr.test” function (“psych”
336 package) to determine if there were correlations between physicochemical properties of the
337 substrates, tree growth and taxa relative abundances for both field and greenhouse experiments.

338 Non-Euclidean distances were calculated from Bray Curtis dissimilarity matrices and implemented in
339 a non-metric multidimensional scaling plot (NMDS, “metaMDS” function, “vegan” package) to
340 visualize both bacterial and fungal community compositional differences between vegetation
341 presence and waste type on the field, and balsam poplar genotypes and substrate type in the
342 greenhouse experiment. A permutational multivariate analysis of variance model (PERMANOVA,
343 “adonis2” function, “vegan” package; (Oksanen *et al.*, 2019)) was also implemented to discern the
344 amount of variation attributed to each factor and their interaction (with 999 permutations). Additional
345 multivariate analyses were performed on communities from each substrate type of the greenhouse
346 experiment separately to better assess the effect of genotype. Variance heterogeneity between the a
347 priori selected groups was tested with the functions “betadisper” and “permutest”, in the “vegan”
348 package. Clusters between treatments were determined by a multilevel pairwise comparison test
349 (“pairwise.adonis2” function, “pairwiseAdonis” package). Correlations between NMDS axes and
350 physicochemical properties of substrates were determined using the “envfit” function (with 999
351 permutations, “vegan” package).

352 One sample (genotype C21 in control substrate) was removed from analyses because its results were
353 considered aberrant. Two samples (genotype W13 in tailings and waste rock) failed to amplify and/or
354 get sequenced for ITS and were therefore excluded from further analyses.

355 **3 Results**

356 **3.1 Assessment of vegetation’s effect on mine wastes under field conditions**

357 **3.1.1 Soil physicochemical properties**

358 Physicochemical analyses of the mine wastes indicated absence of N, low concentration of C and
359 macronutrients such as P and K, and a relatively high concentration of elements like Fe and S in the
360 waste rock from the Westwood site (Table 1). Additionally, the pH of waste rock was highly acidic
361 with values varying between 2.47 and 3.02, whereas in tailings from La Corne Mine site the pH was
362 almost neutral with values between 5.93 and 7.77. For both tailings and waste rock, the vegetated
363 soils contained significantly higher levels of C, N, K, P, Ca, Mg and Mn than the mine wastes (Table
364 1). Vegetation also reduced S and Fe concentrations and increased pH in waste rock, and reduced pH
365 in tailings.

366 **3.1.2 Microbiome analyses**

367 **3.1.2.1 Alpha diversity**

368 Factorial analyses of alpha diversity indices (Figure 1) indicated that vegetation presence (vegetated
369 vs unvegetated soil), waste type (tailings vs waste rock) and the interaction between both factors had
370 a consistently significant effect on bacterial richness (chao1 index: $p < 0.005$) and diversity (Shannon
371 index: $p < 0.001$). For fungal richness and diversity, the interaction between factors was not
372 significant ($p = 0.066$ and 0.355). Pairwise comparison of alpha diversity indices indicated that

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373 vegetation presence significantly ($p < 0.05$) increased bacterial richness in both waste types and
374 bacterial diversity in waste rock; and reduced fungal richness and diversity in both waste types.

375 **3.1.2.2 Beta diversity**

376 Analysis of beta diversity (Figure S4) indicates that bacterial and fungal community structures highly
377 differed between waste types and vegetation presence. The model explains 62% and 35% of the
378 variation in bacterial and fungal community structure, respectively. The main driver of bacterial and
379 fungal community structure was vegetation presence (R^2 = 31.4% and 18.9%, respectively). Waste
380 type also had a significant effect on community structure (R^2 = 14.6% and 9.7%). The interaction
381 between these two factors being significant (R^2 = 14.7% and 6.7%), a multilevel pairwise comparison
382 test was performed on all combinations of waste type by vegetation presence. All combinations
383 clustered separately, indicating that bacterial and fungal community structures differed between each
384 treatment.

385 **3.1.2.3 Taxonomic profiles**

386 Taxonomic profiling of bacterial and fungal communities revealed high heterogeneity as shown by
387 the variability among field replicates. Many bacterial and fungal taxa were only identified at a high
388 taxonomic level. Figure 2 illustrates the relative abundance of the most abundant taxa (>1%, at the
389 genus level) in vegetated soil and mine wastes, at the La Corne Mine site and the Westwood site.

390 Factorial analyses of the relative abundances of bacterial taxa (for taxa >1%, Table S2) showed that
391 waste type had a significant effect on 32 taxa, vegetation presence had a significant effect on 44 taxa,
392 and a significant interaction between both factors was detected for 40 taxa. Pairwise comparisons
393 between all treatments revealed that 50 of the 51 most abundant bacterial taxa (>1%, at the genus
394 level) had a significant difference in at least one treatment ($p < 0.001$). In fungal communities (Table
395 S2), waste type had a significant effect on 11 taxa, vegetation presence had a significant effect on 21
396 taxa and a significant interaction between both factors was detected for 11 taxa. Pairwise comparison
397 between all treatments showed a significant difference in at least one treatment for 21 of the 47
398 fungal taxa ($p < 0.01$).

399 The presence of vegetation on mine wastes reduced the relative abundance of microorganisms
400 associated with acid mine drainage like *Acidiphilium*, *Leptospirillum* and *Sulfobacillaceae_g* on
401 waste rock (Harrison Jr, 1981; Hippe, 2000; Hottenstein *et al.*, 2019) (Figure 2). Additionally, it also
402 reduced the relative abundance of fungal plant pathogens like *Alternaria* and *Ganoderma* on tailings
403 and *Teratosphaeriaceae* on waste rock. Conversely, the presence of vegetation increased the relative
404 abundance of many microorganisms previously found in rhizosphere samples and associated with
405 beneficial ecological functions like the ectomycorriza *Meliniomyces* on tailings and the rhizobacteria
406 *Burkholderia* (Caballero-Mellado *et al.*, 2004) and *Rhodoplanes* (Sun *et al.*, 2015) on both mine
407 wastes.

408 Functions associated with fungal community in all samples comprised ectomycorrhizae (35%),
409 saprotrophs (27%), ericoid mycorrhizae (13%), plant pathogens (3%), white rot (1%) and lichenized
410 (1.8%) and arbuscular (0.05%) mycorrhizae. Factorial analyses of the relative abundances of fungal
411 functions (Table S2) showed that there was no effect of waste type nor interaction between waste
412 type and vegetation presence on functional group prevalence. Pairwise comparisons between all
413 treatments revealed that there was no effect of vegetation on the relative abundance of fungal
414 functions in waste rock. However, the presence of vegetation on tailings significantly increased ($p <$
415 0.001) the relative abundance of ectomycorrhizae and ericoid mycorrhizae; and significantly

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416 decreased the relative abundance of saprotrophs ($p < 0.001$), plant pathogens ($p < 0.001$) and
417 arbuscular mycorrhizae ($p = 0.003$).

418 All bacterial and fungal taxa were significantly correlated with at least one physicochemical property
419 of the substrates (Table S3). Bacterial genera like *Leptospirillum* and *Acidiphilium*, two bacterial
420 genera associated with the oxidoreduction of iron and acid mine drainage (Harrison Jr, 1981; Hippe,
421 2000) were strongly correlated with iron content and the reduction of pH. Similarly,
422 *Sulfobacillaceae*, a bacterial family associated with the oxidation of sulfur and acid mine drainage
423 (Hottenstein *et al.*, 2019) was strongly correlated with sulfur content and the reduction of pH.

424 3.2 Assessment of genotype-by-environment effect under greenhouse experiment

425 3.2.1 Tree growth

426 Tree growth measurements showed a significant effect of genotype for all parameters, an effect of
427 substrate on three parameters and an interaction between both factors for one parameter only (Figure
428 3). Tree growth (Fig. 3A), the number of days before buds start to open at the beginning of the
429 second season (Fig. 3B) and chlorophyll content during the first season (Fig. 3C) differed among
430 plant genotypes ($p < 0.001$) but not between substrate types ($p = 0.155$, 0.323 and 0.628
431 respectively); the interaction between both factors was not significant for these parameters ($p =$
432 0.526, 0.498 and 0.595). Shoot diameter (Fig. 3E) and biomass produced during the second season
433 (Fig. 3F) differed among plant genotypes ($p < 0.001$) as well as between substrate types ($p < 0.001$
434 and 0.008 respectively), but again no significant interaction was detected ($p = 0.937$ and 0.361).
435 Shoot diameter and plant biomass measurements were greater in the nutrient-rich control substrate
436 versus mine substrates. Chlorophyll content during the second season (Fig. 3D) differed between
437 genotypes and it was greater in the waste rock for some genotypes (significant interaction between
438 genotype and substrate type, $p < 0.001$).

439 The genotypes having lower or higher values strongly differed depending on the measured growth
440 parameter (Figure 3). As an example, genotypes W08, W10, W13 and C29 had lower values for most
441 parameters except for their chlorophyll content after the second season of growth in mine substrates,
442 for which they had the highest values. Besides, genotypes W09 and N16 had the highest biomass and
443 highest growth, respectively, but had lower values for other parameters. Genotype N33 had
444 intermediate to high values for all parameters.

445 There was an overall effect of cuttings origin (either the Westwood site: W; the La Corne Mine site:
446 C; or the Natural forest: N) on measured growth parameters when the effect of substrate type was not
447 significant (Figure S5): W genotypes grew significantly less than C and N genotypes (Fig. S4A, $p <$
448 0.001); C genotypes flushed later than W and N genotypes (Fig. S4B, $p < 0.001$); and C genotypes
449 had a higher chlorophyll content during the first season than W genotypes (Fig. S4C, $p < 0.001$).

450 When the effect of substrate type was significant, there was no obvious association between cuttings
451 origin and their growth in the substrate from which they came from. For example, the genotypes that
452 had a higher chlorophyll content in tailings were not only those originating from the La Corne Mine
453 site (Fig. 3D). For shoot diameter (Fig. 3E) and plant biomass (Fig. 3F), there was no significant
454 difference between mine substrates.

455 3.2.2 Soil physicochemical properties

456 A factorial analysis of the physicochemical properties of substrates revealed that all parameters were
457 significantly affected by substrate type, 11 of the 13 parameters had a significant effect of genotype,

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458 and the interaction between substrate type and genotype was significant for 10 parameters (Table S4).
459 To better assess the effect of genotype, pairwise comparisons were made on each substrate type
460 separately (Tables 2 and S5). All physicochemical properties were significantly affected by genotype
461 in at least one substrate. Although significant, the differences between genotypes were small,
462 generally resulting in only two genotypes being different from the others.

463 Genotypes having a significant effect on physicochemical properties varied depending on the
464 substrate type (Table 2). For example, genotypes associated with higher carbon content were W13
465 and C29 in the control substrate; C21 and C25 in tailings; and W13 in waste rock.

466 General trends were observable for some genotypes (Table 2). In all substrates, genotypes W08 and
467 N16 were associated with less favorable soil conditions (lower nutrient and higher sulfur content). In
468 waste rock, genotype C21 led to the lowest content of all elements and the lowest pH, but, in tailings,
469 it was associated with higher nutrient content. In both mine substrates, genotype C29 was associated
470 with higher nutrient content and pH.

471 There was an overall effect of the origin of the cuttings on the physicochemical properties of the
472 substrates. Indeed, several elements showed higher concentrations when genotypes were grown in
473 their original mine waste: in tailings, C (p = 0.003), N (p < 0.001), Ca (p = 0.019) and Mn (p =
474 0.029) contents were higher for genotypes originating from La Corne Mine; in waste rock, C (p =
475 0.022), N (p < 0.001) and K (p = 0.002) contents were higher for genotypes originating from
476 Westwood. Lastly, plant biomass was significantly, albeit weakly, correlated with N and Na contents
477 of the substrates (Table S6).

478 **3.2.3 Microbiome analyses**

479 **3.2.3.1 Alpha-diversity**

480 Factorial analyses of alpha diversity indices indicated that substrate type had a significant effect on
481 bacterial and fungal richness (chao1: p < 0.001) and diversity (Shannon: p < 0.001 and 0.004; Figure
482 4). There was a significant interaction between substrate type and genotype on bacterial diversity (p
483 = 0.031). Pairwise comparison between substrate types indicated that bacterial richness (p < 0.001)
484 and diversity (p < 0.001) were significantly higher in tailings compared to waste rock and control
485 substrate, but that fungal richness (p < 0.001) and diversity (p = 0.001) were higher in the control
486 substrate than in mine substrates. Alpha diversity was analyzed by substrate type to better assess the
487 effect of genotype. Bacterial richness was higher in genotype C29 compared to genotype C21 in the
488 waste rock (p = 0.040; chao1 index, Figure 4). There was no effect of genotype on fungal alpha
489 diversity.

490 **3.2.3.2 Beta-diversity**

491 Bacterial and fungal community structure highly differed between substrate types as shown by
492 variation in beta diversity (Figure 5). The main driver of bacterial and fungal community structure
493 was substrate type (R^2 = 54.9% and 47.0%, respectively), and a multilevel pairwise comparison test
494 revealed that all substrate types, for bacterial and fungal communities, clustered separately (p <
495 0.001). In bacterial beta diversity, genotype and the interaction between substrate type and genotype,
496 explained, respectively, 7.4% and 11.3% of the variation in rhizosphere community structure.
497 Genotype and the interaction were not significant for the fungal community structure. All
498 physicochemical properties of the substrates were significantly (p < 0.001) correlated with bacterial
499 and fungal community structures as shown by the arrows on Figure 5.

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500 Beta diversity was analyzed by substrate type separately to better assess the effect of genotype on
501 bacterial and fungal community structure (Figure S6). There were differences in bacterial community
502 structure between a few genotypes in both mine substrates (Figures S6B, C) and in tailings for fungal
503 community structure (Figure S6E). In all substrates, bacterial and fungal community structure was
504 correlated with at least one physicochemical property (arrows in Figure S6).

505 **3.2.3.3 Taxonomic profiles**

506 Figure 6 illustrates the relative abundance of the most abundant bacterial and fungal taxa (>1%, at the
507 genus level) in the rhizosphere of balsam poplars after two seasons of growth in tailings, waste rock
508 and control substrates. Many bacterial and fungal taxa were only identified at a high taxonomic level.

509 Functions associated with fungal community of the rhizosphere comprised mostly ectomycorrhizae
510 (40%), saprotrophs (22%), plant pathogens (5%), ericoid mycorrhizae (1%) and brown rot (1%).
511 Factorial analyses of the relative abundance of each function (Table S7) revealed that substrate type
512 had a significant effect on the relative abundance of ectomycorrhizae, saprotrophs, plant pathogens,
513 ericoid mycorrhizae and white rot. Pairwise comparisons between genotypes among each substrate
514 type were performed to better assess the effect of genotype. The effect of genotype was significant on
515 the relative abundance of ericoid mycorrhizae in waste rock: they were more abundant in the
516 rhizosphere of genotype N33 compared to genotypes W09, C25 and C29 ($p = 0.005$).

517 Factorial analyses of taxa abundance in bacterial and fungal communities (for taxa >1%, Table S8)
518 showed that the effect of substrate type was significant for all bacterial taxa and most fungal taxa
519 (24/29); the effect of genotype was significant for many bacterial and fungal taxa (17/30 and 7/29,
520 respectively); and the interaction between substrate type and genotype was significant for a few
521 bacterial and fungal taxa (13/30 and 5/29, respectively). Pairwise comparisons between genotypes
522 among each substrate type were made to better assess the effect of genotype. The effect of genotype
523 was significant in at least one substrate for half of bacterial taxa (17/30) and for a few fungal taxa
524 (5/29). The bacterial and fungal taxa significantly affected by genotype were marked with stars in
525 Figure 6.

526 The relative abundance of most bacterial and fungal taxa was significantly correlated with
527 physicochemical properties of substrates (Table S9). In waste rock, bacterial and fungal communities
528 were characterized by acid tolerant taxa like the bacterial family *Xanthomonadaceae* (Callender *et*
529 *al*., 2016) and the fungal genus *Acidea* (Hujslová and Gryndler, 2019), while the tailings were
530 dominated by microorganisms tolerant to stress like the oligotrophic bacterial genus *Geobacter*
531 (Wilkins *et al*., 2008). As for the community composition of the control substrate, it was mostly
532 composed of decomposer microorganisms like the bacterial family *Chitinophagaceae* (Rosenberg,
533 2014) and the fungal genus *Chrysosporium* (Tedersoo *et al*., 2014). A few plant growth parameters
534 were weakly correlated ($|0.3| < r < |0.5|$) with the relative abundance of a few bacterial and fungal
535 taxa (Table S10).

536 There was an overall effect of the origin of the cuttings on the relative abundance of bacterial and
537 fungal taxa (Table S11). For example, the bacterial genus *Bradyrhizobium* was more abundant in the
538 rhizosphere of genotypes originating from the La Corne Mine site compared to the genotypes
539 originating from the Westwood site, in the control substrate ($p = 0.030$).

540 ## 4 Discussion

541 **Vegetation improved physicochemical properties of mine wastes *in situ***

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542 In this study, the impact of naturally grown *P. balsamifera* on two contrasting mine wastes was
543 assessed *in situ*. These mine wastes were considered unfavorable for plant growth because they
544 contain only small concentrations of essential nutrients, have either a very low or very high pH and
545 have poor physical structure and deficient water holding capacity. The pioneer tree *P. balsamifera*
546 has previously been found to naturally grow on mine sites (van Haveren and Cooper, 1992), a
547 phenomenon that was also observed in this study on the highly distinct mine wastes from our two
548 sites. On both sites, well established vegetation significantly improved most of the physicochemical
549 properties of mine wastes, with an increase in carbon and nutrient (N, K, P, Ca and Mg) content, pH
550 values closer to the natural forest soil samples, and a decrease in S and Fe concentrations in waste
551 rock.

552 Increase in C and N comes from organic matter provided by the growth of balsam poplars; organic
553 matter is further degraded by heterotrophic microorganisms in the soil. This increase in organic
554 matter is responsible for the variations in pH as well as S and Fe. Indeed, soil organic matter buffers
555 soil pH by binding to H^+ in acidic soil. Similarly, Fe binds to organic matter making it less available.
556 Consequently, it has been shown that S adsorption decreases when pH is higher, Fe and Al oxides
557 contents are lower and organic matter content is higher, leading to S uptake by plants and leaching
558 (Johnson *et al.*, 1992).

559 **Vegetation caused a beneficial shift in microbial communities of mine wastes**

560 Results from the field experiments are in line with other studies (Chen *et al.*, 2013; Li *et al.*, 2015,
561 2016) that found a succession of microbial communities shifting from lithotrophic to heterotrophic
562 microorganisms during plant growth on mine wastes. These shifts in microbial community structure
563 suggest that initial soil conditions of mine wastes favoring the growth of lithotrophic microorganisms
564 changed during the establishment of balsam poplars on site, confirming the positive influence of
565 these trees on microbial community structure, function and ecosystem health. For example,
566 *Leptospirillum* and *Acidiphilium*, two bacterial genera associated with the oxidoreduction of iron and
567 acid mine drainage (Harrison Jr, 1981; Hippe, 2000), as well as *Sulfobacillaceae*, a bacterial family
568 associated with the oxidation of sulfur and acid mine drainage (Hottenstein *et al.*, 2019), were found
569 to be more abundant in unvegetated than in vegetated zones of the waste rock pile. A previous study
570 also reported that vegetation growth on acid mine tailings lowered the abundance of these key iron
571 and sulfur oxidizing bacteria and lowered acidity (Li *et al.*, 2016).

572 Surprisingly, the presence of vegetation on mine wastes also reduced the relative abundance of fungal
573 taxa typically known as plant pathogens, suggesting that these fungi may have other ecological
574 functions in disturbed lands. Some of these taxa, like *Alternaria*, *Ganoderma* and the
575 *Teratosphaeriaceae* family, have also been previously isolated in mine wastes and various acidic
576 environments (Wong, 1981; Hujslová *et al.*, 2013; Callender *et al.*, 2016; Mosier *et al.*, 2016). It was
577 also surprising that the presence of vegetation on mine wastes reduced the relative abundance of
578 fungal saprotrophs, as it would be expected that an increase in organic matter would also increase the
579 presence of these microorganisms. These results illustrate a well-known limitation of the use of
580 relative abundances in metabarcoding studies (Zhang *et al.*, 2017; Lin *et al.*, 2019). Although the
581 relative abundance of saprotrophs was lower in the vegetated soil samples, the absolute abundance of
582 these microorganisms might still be higher than in unvegetated mine wastes. This issue could be
583 avoided in further studies by using quantitative PCR to estimate the total populations in these
584 environments (Rastogi *et al.*, 2010) or by spiking exogenous bacteria, fungi or synthetic DNA prior
585 to sample processing (Tourlousse *et al.*, 2017).

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586 Furthermore, many rhizobacteria of the orders *Rhizobiales*, *Sphingomonadales* and *Burkholderiales*
587 and the phylum *Planctomycetes*, *Bacteroidetes*, *Actinobacteria* and *Acidobacteria* were found to be
588 more abundant in vegetated soil samples than in unvegetated mine wastes; however, at lower
589 taxonomic levels, the taxa detected differed between waste rock and tailings. These taxa have
590 previously been associated with the rhizosphere microbiome (da Rocha *et al.*, 2013; McBride *et al.*,
591 2014; Madhaiyan *et al.*, 2015; Qiao *et al.*, 2017); plant growth promotion (e.g. through nitrogen
592 fixation (Caballero-Mellado *et al.*, 2004; Dai *et al.*, 2014; Sun *et al.*, 2015; Jeanbille *et al.*, 2016); the
593 production of IAA (Mehnaz *et al.*, 2010); or disease suppression (Xue *et al.*, 2015)), and nutrient
594 cycling (Webb *et al.*, 2014; Santoyo *et al.*, 2016; Wu *et al.*, 2017). Similarly, vegetation increased the
595 relative abundance of ectomycorrhizal taxa, such as *Meliniomyces*, which have previously been
596 isolated from poplars growing in mine wastes (Gaster *et al.*, 2015; Katanić *et al.*, 2015).

597 For both mine sites, *Proteobacteria* were more abundant and the *Proteobacteria*-to-*Acidobacteria*
598 ratio was higher in vegetated soils than in unvegetated mine wastes. This corroborates previous
599 studies showing that this ratio is an indicator of soil trophic levels, and for which *Proteobacteria*
600 were linked to nutrient-rich soils and *Acidobacteria* to nutrient-poor soils (Fierer *et al.*, 2007; Castro
601 *et al.*, 2010). Gottel *et al.* (2011) found similar results in the rhizosphere of *Populus deltoides* in
602 which *Proteobacteria* were slightly more prevalent than *Acidobacteria*.

603 **Vegetation increased bacterial richness and diversity**

604 Mine site restoration aims to mitigate the negative impacts of mining on the environment and human
605 health. However, land restoration is a long process since the affected ecosystems have lost their plant
606 and microbial biodiversity and most of their functions and services (Prach and Tolvanen, 2016). In
607 this study, vegetation increased bacterial richness and diversity in mine substrates, which suggests an
608 improvement in ecosystem productivity and stability (Tilman *et al.*, 2006). On the other hand, a
609 decrease in fungal richness and diversity was observed in the vegetated soils compared to the mine
610 wastes. This might be due to the competitive exclusion of ectomycorrhizal fungi on other fungi,
611 particularly plant pathogens, corroborating other studies that have shown that disturbed lands have a
612 greater fungal diversity than forested lands (Ding *et al.*, 2011).

613 **Substrate type has a stronger effect on community composition than genotype**

614 In the greenhouse experiment, substrate type was shown to be the main driver of bacterial and fungal
615 community structure and diversity in the rhizosphere of balsam poplar. This is a consistent finding
616 among studies about the *Populus* root microbiome (Gottel *et al.*, 2011; Bonito *et al.*, 2014; Veach *et*
617 *al.*, 2019) and other plant species (Marschner *et al.*, 2004; Lebeis *et al.*, 2015; Wagner *et al.*, 2016;
618 Colin *et al.*, 2017; Gallart *et al.*, 2018), indicating that larger-scale edaphic conditions primarily
619 regulate overall rhizosphere microbiomes. Physicochemical properties, including granulometry and
620 pH, and other unmeasured factors, like water holding capacity and variations in temperature and rain
621 between seasons, contribute to those larger-scale edaphic conditions and likely play a role in the
622 *Populus* root microbiome assembly (Chaparro *et al.*, 2012; Philippot *et al.*, 2013). Nevertheless, in
623 this study, plant genotype also influenced physicochemical properties, but this indirect effect was too
624 weak to be reflected on the community assembly. Furthermore, the effect of genotype varied between
625 substrate types, as shown by the significant interaction between both factors for most bacterial and
626 fungal taxa. Bonito *et al.* (2019) also found that soil origin and properties structured microbial
627 communities to a greater degree than host genotype, and that OTUs enriched in genotype samples
628 vary based on the soil properties in which the genotype was grown. This confirms our expectations of
629 a weak genotype effect and indicates that OTUs that are enriched in a sample cannot be used to
630 discriminate plant genotype.

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631 **Tree genotype has low effect over fungal community structure, diversity and functional**
632 **prevalence compared to bacterial community**

633 Similarly to the results obtained for the field experiment, diversity and structure of fungal community
634 are much more conserved between treatments compared to bacterial community. Tree genotype had a
635 significant effect on the relative abundance of 17 of the 30 most abundant bacterial taxa in at least
636 one substrate type but only 5 of the 29 most abundant fungi. Additionally, while all bacterial taxa
637 were affected by substrate type, five fungal taxa were not. Fungal guild designations revealed that
638 there was barely any effect of genotype on the relative abundance of fungal functions: the effect of
639 genotype was only significant for the relative abundance of ericoid mycorrhizae in waste rock. This
640 could be explained by the fact that plants recruit taxonomic groups in order to balance functions
641 (Maherali and Klironomos, 2007). In other words, different genotypes may select different taxa but
642 ultimately they select similar functions.

643 **Tree genotype and its associated microbiome could be linked to improvement of**
644 **physicochemical properties of substrates**

645 Overall, the genotype C29 was associated with higher nutrient content in both mine substrates when
646 compared with the control substrate. This suggests that some genotypes could be selected to improve
647 the physicochemical properties of a broad range of substrate types. Conversely, growth of genotypes
648 W08 and N16 generally led to less favorable physicochemical properties (i.e. lower carbon and
649 nutrient content, lower pH in waste rock) in both mine substrates, suggesting that they are both ill-
650 adapted for revegetation purposes. Moreover, growth of genotype C21 led to the most significant
651 improvement of the physicochemical properties in tailings, but not in waste rock. Interestingly,
652 genotype C21 was collected at the La Corne Mine site, which may suggest a fine scale local
653 adaptation of this genotype that could be further investigated (Boshier *et al.*, 2015).

654 Furthermore, trends in physicochemical properties could be associated with the rhizosphere bacterial
655 richness. Indeed, in waste rock, genotype C29 was associated with higher nutrient content and pH as
656 well as chao1 index of alpha diversity compared to C21, which was associated with the lowest
657 nutrient content, the lowest pH and the lowest chao1 index. These results suggest that an increase in
658 microbial diversity can lead to improved soil health (Garbeva *et al.*, 2004). Additionally, some
659 dominant taxa could be associated with more favorable conditions of the substrates under poplars of
660 particular genotypes. For example, *Rhodoplanes* was previously found in the rhizosphere of rice
661 paddy soils irrigated by acid mine drainage contaminated water and it has been suggested that they
662 may have a beneficial ecological function to enhance soil fertility (Sun *et al.*, 2015). Interestingly, in
663 our study, they were more abundant in the rhizosphere of genotype C23 (8%) compared to genotype
664 W08 (4%) in waste rock, and nutrient content in the pots of genotypes C23 was also higher compared
665 to W08, supporting the idea that they may play a role in soil health.

666 **Further studies will need to assess the degree of standing genetic variation with harsher**
667 **treatments**

668 There was an overall effect of cutting origin on growth measurements, physicochemical properties of
669 the substrates and abundance of some bacterial and fungal taxa in each substrate type. This effect
670 could suggest that there may have been a fine scale adaptation of the genotypes in these novel
671 environments due to high selective environmental pressures. Further research may better assess the
672 effect of this fine scale adaptation on the composition of the microbiome of poplar trees in such novel
673 environments.

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674 Previous studies have shown that changes in the presence-absence or abundance of just a few
675 microbial taxa can affect plant performance because of their broad functions (Zolla *et al.*, 2013;
676 Henning *et al.*, 2016). In this study, it was shown that plant genotype had a significant effect on the
677 abundance of some bacterial and fungal taxa, but there was no obvious correlation between the
678 rhizosphere microbiome and tree growth. This may be because of the relatively lenient conditions of
679 our treatments. Indeed, the mine substrates were amended with a peat mix to help plant growth and
680 reduce stress caused by acidity, non-proper hydric conditions and low nutrient content. Nonetheless,
681 tree growth measurements during the second season (shoot diameter, plant biomass and chlorophyll
682 content) were also influenced by substrate type, showing that longer exposition to the mine substrates
683 could amplify the effect of genotype on the rhizosphere microbiome. Further investigation involving
684 harsher treatments, either directly on the field or with non-amended mine substrates in a greenhouse
685 experiment, would be necessary to better assess the effect of genotype-by-environment interactions
686 on plant performance and its associated microbiome.

687 **Conclusion**

688 This study has shown that balsam poplars are able to improve soil health of mine wastes in field
689 conditions. Moreover, in greenhouse experiments we demonstrated the effect of genotype-by-
690 environment interactions on the structure and diversity of the rhizosphere microbiome as well as the
691 physicochemical properties of the soil. Our results highlight the influence of balsam poplar genotype
692 in contrasting substrate types. We provided evidence that (1) balsam poplars are suitable to initiate a
693 community of microorganisms closer to a functional vegetated ecosystem on various types of mine
694 wastes as well as increasing soil nutrient content and improving pH; (2) substrate type has a stronger
695 effect on rhizosphere microbial community composition than genotype; (3) nevertheless, plant
696 genotype can act as a selective pressure in structuring rhizosphere microbial communities,
697 particularly bacterial taxa; and (4) genotype-by-environment interactions have an impact on the
698 physicochemical properties of substrates and the composition of the rhizosphere microbiome.

699 From a practical point of view, the selection of tree genotypes together with associated microbiomes
700 benefitting their growth in mine wastes is a strategy that could facilitate the ecological restoration of
701 mine sites. This study also highlights the importance of microbial criteria to assess the success of
702 revegetation, as shown by the major changes in microbial community structure and diversity. Future
703 research efforts should take into consideration the interdependence between host identity and
704 associated microbiomes in forest ecosystems, in order to better understand plant-soil feedbacks as
705 well as incorporate microbiome community ecology into mining restoration strategies. Our results
706 contribute to the understanding of the relationships between tree genetics and the associated
707 microbial communities and highlight the potential for host genotype-by-environment interactions to
708 shape the composition of host-associated microbial communities. This study confirms the importance
709 of large-scale conditions and environmental heterogeneity on driving soil microbiome assembly, but
710 additionally validates the contribution of plant host genotype in acting as a selective pressure in the
711 surrounding rhizosphere soil.

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719 master's thesis (Rheault, 2020).

720 ## 6 Author Contributions

721 KR, DL, MG and AS contributed conception and design of the study; KR, DL and MJM contributed
722 to acquisition of data; KR and CM performed the statistical analyses; KR, ET, CM and AS
723 contributed to interpretation of data; KR wrote the first draft of the manuscript; CM and NI wrote
724 sections of the manuscript. All authors contributed to manuscript revision, read and approved the
725 submitted version.

726 ## 7 Conflict of Interest

727 The authors declare that the research was conducted in the absence of any commercial or financial
728 relationships that could be construed as a potential conflict of interest.

729 ## 8 Contribution to the Field

730 Many studies have attempted to characterize the root microbiome of *Populus*. It has been shown that
731 soil type is the main driver of microbial community assembly, since physicochemical properties
732 influence microbial composition and functional group prevalence. However, genetic variations of the
733 host plant are also associated with differential microbial colonization. Differentiating between the
734 effects of soil properties and those of the host plant genotype has not been sufficiently addressed. Our
735 results contribute to the understanding of the relationships between tree genetic background and the
736 associated microbial communities and highlight the potential for host genotype-by-environment
737 interactions to shape the composition of host-associated microbial communities. This study confirms
738 the importance of large-scale conditions and environmental heterogeneity on driving soil microbiome
739 assembly, but additionally validates the contribution of plant host genotype in acting as a selective
740 pressure in the surrounding rhizosphere soil. Initiatives using *P. balsamifera* as a candidate for
741 abandoned mine sites restoration may need to consider the interplay between genotype and the
742 belowground microbiome. Further examination of microbial community dynamics over longer
743 exposition of trees to mine substrates and other degraded lands may provide a clearer understanding
744 of genotype-by-environment interactions. This knowledge will enable the development of more
745 efficient and effective land reclamation strategies.

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1026 11 Data Availability Statement

1027 The Illumina data generated in this study was deposited in the NCBI Sequence Read Archive and is
1028 available under the project number PRJNA615167.

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1031 ## 12 Tables

1032 **Table 1.** Physicochemical properties of substrates from the field. Legend: CEC: Cation exchange
1033 capacity; BCSR: Base cation saturation ratio. Significant differences are highlighted in bold.

1034

	Reference forest soil	Westwood			La Corne Mine		
		Waste rock	Vegetated	p-value	Tailings	Vegetated	p-value
C total (%)	6.218	0.084	0.863	**0.002**	0.042	2.983	**< 0.001**
N total (%)	0.253	< 0.006	0.021	0.053	< 0.006	0.106	**< 0.001**
S total (%)	0.045	0.700	0.134	**< 0.001**	0.017	0.030	0.091
pH	4.9	2.8	4.7	**< 0.001**	6.4	4.5	**< 0.001**
P (mg/kg)	9.83	7.02	23.98	**< 0.001**	0.68	3.80	**0.004**
K ($cmol_c$/kg)	0.418	0.011	0.199	**< 0.001**	0.053	0.202	**< 0.001**
Ca ($cmol_c$/kg)	7.69	8.96	1.80	0.069	0.43	2.93	**< 0.001**
Mg ($cmol_c$/kg)	1.590	0.564	0.676	0.413	0.272	0.902	**< 0.001**
Mn ($cmol_c$/kg)	0.143	0.011	0.040	**0.024**	0.024	0.073	**0.003**
Fe ($cmol_c$/kg)	2.00	6.26	2.68	**< 0.001**	2.85	3.10	0.550
Na ($cmol_c$/kg)	0.035	0.022	0.021	0.805	0.028	0.029	0.929
CEC ($cmol_c$/kg)	13.6	17.0	8.7	0.102	5.3	9.4	**0.003**
BCSR	71.6	46.3	31.3	0.344	14.1	43.2	**< 0.001**

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Table 2. Physicochemical properties of substrates after the greenhouse experiment. See Supplementary Table 5 for the other parameters.

	C total (%)			N total (‰)			P (mg/kg)		
	Control	**Tailings**	**Waste rock**	**Control**	**Tailings**	**Waste rock**	**Control**	**Tailings**	**Waste rock**
W08	9.43 ab	1.47 b	2.50 abc	17.8 ab	0.35 c	3.09 bcd	15.7 b	2.43	6.2 b
W09	8.23 ab	1.63 ab	2.56 ab	17.1 ab	0.52 c	3.98 abc	30.9 ab	4.12	10.9 a
W10	6.08 b	1.93 ab	2.55 abc	11.7 b	1.16 bc	3.19 bcd	38.0 a	6.11	12.6 a
W13	10.96 a	1.83 ab	3.21 a	22.2 a	0.98 bc	4.87 ab	34.6 ab	7.10	10.9 a
N16	10.24 ab	2.21 ab	2.25 bc	21.8 ab	1.87 abc	2.74 cd	27.6 ab	5.07	11.1 a
C21	10.02 ab	2.19 a	1.79 c	19.3 ab	1.91 abc	1.32 d	33.0 ab	5.14	9.2 ab
C23	7.60 ab	2.05 ab	2.61 abc	13.9 ab	1.22 bc	3.50 abcd	37.9 a	6.06	11.5 a
C25	7.81 ab	2.14 a	2.21 bc	15.6 ab	1.47 bc	4.39 abc	35.2 a	3.96	11.9 a
C29	10.59 a	1.95 ab	2.71 ab	21.1 a	3.22 a	5.39 a	37.3 a	7.07	11.9 a
N33	7.16 ab	1.59 ab	2.22 bc	14.3 ab	2.50 ab	4.29 abc	29.8 ab	2.68	10.5 a
p-value	0.009	0.006	0.002	0.009	< 0.001	< 0.001	0.017	0.027	< 0.001

	K ($cmol_c$/kg)			Ca ($cmol_c$/kg)			Mg ($cmol_c$/kg)		
	Control	**Tailings**	**Waste rock**	**Control**	**Tailings**	**Waste rock**	**Control**	**Tailings**	**Waste rock**
W08	4.42 cd	1.14 b	2.16	9.20 c	1.40 b	3.56 c	3.97	0.64 b	0.86 ab
W09	9.61 abcd	1.60 ab	2.20	24.80 a	2.85 ab	5.93 ab	7.07	1.12 a	1.28 a
W10	11.06 a	2.59 ab	2.00	21.50 abc	3.45 a	5.46 abc	6.43	1.45 a	1.12 ab
W13	8.75 bc	1.88 ab	2.00	23.90 ab	3.49 a	6.47 ab	7.26	1.30 a	1.28 a
N16	7.56 d	2.22 ab	1.67	20.00 abc	4.66 a	4.66 abc	6.49	1.62 a	1.18 ab
C21	9.07 abcd	2.67 a	1.37	19.50 abc	4.10 a	3.10 c	6.13	1.45 a	0.67 b
C23	10.11 ab	1.98 ab	1.72	17.60 bc	3.35 a	5.99 ab	5.70	1.22 a	1.24 a
C25	9.35 abcd	2.84 a	1.58	20.90 abc	3.59 a	4.27 bc	6.40	1.46 a	1.01 ab
C29	7.10 d	1.67 ab	2.05	19.70 abc	4.06 a	6.74 a	5.95	1.22 a	1.28 a
N33	8.61 abcd	1.83 ab	1.57	21.10 abc	3.19 a	4.88 abc	5.77	1.24 a	1.06 ab
p-value	0.013	0.013	0.068	0.005	< 0.001	< 0.001	0.094	< 0.001	0.003

	pH			S total (‰)			Fe ($cmol_c$/kg)		
	Control	**Tailings**	**Waste rock**	**Control**	**Tailings**	**Waste rock**	**Control**	**Tailings**	**Waste rock**
W08	5.81	5.83	4.66 ab	1.04 ab	0.52	10.60 a	0.450	0.910 d	2.100
W09	5.59	5.51	4.86 a	1.27 ab	0.54	10.30 a	1.054	1.800 ab	2.320
W10	5.68	5.58	4.73 ab	0.62 ab	0.70	12.70 a	0.869	1.890 a	2.450
W13	5.77	5.64	4.92 a	1.84 a	0.14	10.40 a	0.957	1.720 abc	2.170
N16	5.64	6.03	4.84 a	1.21 ab	0.05	10.20 a	0.813	1.220 cd	2.130
C21	5.39	5.61	4.32 b	1.03 ab	0.89	11.20 a	0.922	1.420 bc	2.660
C23	5.63	5.64	4.83 a	1.19 ab	0.48	3.40 b	1.089	1.580 abc	2.700
C25	5.65	5.48	4.66 ab	0.30 b	0.11	3.50 b	1.028	1.430 abc	2.140
C29	5.54	5.62	4.92 a	0.34 b	0.02	2.60 b	0.973	1.650 abc	2.270
N33	5.44	5.53	4.75 ab	0.03 b	0.03	4.10 b	0.998	1.780 ab	2.260
p-value	0.282	0.111	0.015	0.011	0.684	< 0.001	0.354	< 0.001	0.497

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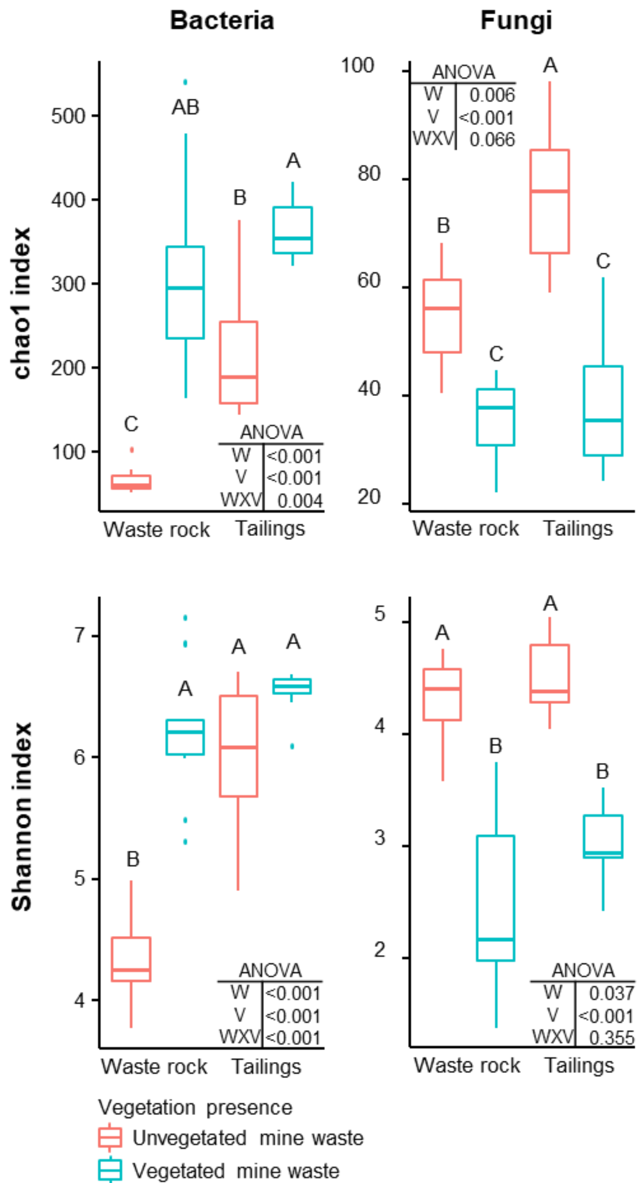
1044 **13 Figures**



1045

1046 **Figure 1.** Alpha diversity indices of bacterial and fungal community profiles in field samples. A two-
1047 way ANOVA was used to discern how waste type, vegetation presence and their interaction
1048 influenced alpha diversity indices. In the ANOVA table, W is waste type; V is vegetation presence;
1049 and WXV is the interaction between waste type and vegetation presence. Treatments are composed
1050 of two factors: waste rock and tailings as waste type; and vegetated or unvegetated mine waste as
1051 vegetation presence. Shared letters between treatments means there is no significant difference
1052 between these treatments, as determined by Tukey HSD post-hoc pairwise comparison test ($n \geq 8$).
1053 Significance level is $p < 0.05$.

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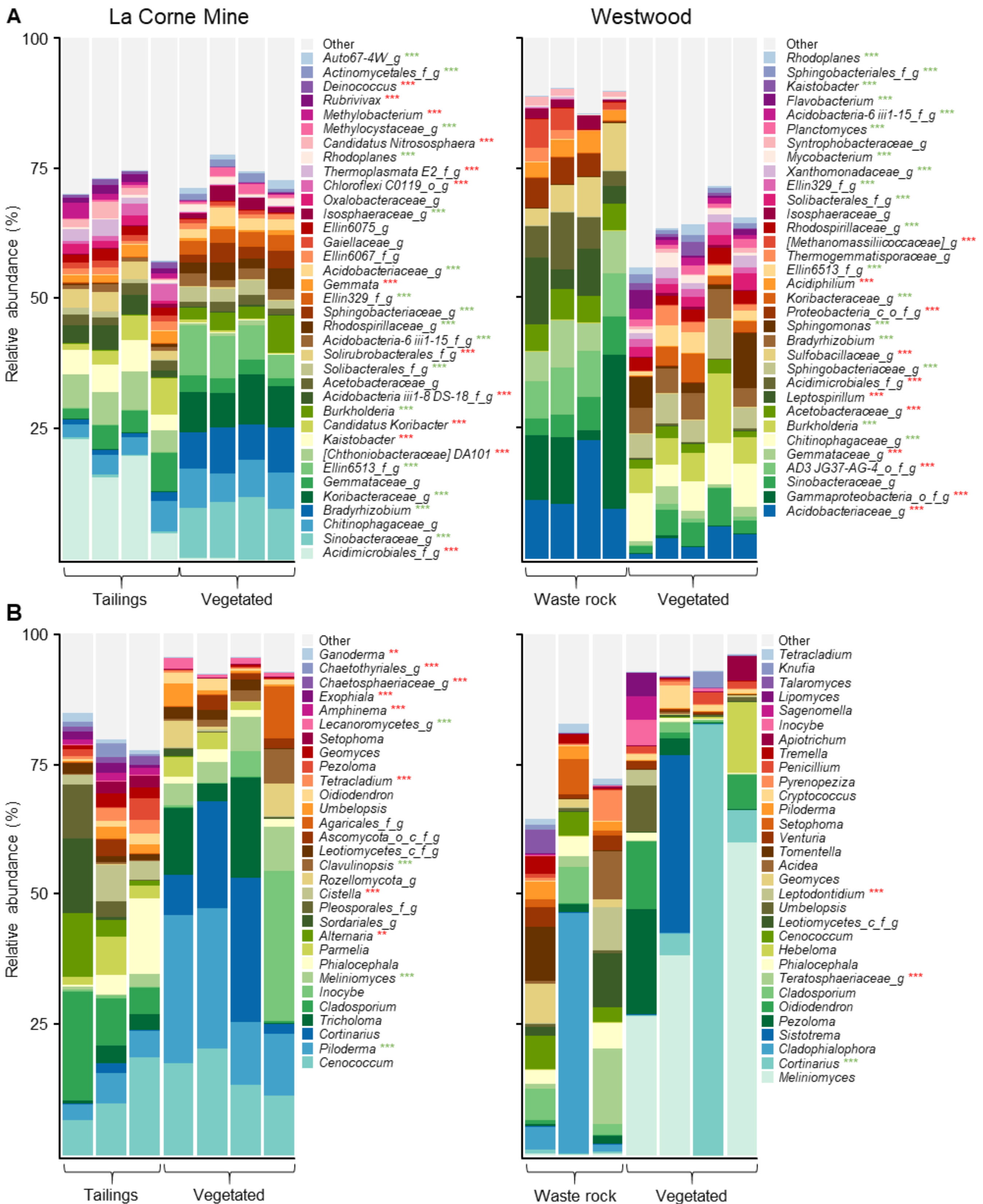

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1056 **Figure 2.** Taxonomic profiles of bacterial and fungal communities in vegetated and unvegetated
1057 samples of waste rock from the Westwood site and tailings from the La Corne Mine site. Taxonomic
1058 profiles of bacterial communities **(A)** and fungal communities **(B)** at the genus level. Only bacterial
1059 and fungal taxa with a relative abundance >1% in at least one treatment are shown. Green and red
1060 stars represent a significant increase and decrease, respectively, in the relative abundance of the taxa
1061 in the vegetated soil compared to the unvegetated soil as determined by a Tukey HSD post-hoc
1062 pairwise comparison test ($n \geq 3$). Significance level is represented as follows: $p < 0.05$ *, $p < 0.01$ **,
1063 $p < 0.001$ ***.

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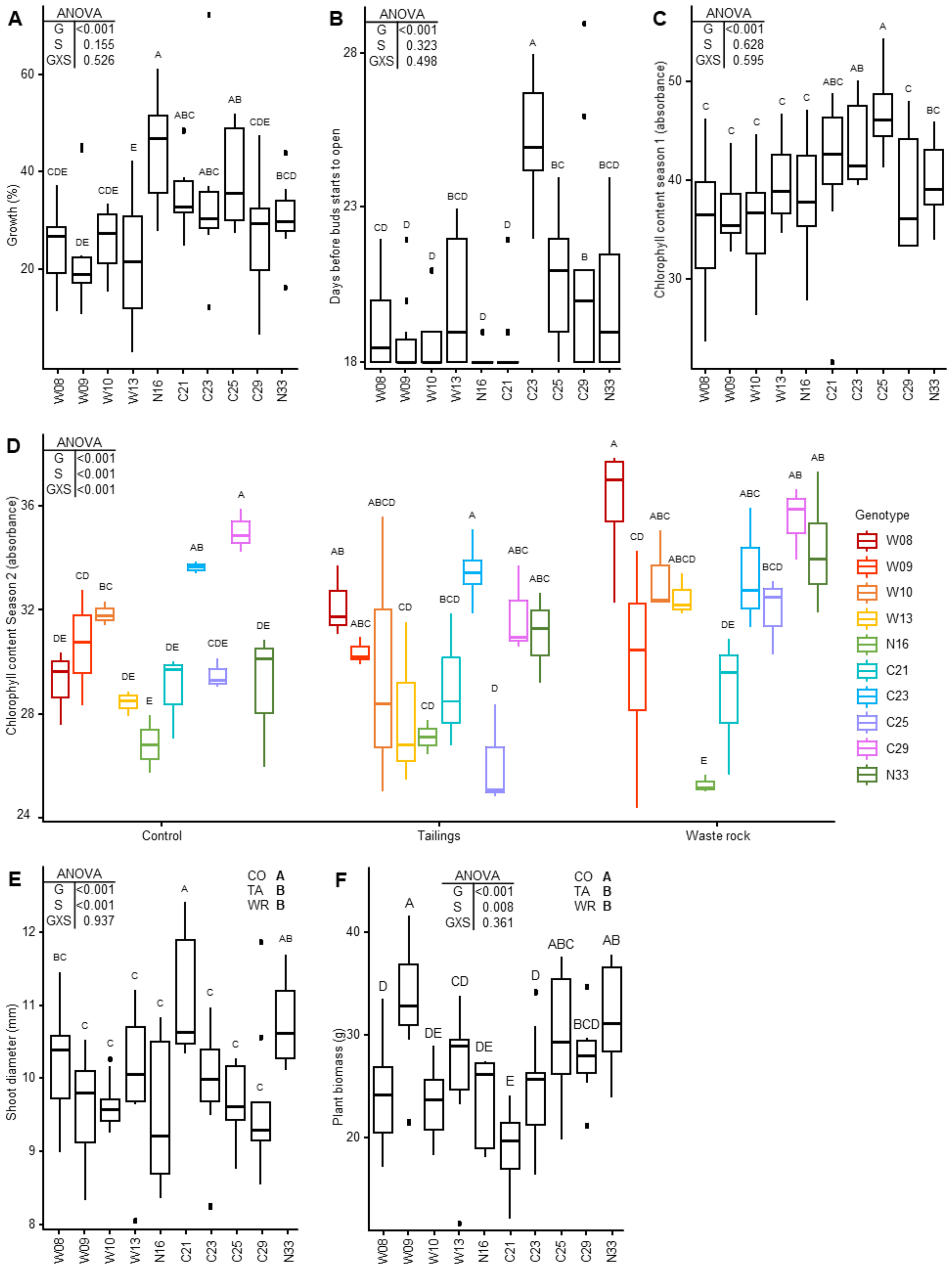

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1066 **Figure 3.** Effect of genotype and substrate type on tree growth measurements. Tree growth during
1067 the first season **(A)**; chlorophyll content at the end of the first **(B)** and second **(C)** seasons; blooming
1068 during the second season **(D)**; shoot diameter **(E)**; and biomass produced during the second season
1069 **(F)**. A two-way ANOVA was used to discern how genotype, substrate type and their interaction
1070 influenced tree growth measurements. Shared letters between treatments means there is no significant
1071 difference between these treatments as determined by Tukey HSD post-hoc pairwise comparison test
1072 ($n \geq 3$). In the ANOVA tables, G is genotype; S is substrate type; and GXS is the interaction between
1073 genotype and substrate type. In panels **(E)** and **(F)** CO is control; TA is tailings; and WR is waste
1074 rock. Significance level is $p < 0.05$. In panels **(A)**, **(B)** and **(C)**, because only the effect of genotype is
1075 significant, measures from all substrate types were pooled. In panel **(D)**, because the interaction of
1076 both factors is significant, all treatments were shown separately. In panels **(E)** and **(F)**, pooled
1077 measures from all substrate types are shown for each genotype as a boxplot and the effect for each
1078 substrate type is shown in the upper right corner of the plots.

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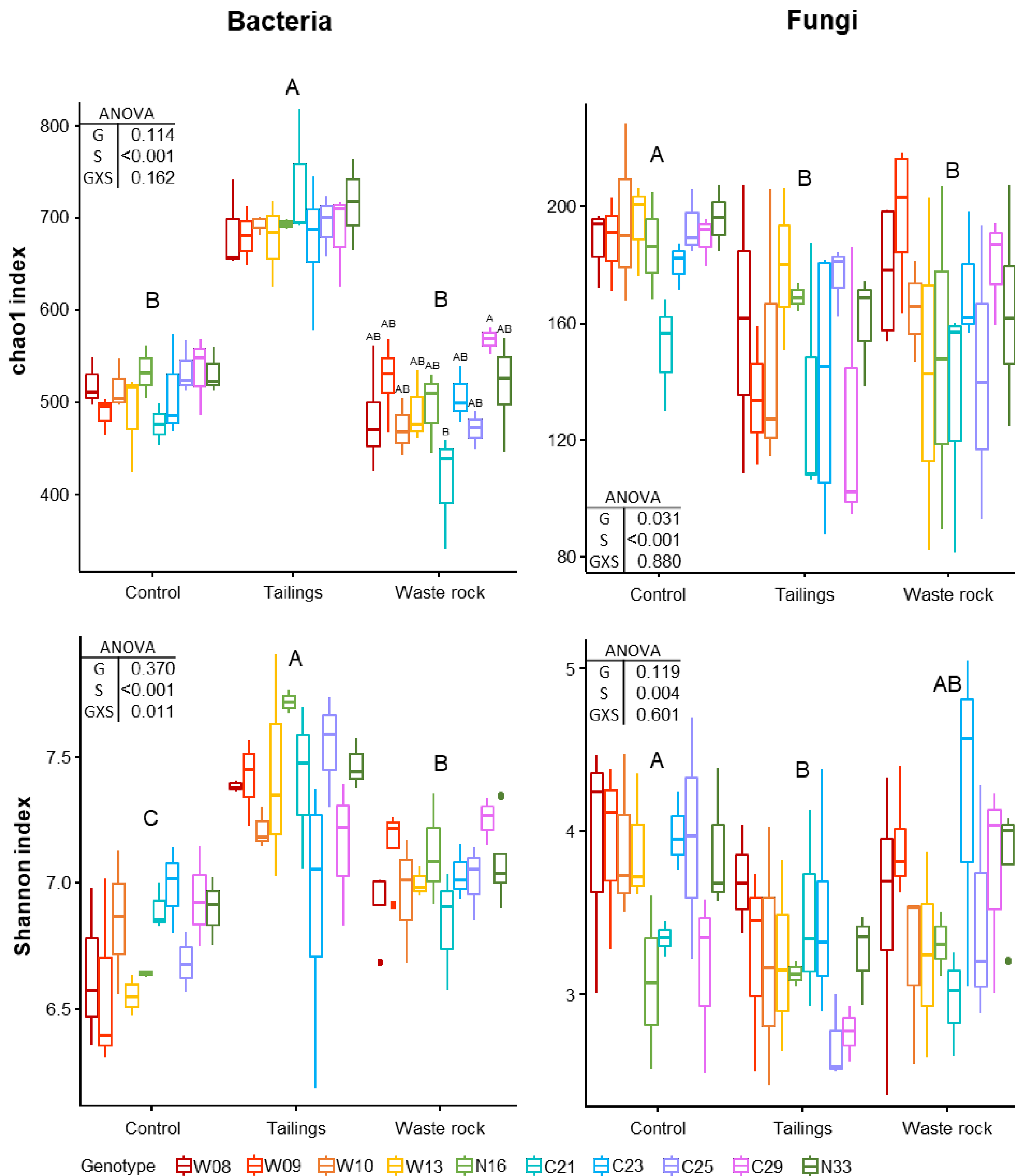

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1083 **Figure 4.** Alpha diversity indices of bacterial and fungal community profiles in greenhouse samples.
1084 A two-way ANOVA was used to discern how genotype, substrate type and their interaction
1085 influenced alpha diversity indices. In the ANOVA table, G is genotype; S is substrate type; and GXS
1086 is the interaction between genotype and substrate type. Shared letters between treatments means there
1087 is no significant difference between these treatments, as determined by Tukey HSD post-hoc pairwise
1088 comparison test ($n \geq 3$). Significance level is $p < 0.05$. Genotype had a significant effect on bacterial
1089 richness in waste rock only (chao1 index; $p = 0.040$).

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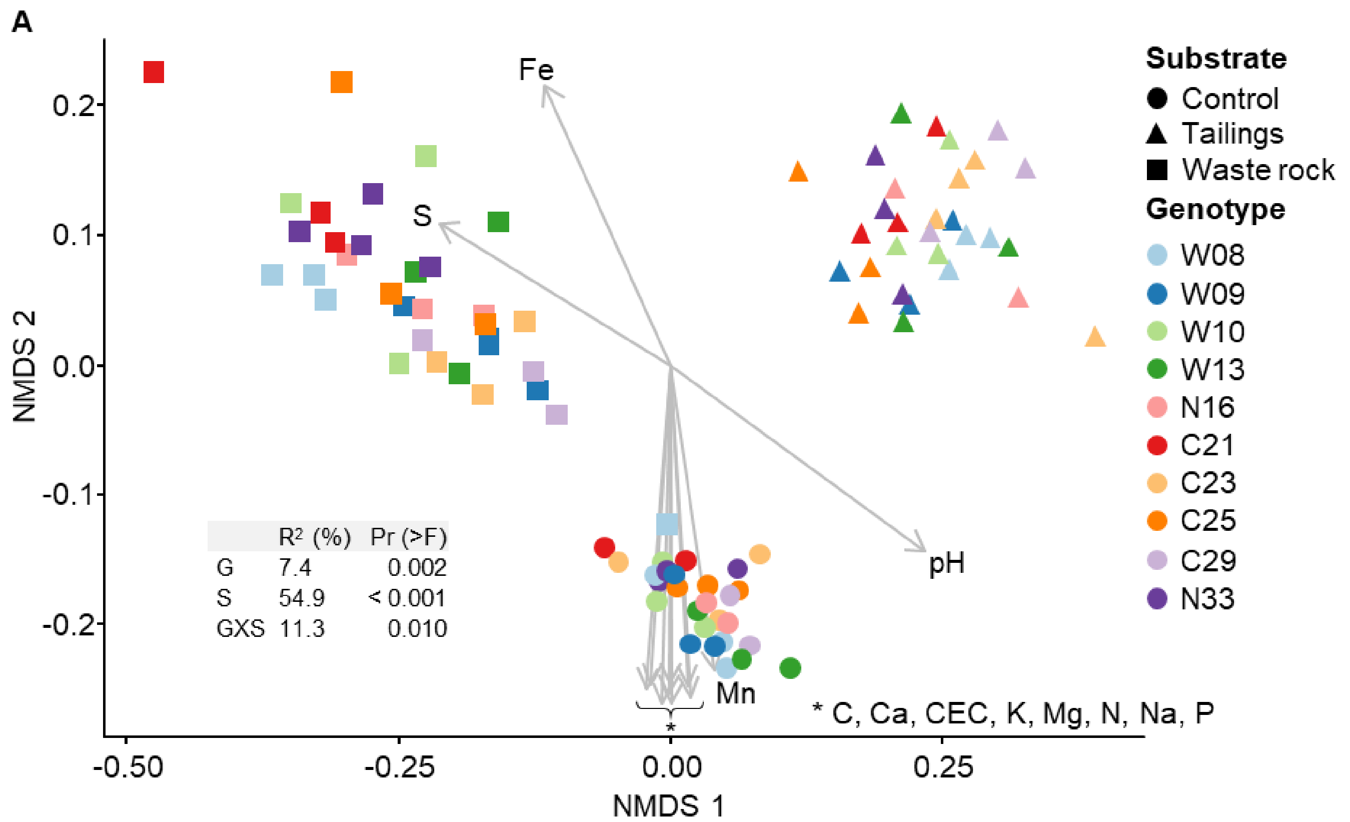

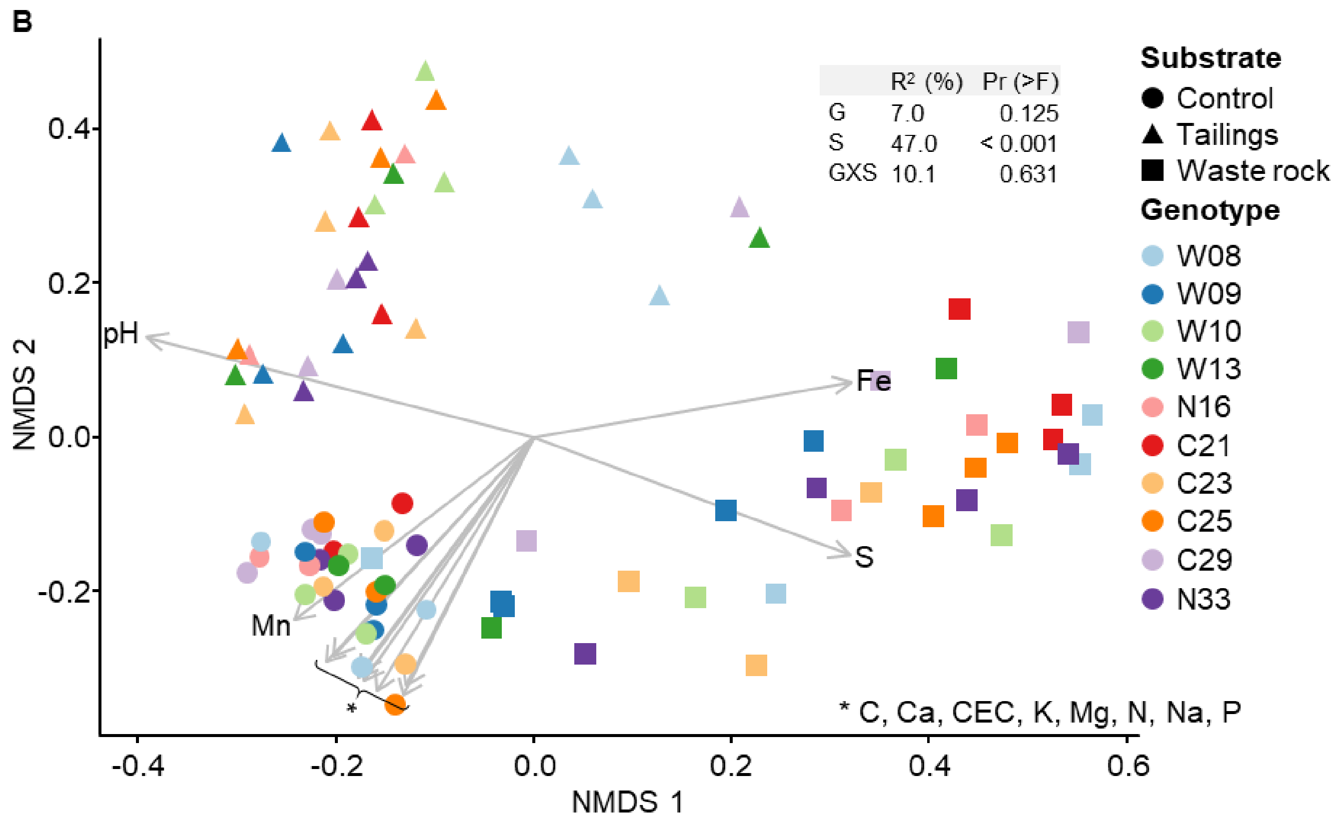

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1092 **Figure 5.** Non-metric multidimensional scaling (NMDS) ordination of variation in bacterial and
1093 fungal community structure of balsam poplar rhizosphere in the greenhouse experiment. Bacterial
1094 community **(A)** and fungal community **(B)**. Points represent samples and arrows represent the
1095 significant ($p < 0.001$) correlations between NMDS axes and the physicochemical properties of the
1096 substrates. In PERMANOVA tables, G is for genotype; S for substrate type and GXS for the
1097 interaction between genotype and substrate type. The model explains 73.6% and 64.1% of the
1098 variation in bacterial and fungal community structure, respectively ($n \geq 3$).

1099

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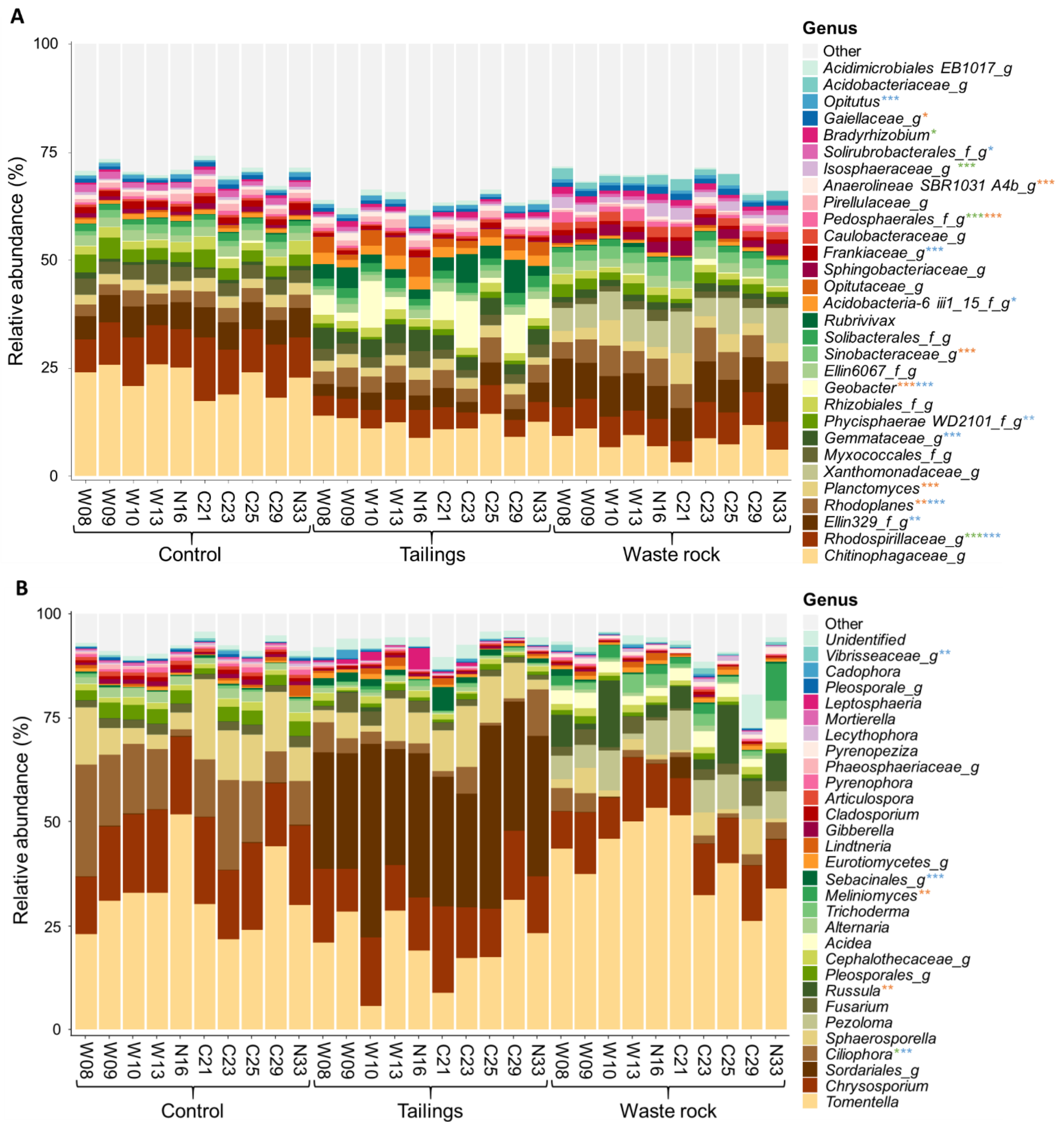

1100

1101 **Figure 6.** Taxonomic profiles of bacterial and fungal communities in treatments from the greenhouse
1102 experiment. Bacterial **(A)** and fungal **(B)** communities at the genus level. Only bacterial and fungal
1103 taxa with a relative abundance >1% in at least one treatment are shown. Replicates for each genotype
1104 were pooled for visual simplification ($n \geq 3$). Stars represent levels of significant difference between
1105 genotypes in the three substrates analyzed separately: green for control, blue for tailings and orange
1106 for waste rock. Significant differences were determined by a Tukey HSD post-hoc pairwise
1107 comparison test. Significance level is represented as follows: $p < 0.001$ ***, $p < 0.01$ **, $p < 0.05$ *.