Risueño, Y., Petri, C., Conesa H.M. 2021. A critical assessment on the short-term response of microbial relative composition in a mine tailings soil amended with biochar and manure compost. *Journal of Hazardous Materials*. 417:126080. https://doi.org/10.1016/j.jhazmat.2021.126080

A critical assessment on the short-term response of microbial relative composition in a mine tailings soil amended with biochar and manure compost

Yolanda Risueño[1*], César Petri[2], Héctor M. Conesa[1]

Yolanda Risueño[1*]

[1]Universidad Politécnica de Cartagena, Escuela Técnica Superior de Ingeniería Agronómica, Departamento de Ingeniería Agronómica. Paseo Alfonso XIII, 48. 30203 Cartagena (Spain)

*corresponding author

Telephone: +34 968 327034; Fax: +34 968 327046

e-mail: yolanda.risueno@upct.es

César Petri[2]

[2]IHSM-UMA-CSIC La Mayora, Departamento de Fruticultura Subtropical y Mediterránea. Avenida Dr. Wienberg, s/n. 29750 Algarrobo-Costa, Málaga (Spain)

e-mail: cesar.petri@csic.es

Héctor M. Conesa[1]

[1]Universidad Politécnica de Cartagena, Escuela Técnica Superior de Ingeniería Agronómica, Departamento de Ingeniería Agronómica. Paseo Alfonso XIII, 48. 30203 Cartagena (Spain)

e-mail: hector.conesa@upct.es

Abstract:

Phytomanagement of tailings requires the use of soil conditioners to favour plant establishment, but their benefits on soil microbial composition need to be assessed. The goal of this work was to evaluate the effect of two organic amendments, manure compost and biochar, on soil bacterial and fungal composition at metallic mine tailings. The addition of compost caused stronger effects in most of soil parameters and microbial composition than biochar, especially at the initial stage of the experiment. However, the higher dependence on labile organic carbon for some bacterial groups at the treatments containing compost determined their decay along time (*Flavobacteriales*, *Sphingobacteriales*) and the appearance of other taxa more dependent on recalcitrant organic matter (*Xanthomonadales*, *Myxococcales*). Biochar favoured bacterial decomposers (*Actinomycetales*) specialized in high lignin and other recalcitrant carbon compounds. Unlike bacteria, only a few fungal orders increased their relative abundances in the treatments containing compost (*Sordariales* and *Microascales*) while the rest showed a decrease or remained unaltered. The mix biochar-compost may result the best option to support a more diverse microbial population in terms of soil functionality that is able to decompose both labile and recalcitrant carbon compounds. This may favour the resilience of the system against environmental stressors.

Keywords:

mine tailings; metals; phytostabilization; microbial composition; soil amendments

Introduction

Mine tailings are wastes composed by the left-over of ore-processing activities and they are considered as the main responsible for environmental health impacts in former metallic mining areas (Conesa and Schulin, 2010). The bare areas of these tailings are usually impacted by wind and water erosion, which may spread metal(loid) enriched particles to their surroundings. Phytomanagement by phytostabilisation is considered a suitable alternative to decrease the environmental risks associated to mine tailings (Robinson et al., 2009). This technique consists of the generation of a stable vegetation cover at the tailings surfaces which decreases the erosion through the fixation of soil particles within plant rhizospheres (Mendez and Maier, 2008; Wong, 2003).

Soil conditions within tailings are unfavourable for plant growth (*e.g.* high metal(loid) concentrations, low fertility, high salinity). To overcome this issue, the addition of soil conditioners that ameliorate soil constraints are usually proposed (Clemente et al., 2010; Párraga-Aguado et al., 2015). Among the available options, those including organic amendments are highly appreciated because of their beneficial effects in soil fertility and metal(loid) mobility reduction (Clemente et al., 2010; Pardo et al., 2014a, 2014b). The final performance of these amendments depends on their nature, the rate of addition and the edaphic properties of the polluted soil. The combined use of organic amendments like compost and biochar has been suggested as an interesting option in mine tailings because of their complementarity: composts are known to provide readily available nutrients for plants; biochar is a type of organic material obtained by pyrolysis that shows high capacity to complex metals (because of its high specific surface area and negative sorption sites) and high stability to be degraded (due to its high content of recalcitrant organic carbon) (Rodríguez-Vila et al., 2017; Forján et al., 2018). Although there is a general acceptance of the benefits associated to the addition of organic amendments in tailings, recent works consider necessary to carry out a specific assessment to avoid any undesirable effects such as increases of metal(loid) available pools or negative impacts in plant ecological relationships (Martínez-Oró et al., 2019; Pardo et al., 2014a).

Recent works on the phytomanagement of mine tailings have pointed out the critical role of soil microbiology for the successful establishment of vegetation (Kolaříková et al., 2017; Sun et al., 2018). Soil microorganisms may interact with plants rhizospheres, acting like filters to decrease the phytotoxic effects of metal(loid)s and like supporters of C-N cycles, providing available nutrients for plant growth (Thavamani et al., 2017). The adverse edaphic conditions at tailings may restrict the native microbial composition to those tolerant or highly resistant groups but with limited soil functional capabilities (Risueño et al., 2020a). Nevertheless, and in spite of these limitations, those bacterial or fungal groups could play an important role in favouring the edaphic successional processes, which might support the establishment of a self-sustaining vegetation (Colin et al., 2019; Kolaříková et al., 2017; Sun et al., 2018). A key factor in these systems could be to accelerate these successional processes driven by soil microbiology through the application of organic amendments. The assessment of the effects of soil amendments is then critical, because the autochthonous microbiology of the tailings, already adapted to high metal(loid)s concentrations, salinity and low fertility conditions, might be negatively impacted by those microorganisms contained in the amendments or by the changes in soil conditions which the amendments might generate (Grandlic et al., 2008). In addition, it is important to assess whether the microbiome contained in the amendment is negative impacted by the edaphic conditions of the tailings or not.

The goal of this work was to assess the effect of the single application of two organic amendments, manure compost, as a source of labile carbon, and biochar, as a recalcitrant organic carbon material,, and their combination, in the soil bacterial and fungal composition within a metal enriched mine tailings soil. For that purpose, a dynamic mesocosm experiment was carried out comparing four different treatments: bulk tailings, bulk tailings + compost, bulk tailings + biochar and bulk tailings + compost + biochar. Soil parameters data (pH, electrical conductivity, dissolved organic carbon, dissolved nitrogen, water extractable ions and metals) and soil microbial composition data (bacteria and fungi) were collected and analysed at three different times from the beginning of the experiment: 1 month after, 3 months after and 6 months after.

Material and methods

Characterization of mine tailings soil and amendments (compost and biochar)

The mine tailings substrate was taken at a former mine tailings disposal site located at the former Mining District of Cartagena-La Unión (southeast of Spain, 0-385 m a.s.l.; 37º37' N, 0 º 49' W– 37 º 35'N, 0 º 50' W, ~ 50 km^2) (Figure SM1, Supplementary Material). Former mining activities focused on metallic sulphur minerals such as galena, pyrite or sphalerite. The local climate is semiarid, with annual rainfall of 250-300 mm, and average temperature of 18 ºC. Additional information on the environmental impacts of the mining activity in this area is available in Conesa and Schulin (2010). The amendments used in this study consisted of biochar (B) and composted manure (C). The biochar, acquired from *Proininso S.A.* (Málaga, Spain), was produced from tree wood (a mix of pine, oak and eucalyptus) after pyrolyzation at 900 ºC. The composted manure, provided by local farmers, consisted of a mixture of chicken, horse and sheep dung that was composted during three months in open air piles.

Mine tailings soil and amendments were sieved through a 2 mm mesh. Some of the properties of the tailings substrate and the two amendments are shown in Table SM1 (Supplementary Material). Tailings substrate showed neutral pH (7.38), moderate electrical conductivity (2.07 dS m^{-1}), mainly due to sulphate contribution (4130 mg kg^{-1} water extractable SO_4^{2-}), low values of organic matter (*e.g.* < 0.5 % total organic carbon) and high total metal concentrations (*e.g.* > 10 000 mg kg^{-1} Zn, Mn and Pb). Compost showed alkaline pH (8.17), high electrical conductivity (9.75 dS m^{-1}), mainly due to the contribution of chloride concentrations (~4500 mg kg^{-1} water extractable Cl^-), high total Ca (~ 22 %), K (~ 4 %), organic carbon (~ 25 %) and nitrogen (~ 2.5 %) concentrations, and some moderate total metal concentrations (*e.g.* 1255 mg kg^{-1} Zn, 1295 mg kg^{-1} Mn). Biochar was strongly alkaline (pH 9.90), with moderate electrical conductivity (2.65 dS m^{-1}), high total Ca (~7%), K (~1.8%) and organic carbon (~ 83 %) concentrations, low total nitrogen (0.70 %) and low total metal concentrations (*e.g.* 100 ± 1 mg kg^{-1} Zn, < 10 mg kg^{-1} Pb). When comparing both amendments, in terms of labile organic matter, the compost showed higher concentrations of dissolved organic carbon (4490 mg kg^{-1} DOC) than the biochar (790 mg kg^{-1} DOC). This difference also

occurred for the dissolved nitrogen concentration (1244 mg kg^{-1} DN in the compost; 8.7 mg kg^{-1} DN in the biochar).

Experimental set-up

Four treatments were carried out: S, corresponded to the bulk tailings substrate; SC consisted of bulk tailings soil and 4% (weight) of compost; SB was composed by bulk tailings soil and 4% (weight) of biochar; and SCB was the treatment combining bulk tailings with 4% of biochar and 4% of compost. The elemental composition of each substrate treatment is available in Table SM2 (Supplementary Material). Plastic pots (13 cm diameter, 15 cm height), nine for each treatment, were filled with ~ 1.5 kg of treatment substrates and randomly distributed in a climate chamber with controlled temperature/light/humidity (23 ºC during 16h light and 16 ºC during 8h darkness; 60% constant relative humidity). Pots were watered approximately at half of field capacity throughout the experiment with distilled water. Soil samples were collected at three different points: after one month (T1 samples) from the beginning of the experiment, after three months (T3 samples) and after six months (T6 samples). At each sampling point, a composite soil sample each three pots (~ 12 g) was collected in a sterilized plastic cylinder, resulting in three composite soil samples per treatment and sampling time. An aliquot of each sample was stored at -20 ºC for microbial analysis. The rest was used for soil parameters determination.

Soil parameters analyses

For all samples, a 1:2.5 soil (g):water (ml) extraction was performed by shaking for 2 hours. These extracts were filtered through nylon membrane 0.45 µm syringe filters (WICOM) and used for measuring pH, Electrical Conductivity (EC), water extractable ions (K^+, Mg^{2+}, Ca^{2+}, Cl^- and SO_4^{2-}) by using an Ion Chromatographer (Metrohm), Dissolved Organic Carbon (DOC) and Dissolved Nitrogen (DN) by using a TOC-automatic analyser (TOC-VCSH Shimadzu) and metals (Cu, Mn, Pb, Zn) by using an ICP-MS (Agilent 7500A).

DNA extraction, PCR amplification and sequencing

Microbial (bacteria and fungi) DNA was extracted from 0.25 g soil samples using the PowerSoil DNA Isolation Kit (MOBIO), according to the manufacturer's instructions. The

isolated DNA was quantified using a NanoDrop 2000 spectrophotometer. Library preparation and Illumina sequencing were carried out at the IPBLN Genomics Facility (CSIC, Granada, Spain). Raw sequence data in FASTQ format (16S and ITS2) were subjected to quality control analysis with FastQC software and prepared for taxonomic classification using the Mothur software (version 1.43.0) (Schloss et al., 2009) and following the standard operating protocol proposed by Kozich et al. (2013). Phyla (both bacteria and fungi) that showed > 5% abundance in at least one sampling and orders that showed > 3% in at least two samplings, were considered.

The relative abundance percentages at order level were used to calculated the Shannon-Weaver index (H') (Shannon and Weaver, 1963) as it follows:

$$H'p = -\sum_{i=1}^{S} p_i \, ln \, p_i$$

Where p_i is the relative frequency of the order "i" at each sample and S is the number of bacteria or fungi orders at each sample.

Statistical analyses

Statistical analyses were performed with the software IBM SPSS Statistics 24. Homogeneity of variances was tested using the Levene's test and data were transformed as needed to fit to a normal distribution. The ANOVA of repeated measures was performed in order to evaluate differences along time (within subject factor) and among treatments (between subject factor). Multiple comparisons with Bonferroni test were applied for evaluating the whole effect of the treatments while pairwise comparisons with Bonferroni test were performed at specific sampling times. The bacterial and fungal relative abundance percentages were analysed by Canonical Correspondence Analysis (CCA) for evaluating the relationship between soil fertility and microbiological groups. The CANOCO software for Windows v4.02 was used for CCA (Ter Braak and Smilauer, 1999).

Results

Changes of edaphic parameters between treatments and samplings

178 The evolution of the analysed soil parameters along the experiment is shown in Figure 1
179 and Figure SM2. The *time* factor caused a significant effect in pH ($p < 0.05$) but there was no
180 effect of the treatments on pH variation along time (*time*treatment* factor, $p > 0.05$). Initially,
181 the treatments without biochar, S and SC, showed lower pH values (pH ~ 7.7, $p < 0.05$, Figure
182 1A) than those measured at the biochar containing treatments, SB and SBC (pH ~ 7.9-8.0).
183 However, after three months, all the treatments showed similar pH values (7.4-7.7, $p > 0.05$). In
184 contrast to pH values, the *time* or *time*treatment* factors did not affect significantly the EC
185 values ($p > 0.05$). At T3 sampling point, treatments containing compost (SC and SCB) showed
186 higher EC values (~2.5 dS m^{-1}, $p<0.05$, Figure 1B)) than the other two treatments (S and SB,
187 ~2.1 dS m^{-1}). Similarly to EC values, the water extractable K^+, Ca^{2+} and SO_4^{2-} concentrations
188 (Figure SM2 A, B and D) were not affected by the *time* or *time*treatment* factors. All the
189 treatments showed similar SO_4^{2-} and Ca^{2+} concentrations (4000-4300 mg kg^{-1} SO_4^{2-} and ~2000
190 mg kg^{-1} Ca^{2+}), in contrast to K^+, whose concentrations were different among treatments (SBC >
191 SC > SB > S, $p < 0.05$) at each sampling time. The Cl^- and Mg^{2+}concentrations showed a
192 significant effect of the *time* factor but not of its interaction *time*treatment*. The Cl^-
193 concentrations of the treatments without compost were below the detection limit (12 mg kg^{-1})
194 while those containing compost showed concentrations between 70-200 mg kg^{-1} (Figure
195 SM2C). For Mg^{2+}, the concentrations in the treatments containing compost, SC and SCB, were
196 at least 1.2-fold higher ($p <0 .05$) than those obtained for the S and SB treatments (Figure
197 SM2E). The *time* and *time*treatment* factors showed a significant effect ($p < 0.05$) on the DN
198 and DOC parameters. At the three sampling points, the treatments containing compost (SC and
199 SCB) showed higher DN and DOC concentrations (~4-fold higher values, $p < 0.05$) than the
200 other two treatments (S and SB, Figure 1 C and D).
201 Lead water extractable concentrations were below the detection limit along the
202 experiment for the four treatments (< 10 µg kg^{-1}). The *time* factor significantly affected ($p <$
203 0.05) the Cu, Mn and Zn concentrations. The effect of the interaction *time*treatment* was only
204 significant for Mn ($p < 0.05$). For Cu (Figure 2A), the treatments containing compost (SC and
205 SCB) showed at least 1.5-fold higher concentrations ($p < 0.05$) than the other two treatments (S

and SB) except at the T6, where S and SB concentrations came closer to SC ($p > 0.05$). After one month from the beginning of the experiment (T1 samples), the treatments S and SB showed significant lower Mn concentrations (at least 3-fold lower values, $p < 0.05$, Figure 2B) than those treatments containing compost (SC and SCB). These differences among treatments were not detected along the whole experiment (T3, T6, $p > 0.05$), due to the decrease of Mn concentrations ($p < 0.05$) in the treatments containing compost (SC and SCB). Regarding Zn (Figure 2C), the T1 samples, showed similar Zn concentrations 210-266 μg kg^{-1} ($p < 0.05$) in all the treatments. Nevertheless, at T3 and T6, Zn concentrations decreased significantly ($p < 0.05$) in the treatments containing biochar (SB and SBC, 100-120 μg kg^{-1}) in comparison to the other treatments (S and SC, ~200 μg kg^{-1}).

Shannon diversity index for bacteria and fungi

The *time* and *time*treatment* factors caused a significant effect ($p < 0.05$, ANOVA of repeated measures) in the values of the Shannon diversity index for the bacterial orders (Figure SM3A). At the initial sampling (T1), the diversity index of the SB treatment showed significant lower values ($p < 0.05$) than the rest of the treatments (S, SC and SCB, $p > 0.05$). However, the SB treatment showed a significant increase along the experiment reaching similar diversity values ($p > 0.05$) to those obtained for the S treatment at the T6 samples. The treatments containing compost (SC and SCB) showed higher diversity ($p <0 .05$) by the end of the experiment (T6 samples) than the other two treatments (S and SB) (Figure SM3).

In relation to fungi (Figure SM3B), the modifications of the Shannon index values were significantly affected by the *time* factor ($p < 0.05$, ANOVA of repeated measures) but not by the *time*treatment* interaction ($p > 0.05$). Unlike bacteria, fungal diversity did not show differences among treatments at any sampling time ($p > 0.05$).

Influence of treatments on bacterial composition

Bacterial groups belonging to *Proteobacteria, Actinobacteria, Acidobacteria, Bacteroidetes, Planctomycetes* and *Gemmatimonadetes* phyla were the most abundant in our study (Figure 3). There was a significant effect of the *time* factor in the variations of relative abundance percentages for all the bacterial phyla ($p < 0.05$, ANOVA of repeated measures). In

addition, the interaction *time*treatment* affected significantly almost all phyla relative abundances ($p < 0.05$), with the exception of the *Actinobacteria* phylum ($p > 0.05$).

The *Proteobacteria* phylum showed relative abundances higher than 20% across all the treatments and samplings (Figure 3A). The treatments with compost (SC and SCB) showed higher abundances ($p < 0.05$) than the other two treatments without compost (S and SB). For each treatment, the percentages of *Proteobacteria* abundance at the T6 samples did not differ ($p < 0.05$) from those obtained at the first sampling time (T1). The main orders which contribute to this phylum included *Rhizobiales* and *Sphingomonadales* (*Alphaproteobacteria* class), *Betaproteobacteria uncl.* and *Burkholderiales* (*Betaproteobacteria* class), *Myxococcales* (*Deltaproteobacteria* class) and *Pseudomonadales*, *Gammaproteobacteria uncl.* and *Xanthomonadales* orders (*Gammaproteobacteria* class) (Figure SM4). These orders showed a contrasting variation in their abundances along the experiment: while some orders did not vary their relative abundance (*e.g. Sphingomonadales* and *Rhizobiales*, Figure SM4A and C); other groups showed significant decreases or increases of relative abundances (*e.g. Pseudomonadales* and *Burkholderiales* and *e.g. Myxococcales*, respectively, Figure SM4B, H and E).

Actinobacteria was the only phylum in which no significant ($p > 0.05$) interaction *time*treatment* occurred. In contrast to what occurred for *Proteobacteria*, the treatments without the presence of compost (S and SB) showed higher relative abundance percentages of *Actinobacteria* (about double, $p<0.05$) than those treatments containing compost (SC and SCB, Figure 3B). Three main orders were recorded within this phylum: *Actinomycetales*, which decreased its abundance percentage ($p < 0.05$) along the experiment in all treatments (Figure SM5B); *Acidimicrobiales*, which showed the opposite behaviour, a significant increase of relative abundance ($p < 0.05$) along the experiment in all treatments (Figure SM5A); and *Solirubrobacterales*, which showed a significant increase ($p < 0.05$) but only in two treatments, SC and SB (Figure SM5H).

For the *Acidobacteri*a phylum, at the first sampling (T1), the treatments without compost (S and SB) showed higher relative abundances ($p < 0.05$) than the other two treatments (SC and SCB, Figure 3C). Along the experiment (from T1 to T6) there was a significant

increase ($p < 0.05$) of the abundance percentages in the three amended treatments (SC, SB and SCB), while the bulk tailing treatment (S) maintained similar data to those obtained at T1. For instance, SC showed three times higher abundance values in T6 in relation to T1. The main identified order corresponded to the *Gp7* (Figure SM5G).

In the case of the *Bacteroidetes* phylum, the treatments without compost, S and SB, showed lower relative abundance values (at least 10-fold lower, $p < 0.05$) than the treatments containing compost (SC and SCB) along the whole experiment (Figure 3D). There was no variation in the relative abundance values throughout the experiment ($p > 0.05$) in the two treatments without compost (S and SB). However, a significant decrease in the abundance percentages occurred in the two treatments containing compost (SC and SCB) from T1 to the final T6 samples ($p < 0.05$). This tendency was similar for the three main bacterial orders (Figure SM5D, E and I) belonging to this phylum: *Sphingobacteriales*, *Flavobacteriales* and *Cytophagales*.

For the *Planctomycetes* phylum (Figure 3E), and its main contributing order *Planctomycetales* (Figure SM5F), the relative abundance in the treatments containing compost, SC and SCB, raised along with the experiment, from similar abundance data ($p > 0.05$) to those obtained for the S treatment at T1 to 2-fold-higher percentages ($p < 0.05$) than the treatments without compost, S and SB, at T6.

The *Gemmatimonadetes* phylum (Figure 3F), and its order *Gemmatimonadales* (Figure SM5C), showed significantly higher abundance percentages ($p < 0.05$) in the treatments without compost (S and SB) than the other two treatments (SC and SCB). There was a significant increase in the relative abundance (about half higher, $p < 0.05$) from T1 to T6 for the S, SC and SB treatments.

Results of the CCA for bacterial orders are shown in the Figure 4 and Tables SM3 and SM4. Data analyses resulted significant (Monte-Carlo test, $p < 0.05$) and the first axis explained 63.1 % of the variance (Table SM3). The positive side of the CCA1-axis was defined by increasing concentrations of DOC, DN, extractable elements (K^+, Cu and Mn) and EC values ($r > 0.64$ for DN, DOC, EC, K^+, Cu and Cu). In contrast, the negative side of the CCA1-axis was

conditioned by lower values of the aforementioned parameters. In this way, the CCA1-axis (Figure 4) segregated the bacterial orders between those samples coming from the treatments containing compost, SC (rectangles) and SCB (stars), which were mostly depicted on the positive side of CCA1-axis, and those belonging to the treatments with no addition of compost, S (circles) and SB (diamonds), which were depicted on the negative side of the CCA1-axis.

The CCA2–axis (Figure 4 and Tables SM3 and SM4) was mainly defined by pH ($r = -0.76$) and segregated the samples attending to the sampling time (T1, T3 and T6). For instance, all samples from T1 (white symbols) were depicted on the negative side of the CCA2-axis while the samples of T6 (black symbols) were depicted on the positive side of the CCA2-axis. This segregation was higher for the treatments containing compost (SC, SCB) and the single biochar (SB) treatment than for the bulk soil treatment (S).

Influence of treatments on fungal composition

Fungal taxa belonging to the *Ascomycota*, *Basidiomycota* and *Chytridiomycota* phyla were detected in the four treatments (Figure 5). There was a significant effect of the *time* factor in the variations of relative abundance percentages for the *Basidiomycota* phyla ($p < 0.05$, ANOVA of repeated measures) but not for the other two phyla. The interaction *time*treatment* did not result significant for any phyla ($p > 0.05$, ANOVA of repeated measures).

The *Ascomycota* phylum showed relative abundances higher than 60 % in all treatments at every sampling time (Figure 5A). Significant differences among treatments occurred at the last sampling time (T6), when the SC treatment (~ 63 %) showed lower abundance ($p < 0.05$) than the treatments without compost (S and SB, ~ 80 % abundance). This was mainly due to the contribution of three orders, *Eurotiales*, *Pleosporales* and *Chaetothyriales*, which showed higher abundances in the treatments without compost (S and SB) than the other two treatments (SCB and SC, Figure SM6B, C and G). In contrast, the *Sordariomycetes uncl.*, *Sordariales* and *Microascales* orders, showed at least 2-fold higher relative abundances ($p < 0.05$) at the treatments containing compost than the treatments without compost (S and SB, Figure SM6D, E

316 and H). The *Hypocreales* and *Ascomycota uncl.* orders did not show a clear pattern throughout
317 the experiment (Figure SM6A and F).

318 The *Basidiomycota* phylum showed a decrease of relative abundances along time ($p <$
319 0.05) in the S, SC and SCB treatments (Figure 5B). By contrast, the abundance percentages of
320 the SB treatment remained unaltered throughout the experiment and they were higher than the
321 rest of the treatments (~ 10 %, $p < 0.05$) at the last sampling time (T6). The main orders
322 identified within this phylum were *Agaricales* and *Thelephorales* (Figure SM6 I and J). The
323 latter showed higher relative abundances in the S and SB treatments (around 5%) at the first
324 sampling (T1) compared with the SC and SCB samples. However, they showed a sharp
325 decreased (half of that value) along the experiment. As it occurred for the corresponding
326 phylum data, both *Agaricales* and *Thelephorales* showed 2-fold higher relative abundance
327 percentages ($p < 0.05$) in the SB treatment than the rest of the treatments.

328 The *Chytridiomycota* phylum (Figure 5C) was only composed by the *Spizellomycetales*
329 order (Figure SM6K). This fungal group did not show significant differences among treatments
330 along the experiment ($p > 0.05$).

331 Results of the CCA of fungal orders were significant (Monte-Carlo test, $p < 0.05$) and
332 the first axis explained 78.3 % of the variance (Figure 6 and Tables SM3 and SM4). The
333 positive side of the CCA1-axis was mainly defined by increasing values of EC, fertility
334 parameters (K^+, DOC and DN), and water extractable Cu concentrations (*e.g.* $r = 0.90$ for EC
335 and K^+). The samples coming from the treatments containing compost, SC (rectangles) and SCB
336 (stars), were mostly depicted on the positive side of CCA1-axis, and those belonging to the
337 treatments without compost, S (circles) and SB (diamonds) were depicted on the negative side
338 of the CCA1-axis.

339 On the other hand, the positive side of the CCA2–axis was mainly defined by high pH
340 values ($r = 0.78$) and to a lesser extent by increasing values of Mn concentrations ($r = 0.39$),
341 while the negative side was mainly conditioned by lower values of these two parameters. So, a

gradient was generated along the CCA2-axis, which segregated the samples from the amended treatments (SB-diamonds, SC-rectangles and SCB-stars) attending to the sampling times (T1, T3 and T6, Figure 6): the samples from T1 were depicted on the positive side of the CCA2-axis, while the corresponding T3 and T6 samples appeared consecutively on the negative side of the CCA2-axis. This segregation was not so evident for the samples of the bulk tailings treatment (circles).

Discussion

Effects of amendments on soil parameters

Soil microbial composition can be easily modified by the addition of amendments such as compost or biochar (Abujabhah et al., 2016). The extent of these changes depends on soil properties such as pH, moisture and especially, organic carbon (Asemaninejad et al., 2021; Zornoza et al., 2015). In our experiment, the addition of compost caused stronger effects in most of soil parameters than the biochar, especially in the SC treatment (Figure 1, 2 and SM2). This might be explained by the larger pool of readily available compounds released by the compost, especially at the T1. After this initial flush, there was a gradual decrease in the DOC and or extractable Mn and Zn concentrations, probably because the labile pools became shorter with the time (Parraga-Aguado et al., 2015). In contrast, the higher recalcitrant organic matter content (low labile pool and higher stability) and lower metal load of the biochar in relation to the compost conditioned lower modifications in those parameters at the SB treatment (Rodríguez-Vila et al., 2016; You et al., 2018). In the treatment combining biochar-compost, most of the studied parameters were mainly influenced by compost (e.g. DOC) and results differed from those at the single composted treatment in the DN (that was lower) and K^+ (that was higher) values. Lower DN concentrations in the combined biochar-compost treatment could be attributed to the retention of water-soluble nitrogen by biochar (Haider et al., 2016).

Effects of amendments in bacterial composition

In terms of phylum abundance, the compost caused a significant increase in the percentages of *Proteobacteria* and *Bacteroidetes* (Figure 3). Similar increases in the relative

abundance of these two phyla were also reported by Cao et al. (2020) in compost amended coal mining soils.

The controlled conditions of the growth chamber (temperature, light and humidity) might influence the relative abundances observed for several bacterial groups along the experiment. This could explain why several bacterial orders (Figures SM4 and SM5) showed similar trends in all treatments (including the bulk tailings, S). This was the case of *Myxococcales, Burkholderiales* and *Gammaproteobacteria uncl.* from the *Proteobacteria* phylum, *Acidimicrobiales* and *Actinomycetales* from the *Actinobacteria* phylum and *Gemmatimonadales* from the *Gemmatimonadetes* phylum.

In the initial T1 sampling, the presence of compost showed a stronger effect in soil microbial composition than the biochar. This was clearly shown in Figure 4, where the main gradient defined by the CCA1-axis segregated between samples with and without compost. The initial effect of biochar on the bacterial composition in the SB treatment was restricted to a lower number of bacterial orders than the compost, resulting also in a lower microbial diversity index (Figure SM3). The modifications of bacterial composition promoted by biochar are highly dependent on its type of raw materials and conditions of pyrolysis, which determine the final effects in soils (Abujabhah et al., 2016)**.** In this case, the conditions of production of the biochar at high temperature (900 ºC) generated a highly stable material with low carbon accessibility for microorganisms (You et al., 2018). The biochar only caused a significant increase of the relative abundance of the order *Actinomycetales,* belonging to the phylum *Actinobacteria*. This could be due to the specific ability of *Actinomycetales* taxa for degrading hard to decompose organic materials (Bhatti et al., 2017; Tian et al., 2014) and dealing with high concentrations of inorganic contaminants (Risueño et al., 2020a), which could have provided a competitive advantage in relation to the rest of bacteria.

Initially (at T1), the treatments containing compost showed a bacterial composition characterized by orders which are related to the degradation of organic matter (Figures 4, SM4 and SM5), such as *Sphingobacteriales, Flavobacteriales* and *Cytophagales* from the *Bacteroidetes* phylum (Fierer et al., 2007), *Xanthomonadales, Pseudomonadales,*

Burkholderiales and *Gammaproteobacteria uncl* from the *Proteobacteria* phylum (Kersters et al., 2006; Tang et al., 2019) or *Planctomycetales* from the *Planctomycetes* phylum (Wiegand et al., 2018). However, some of these orders such as *Flavobacteriales, Sphingobacteriales, Pseudomonadales,* and *Burkholderiales* showed a decrease of their abundance percentages throughout the experiment. The reasons of this drop might include a toxic effect of the tailings edaphic properties (*e.g.* salinity, metals) and/or a correlation ($r > 0.5$, $p < 0.01$) with the decreasing concentrations of dissolved organic carbon which serves as a source of energy. Several studies have shown the relevance of the dynamics of soil organic matter as a critical driver which determines the shifts in microbial composition during the reclamation of mining soils (Asemaninejad et al., 2021; Zornoza et al., 2015). For instance, most taxa included in the *Bacteroidetes* phylum are considered copiotrophic and their relative abundances are highly dependent of the availability of labile carbon (Fierer et al., 2007). In our study, the relative abundances of the orders belonging to the *Bacteroidetes* phylum, *Flavobacteriales, Sphingobacteriales and Cytophagales,* were positively correlated with the decreasing DOC concentrations ($r > 0.8$, $p < 0.01$). In the case of the *Cytophagales* order, the decrease of the abundance percentages along the time was less sharp, probably because of its less dependence on labile organic matter and better adaptation to degrade more complex organic compounds (Kirchman, 2002; Reichenbach, 2006).

Several bacterial taxa maintained the initial increase of abundance percentages promoted by the compost addition, showing certain level of adaptation to the tailings properties and decreasing DOC concentrations throughout the experiment. This group includes the *Proteobacteria* orders belonging to the *Gammaproteobacteria* class, *Xanthomonadales,* and *Gammaproteobacteria uncl.,* which have been observed as representative of the microbiome in mature edaphic successional stages of reclaimed mine tailings (Colin et al., 2019). Some orders belonging to *Gammaproteobacteria* class are also known by their lithotrophic nature (Wakelin et al., 2012) and capacity for adaptation to extreme soil conditions such as low nutrient concentrations or high salinity, including barren tailing materials (Sun et al., 2018). The high abundances of these *Gammaproteobacteria* orders can be also supported by their well-known

capacity of dealing with high SO_4^{2-} soil concentrations (Edwardson and Hollibaugh, 2017; Shimkets et al., 2006) for what they showed a significant correlation ($r > 0.6$, $p < 0.01$). The *Myxococcales* order belonging to the *Deltaproteobacteria* class showed a stability in its abundance percentages throughout the experiment. This can be explained by their ability to break down a high variety of organic compounds including those considered as hard to decompose (Shimkets et al., 2006) which could result in a lower dependence on labile organic matter (no significant correlation between abundance percentages and DOC). The *Planctomycetales* order (*Planctomycetes* phylum) showed higher abundances in the T6 than in the T1 samples of the treatments containing compost. This may be due to its lower competitive capacity within nutrient rich media (T1) (Burges et al., 2020) and its ability to decompose more complex organic matter compounds (Probandt et al., 2017; Wiegand et al., 2018), as it was expected to occur by the end of the experiment, at T6.

The abundance of the *Proteobacteria* orders, *Rhizobiales* and *Sphingomonadales*, were unaffected by the amendments. The bacterial taxa belonging to the *Rhizobiales* order play an important role in the dynamics of N in the soil (e.g. N_2 fixation) but their activity is strongly dependent on the presence of plants (Burges et al., 2020). The *Sphingomonadale*s order is also related to plant rhizospheres and has been previously reported as a plant growth promoting bacteria in extreme environmental conditions including mine tailings (Risueño et al., 2020b) or saline soils (Oliveira et al., 2014)**.** That strong dependence on plant rhizospheres of these two orders may explain the no variation of their abundance percentages during the experiment.

A group of bacterial orders was negatively impacted by the addition of compost. These orders include oligotrophic bacteria whose abundance is favoured in extreme environments but negatively affected in nutrient rich systems, where fast-growing bacteria show a better competitive behaviour (Burges et al., 2020). Similarly to what occurred at our experiment, Wang et al. (2017) showed a decrease in the relative abundance of *Gp7* when adding organic fertilizers into an orchard soil, while Burges et al. (2020) observed a decrease of *Gemmatimonadales* abundance percentages after adding compost in a metal polluted soil.

452 Risueño et al. (2020a) reported *Acidimicrobiales* as one of the most abundant orders in bulk
453 tailings areas but it was displaced by other organotrophic bacteria in the presence of vegetation.

454 ***Effects of amendments in fungal composition***

455 Several studies on the reclamation of polluted sites have shown *Ascomycota* and
456 *Basidiomycota* as the most abundant phyla in these systems (Bastida et al., 2017; Ma et al.,
457 2013). The phylum *Ascomycota* is known to be the largest fungal group in ecosystems and
458 includes from saprotrophs, necrotrophic or biotrophic parasites of plants and animals to
459 endophytes or mutualistic symbionts (Webster and Weber, 2007). This wide range of life
460 conditions for the taxa of this phylum allows their adaptation to several polluted environments
461 such as mine impacted areas (Narendrula-Kotha and Nkongolo, 2017; Op De Beeck et al., 2015;
462 Rosenfeld et al., 2018). On the other hand, the taxa belonging to the *Basidiomycota* phylum are
463 more abundant in non-polluted areas or associated to vegetation in restored areas (Op De Beeck
464 et al., 2015), probably because of their more specific lifestyles as ectomycorrhizal fungi or
465 saprotrophs (Webster and Weber, 2007). In addition, the saprophytic fungal taxa of the
466 *Basidiomycota* phylum are involved in the decomposition of recalcitrant organic materials
467 which normally cannot be accessed by those taxa of the *Ascomycota* phylum (Lundell et al.,
468 2010). Finally, the *Chytridiomycota* phylum was also identified although in lower abundance
469 percentages (Figure 5C). The taxa from this phylum can also colonize a wide variety of
470 environments, being considered the order identified in this study, *Spizellomycetales*, of
471 saprotrophic behaviour (Webster and Weber, 2007).

472 Similar to what occurred with bacteria, the addition of compost showed a stronger effect
473 on fungal relative abundance than the biochar. This was clearly shown in Figure 6, where the
474 main gradient defined by the X-axis segregated the samples with and without compost.
475 However, unlike bacteria, only a few orders showed an increase of the relative abundances in
476 the treatments containing compost (*Sordariomycetes uncl.*, *Sordariales* and *Microascales*) while
477 the rest showed a decrease (*Eurotiales, Pleosporales, Chaetothyriales* and *Telephorales*) o no
478 clear effect (*Hypocreales, Ascomycota uncl. Agaricales* and *Spizellomycetales*). Similar to what

occurred in our experiment, Siles et al. (2014) reported an increase in the abundance percentages of *Sordariomycetes* uncl. and *Sordariales* and a decrease of *Eurotiales* and *Pleosporales* in a pot trial which used olive residues as an amendment. These authors explained the contrasting behaviour of these fungal orders by their response to specific compounds contained in the amendment, whether beneficial or toxic. In other fungal orders, such as the case of *Telephorales* the decrease in the abundance percentages due to compost could be related to a lower competitive behaviour in relation to other fungal taxa (Courty et al., 2010). This revealed the strong relationship between fungal groups and the characteristics of the C source, which can strongly shape fungal composition (Bastida et al., 2015, 2013). In addition, the treatments containing compost showed significant higher electrical conductivity (Figure 1B), which may have also a strong effect in selecting those salinity tolerant taxa (Zeng et al., 2020).

No significant effects of biochar on abundance percentages was found for any order. Several authors have reported no effects (Elzobair et al., 2016) or even a depletion of fungal activity (Warnock et al., 2010; Liao et al., 2016) after applying different types of biochar in agricultural soils. In spite on these results, other studies have determined substantial positive effects on the application of biochar on fungal growth (e.g. Abujabhah et al., 2016; Warnock et al., 2007). These discrepancy of results reveal the importance of the biochar properties (nature, C:N ratio, temperature during pyrolysis) in the final fungal taxa composition (Elzobair et al., 2016) along with the necessity of performing controlled comparative experiments to elucidate the feasibility of the organic amendments in the enhancement of microbiological activity.

Conclusions

The results derived from the application of two organic amendments, manure compost and biochar, and their combination revealed contrasting effects of each type of amendment on some edaphic parameters and in turn, on microbial composition. While the presence of compost determined the occurrence of microbial groups with strong dependence on labile carbon, biochar favoured decomposers specialized in high lignin or other recalcitrant carbon compounds. The combined treatment biochar-compost may result the best option to support a

more diverse microbial population in terms of soil functionality that is able to decompose both labile and recalcitrant carbon compounds. This may favour the resilience of the system against environmental stressors. These conditions may allow the development of some microbial groups which also play an important role on plant establishment. Future research should focus on evaluating how the microbial community resulted from amended tailings is able to fit within the rhizospheric microbiome of the plant species employed in the phytomanagement of these systems.

Acknowledgements

Financial support for this research was provided by *FEDER/Ministerio de Ciencia e Innovación -Agencia Estatal de Investigación*- Project CGL2017-82264-R. The experiment was performed at the facilities of the *Instituto of Biotecnología Vegetal* (IBV) of the *Universidad Politécnica de Cartagena*. We thank Borja Rojas from IBV for its help in bioinformatics processing and Dr. Francisco J. Jiménez from *BIOCYMA, Consultora en Calidad y Medio Ambiente* (Murcia) for his help in field sampling.

References

Abujabhah, I.S., Bound, S.A., Doyle, R., Bowman, J.P., 2016. Effects of biochar and compost amendments on soil physico-chemical properties and the total community within a temperate agricultural soil. Appl. Soil Ecol. 98, 243–253. https://doi.org/10.1016/j.apsoil.2015.10.021

Asemaninejad, A., Langley, S., Mackinnon, T., Spiers, G., Beckett, P., Mykytczuk, N., Basiliko, N., 2021. Blended municipal compost and biosolids materials for mine reclamation: Long-term field studies to explore metal mobility, soil fertility and microbial communities. Sci. Total Environ. 760, 143393. https://doi.org/10.1016/j.scitotenv.2020.143393

Bastida, F., Hernández, T., Albaladejo, J., García, C., 2013. Phylogenetic and functional changes in the microbial community of long-term restored soils under semiarid climate. Soil Biol. Biochem. 65, 12–21. https://doi.org/10.1016/j.soilbio.2013.04.022

Bastida, F., Selevsek, N., Torres, I.F., Hernández, T., García, C., 2015. Soil restoration with organic amendments: Linking cellular functionality and ecosystem processes. Sci. Rep. 5, 1–12. https://doi.org/10.1038/srep15550

Bastida, F., Torres, I.F., Hernández, T., García, C., 2017. The impacts of organic amendments: Do they confer stability against drought on the soil microbial community? Soil Biol. Biochem. 113, 173–183. https://doi.org/10.1016/j.soilbio.2017.06.012

Bhatti, A.A., Haq, S., Bhat, R.A., 2017. Actinomycetes benefaction role in soil and plant health. Microb. Pathog. 111, 458–467. https://doi.org/10.1016/j.micpath.2017.09.036

Burges, A., Fievet, V., Oustriere, N., Epelde, L., Garbisu, C., Becerril, J.M., Mench, M., 2020. Long-term phytomanagement with compost and a sunflower – Tobacco rotation influences the structural microbial diversity of a Cu-contaminated soil. Sci. Total Environ. 700, 134529. https://doi.org/10.1016/j.scitotenv.2019.134529

Cao, Y., Zhou, B., Wang, X., Meng, H., Zhang, J., Li, L., Hong, J., 2020. Different fertilization treatments in coal mining-affected soils change bacterial populations and enable soil reclamation. Ann. Microbiol. 70, 47. https://doi.org/10.1186/s13213-020-01589-2

Clemente, R., Hartley, W., Riby, P., Dickinson, N.M., Lepp, N.W., 2010. Trace element

mobility in a contaminated soil two years after field-amendment with a greenwaste compost mulch. Environ. Pollut. 158, 1644–1651. https://doi.org/10.1016/j.envpol.2009.12.006

Colin, Y., Goberna, M., Verdú, M., Navarro-Cano, J.A., 2019. Successional trajectories of soil bacterial communities in mine tailings: The role of plant functional traits. J. Environ. Manage. 241, 284–292. https://doi.org/10.1016/j.jenvman.2019.04.023

Conesa, H.M., Schulin, R., 2010. The Cartagena-La Unión mining district (SE spain): A review of environmental problems and emerging phytoremediation solutions after fifteen years research. J. Environ. Monit. 12, 1225–1233. https://doi.org/10.1039/c000346h

Courty, P.E., Buée, M., Diedhiou, A.G., Frey-Klett, P., Le Tacon, F., Rineau, F., Turpault, M.P., Uroz, S., Garbaye, J., 2010. The role of ectomycorrhizal communities in forest ecosystem processes: New perspectives and emerging concepts. Soil Biol. Biochem. 42, 679–698. https://doi.org/10.1016/j.soilbio.2009.12.006

Edwardson, C.F., Hollibaugh, J.T., 2017. Metatranscriptomic analysis of prokaryotic communities active in sulfur and arsenic cycling in Mono Lake, California, USA. ISME J. 11, 2195–2208. https://doi.org/10.1038/ismej.2017.80

Elzobair, K.A., Stromberger, M.E., Ippolito, J.A., Lentz, R.D., 2016. Contrasting effects of biochar versus manure on soil microbial communities and enzyme activities in an Aridisol. Chemosphere 142, 145–152. https://doi.org/10.1016/j.chemosphere.2015.06.044

Fierer, N., Bradford, M.A., Jackson, R.B., 2007. Toward anecological classification of soil bacteria. Ecology 88, 1354–1364. https://doi.org/10.1890/05-1839

Grandlic, C.J., Mendez, M.O., Chorover, J.O.N., 2008. Plant Growth-Promoting Bacteria for Phytostabilization of Mine Tailings. Environ. Sci. Technol. 42, 2079–2084. https://doi.org/10.1021/es072013j

Haider, G., Steffens, D., Müller, C., Kammann, C.I., 2016. Standard Extraction Methods May Underestimate Nitrate Stocks Captured by Field-Aged Biochar. J. Environ. Qual. 45, 1196–1204. https://doi.org/10.2134/jeq2015.10.0529

Kersters, K., De Vos, P., Gillis, M., Swings, J., Vandamme, P., Stackebrandt, Erko, 2006.

578 Introduction to the Proteobacteria, in: Dworkin, M., Falkow, S., Rosenberg, E., Schleifer,
579 K.-H., Stackebrandt, E. (Eds.), The Prokaryotes: A Handbook on the Biology of Bacteria.
580 Springer., New York, NY, USA, pp. 3–37. https://doi.org/10.1007/0-387-30745-1_1
581 Kirchman, D.L., 2002. The ecology of Cytophaga-Flavobacteria in aquatic environments.
582 FEMS Microbiol. Ecol. 39, 91–100. https://doi.org/10.1016/S0168-6496(01)00206-9
583 Kolaříková, Z., Kohout, P., Krüger, C., Janoušková, M., Mrnka, L., Rydlová, J., 2017. Root-
584 associated fungal communities along a primary succession on a mine spoil: Distinct
585 ecological guilds assemble differently. Soil Biol. Biochem. 113, 143–152.
586 https://doi.org/10.1016/j.soilbio.2017.06.004
587 Kozich, J.J., Westcott, S.L., Baxter, N.T., Highlander, S.K., Schloss, P.D., 2013. Development
588 of a Dual-Index Sequencing Strategy and Curation Pipeline for Analyzing Amplicon
589 Sequence Data on the MiSeq Illumina Sequencing Platform. Appl. Environ. Microbiol. 79,
590 5112–5120. https://doi.org/10.1128/aem.01043-13
591 Liao, N., Li, Q., Zhang, W., Zhou, G., Ma, L., Min, W., Ye, J., Hou, Z., 2016. Effects of biochar
592 on soil microbial community composition and activity in drip-irrigated desert soil. Eur. J.
593 Soil Biol. 72, 27–34. https://doi.org/10.1016/j.ejsobi.2015.12.008
594 Lundell, T.K., Mäkelä, M.R., Hildén, K., 2010. Lignin-modifying enzymes in filamentous
595 basidiomycetes - Ecological, functional and phylogenetic review. J. Basic Microbiol. 50,
596 5–20. https://doi.org/10.1002/jobm.200900338
597 Ma, A., Zhuang, X., Wu, J., Cui, M., Lv, D., Liu, C., Zhuang, G., 2013. Ascomycota Members
598 Dominate Fungal Communities during Straw Residue Decomposition in Arable Soil. PLoS
599 One 8, 1–9. https://doi.org/10.1371/journal.pone.0066146
600 Martínez-Oró, D., Párraga-Aguado, I., Querejeta, J.I., Álvarez-Rogel, J., Conesa, H.M., 2019.
601 Nutrient limitation determines the suitability of a municipal organic waste for
602 phytomanaging metal(loid) enriched mine tailings with a pine-grass co-culture.
603 Chemosphere 214, 436–444. https://doi.org/10.1016/j.chemosphere.2018.09.147
604 Mendez, M.O., Maier, R.M., 2008. Phytoremediation of mine tailings in temperate and arid
605 environments. Rev. Environ. Sci. Biotechnol. 7, 47–59. https://doi.org/10.1007/s11157-

606 007-9125-4
607 Narendrula-Kotha, R., Nkongolo, K.K., 2017. Bacterial and fungal community structure and
608 diversity in a mining region under long-term metal exposure revealed by metagenomics
609 sequencing. Ecol. Genet. Genomics 2, 13–24. https://doi.org/10.1016/j.egg.2016.11.001
610 Oliveira, V., Gomes, N.C.M., Cleary, D.F.R., Almeida, A., Silva, A.M.S., Simões, M.M.Q.,
611 Silva, H., Cunha, Â., 2014. Halophyte plant colonization as a driver of the composition of
612 bacterial communities in salt marshes chronically exposed to oil hydrocarbons. FEMS
613 Microbiol. Ecol. 90, 647–662. https://doi.org/10.1111/1574-6941.12425
614 Op De Beeck, M., Ruytinx, J., Smits, M.M., Vangronsveld, J., Colpaert, J. V., Rineau, F., 2015.
615 Belowground fungal communities in pioneer Scots pine stands growing on heavy metal
616 polluted and non-polluted soils. Soil Biol. Biochem. 86, 58–66.
617 https://doi.org/10.1016/j.soilbio.2015.03.007
618 Pardo, T., Bernal, M.P., Clemente, R., 2014a. Efficiency of soil organic and inorganic
619 amendments on the remediation of a contaminated mine soil: I. Effects on trace elements
620 and nutrients solubility and leaching risk. Chemosphere 107, 121–128.
621 https://doi.org/10.1016/j.chemosphere.2014.03.023
622 Pardo, T., Clemente, R., Alvarenga, P., Bernal, M.P., 2014b. Efficiency of soil organic and
623 inorganic amendments on the remediation of a contaminated mine soil: II. Biological and
624 ecotoxicological evaluation. Chemosphere 107, 101–108.
625 https://doi.org/10.1016/j.chemosphere.2014.03.017
626 Parraga-Aguado, I., González-Alcaraz, M.N., Schulin, R., Conesa, H.M., 2015. The potential
627 use of Piptatherum miliaceum for the phytomanagement of mine tailings in semiarid areas:
628 Role of soil fertility and plant competition. J. Environ. Manage. 158, 74–84.
629 https://doi.org/10.1016/j.jenvman.2015.04.041
630 Probandt, D., Knittel, K., Tegetmeyer, H.E., Ahmerkamp, S., Holtappels, M., Amann, R., 2017.
631 Permeability shapes bacterial communities in sublittoral surface sediments. Environ.
632 Microbiol. 19, 1584–1599. https://doi.org/10.1111/1462-2920.13676
633 Reichenbach, H., 2006. The Order Cytophagales, in: Dworkin, M., Falkow, S., Rosenberg, E.,

Schleifer, K., Stackebrandt, E. (Eds.), The Prokaryotes. Springer, New York, NY, pp. 549–590. https://doi.org/https://doi.org/10.1007/0-387-30747-8_20

Risueño, Y., Petri, C., Conesa, H.M., 2020a. The importance of edaphic niches functionality for the sustainability of phytomanagement in semiarid mining impacted ecosystems. J. Environ. Manage. 266, 110613. https://doi.org/10.1016/j.jenvman.2020.110613

Risueño, Y., Petri, C., Conesa, H.M., 2020b. Edaphic factors determining the colonization of semiarid mine tailings by a ruderal shrub and two tree plant species: Implications for phytomanagement. Chemosphere 259, 127425. https://doi.org/10.1016/j.chemosphere.2020.127425

Robinson, B.H., Bañuelos, G., Conesa, H.M., Evangelou, M.W.H., Schulin, R., 2009. The Phytomanagement of Trace Elements in Soil. CRC. Crit. Rev. Plant Sci. 28, 240–266. https://doi.org/10.1080/07352680903035424

Rodríguez-Vila, A., Asensio, V., Forján, R., Covelo, E.F., 2016. Carbon fractionation in a mine soil amended with compost and biochar and vegetated with Brassica juncea L. J. Geochemical Explor. 169, 137–143. https://doi.org/10.1016/j.gexplo.2016.07.021

Rosenfeld, C.E., James, B.R., Santelli, C.M., 2018. Persistent bacterial and fungal community shifts exhibited in seleniumcontaminated reclaimed mine soils. Appl. Environ. Microbiol. 84, 1–18. https://doi.org/10.1128/AEM.001394-18

Schlesner, H., 1994. The Development of Media Suitable for the Microorganisms Morphologically Resembling Planctomyces spp., Pirellula spp., and other Planctomycetales from Various Aquatic Habitats Using Dilute Media. Syst. Appl. Microbiol. 17, 135–145. https://doi.org/10.1016/S0723-2020(11)80042-1

Schloss, P.D., Westcott, S.L., 2011. Assessing and improving methods used in operational taxonomic unit-based approaches for 16S rRNA gene sequence analysis. Appl. Environ. Microbiol. 77, 3219–3226. https://doi.org/10.1128/AEM.02810-10

Shannon, C.E., Weaver, W., 1963. The Mathematical Theory of Communications. University of Illinois Press, Chicago, United States.

Shimkets, L.J., Dworkin, M., Reichenbach, H., 2006. The Myxobacteria., in: Dworkin, M.,

662 Falkow, S., Rosenberg, E., Schleifer, K., Stackebrandt, E. (Eds.), The Prokaryotes. New
663 York, NY, USA: Springer, New York, NY, pp. 31–115. https://doi.org/10.1007/0-387-
664 30747-8_3
665 Siles, J.A., Rachid, C.T.C.C., Sampedro, I., García-Romera, I., Tiedje, J.M., 2014. Microbial
666 diversity of a Mediterranean soil and its changes after biotransformed dry olive residue
667 amendment. PLoS One 9, e103035. https://doi.org/10.1371/journal.pone.0103035
668 Sun, X., Zhou, Y., Tan, Y., Wu, Z., Lu, P., Zhang, G., Yu, F., 2018. Restoration with pioneer
669 plants changes soil properties and remodels the diversity and structure of bacterial
670 communities in rhizosphere and bulk soil of copper mine tailings in Jiangxi Province,
671 China. Environ. Sci. Pollut. Res. 25, 22106–22119. https://doi.org/10.1007/s11356-018-
672 2244-3
673 Tang, H., Xiao, X., Xu, Y., Li, C., Cheng, K., Pan, X., Li, W., 2019. Utilization of carbon
674 sources in the rice rhizosphere and nonrhizosphere soils with different long-term
675 fertilization management. J. Basic Microbiol. 59, 621–631.
676 https://doi.org/10.1002/jobm.201800736
677 Ter Braak, C.J., Smilauer, P., 1999. Canoco for Windows v. 4.02.
678 Thavamani, P., Samkumar, R.A., Satheesh, V., Subashchandrabose, S.R., Ramadass, K., Naidu,
679 R., Venkateswarlu, K., Megharaj, M., 2017. Microbes from mined sites: Harnessing their
680 potential for reclamation of derelict mine sites. Environ. Pollut. 230, 495–505.
681 https://doi.org/10.1016/j.envpol.2017.06.056
682 Tian, J.H., Pourcher, A.M., Bouchez, T., Gelhaye, E., Peu, P., 2014. Occurrence of lignin
683 degradation genotypes and phenotypes among prokaryotes. Appl. Microbiol. Biotechnol.
684 98, 9527–9544. https://doi.org/10.1007/s00253-014-6142-4
685 Wakelin, S.A., Anand, R.R., Reith, F., Gregg, A.L., Noble, R.R.P., Goldfarb, K.C., Andersen,
686 G.L., Desantis, T.Z., Piceno, Y.M., Brodie, E.L., 2012. Bacterial communities associated
687 with a mineral weathering profile at a sulphidic mine tailings dump in arid Western
688 Australia. FEMS Microbiol. Ecol. 79, 298–311. https://doi.org/10.1111/j.1574-
689 6941.2011.01215.x

690 Wang, L., Li, J., Yang, F., Yaoyao, E., Raza, W., Huang, Q., Shen, Q., 2017. Application of
691 Bioorganic Fertilizer Significantly Increased Apple Yields and Shaped Bacterial
692 Community Structure in Orchard Soil. Microb. Ecol. 73, 404–416.
693 https://doi.org/10.1007/s00248-016-0849-y
694 Warnock, D.D., Lehmann, J., Kuyper, T.W., Rillig, M.C., 2007. Mycorrhizal responses to
695 biochar in soil - Concepts and mechanisms. Plant Soil 300, 9–20.
696 https://doi.org/10.1007/s11104-007-9391-5
697 Warnock, D.D., Mummey, D.L., McBride, B., Major, J., Lehmann, J., Rillig, M.C., 2010.
698 Influences of non-herbaceous biochar on arbuscular mycorrhizal fungal abundances in
699 roots and soils: Results from growth-chamber and field experiments. Appl. Soil Ecol. 46,
700 450–456. https://doi.org/10.1016/j.apsoil.2010.09.002
701 Webster, J., Weber, R., 2007. Introduction to Fungi, 3rd ed. Cambridge, UK: Cambridge
702 University Press. https://doi.org/doi:10.1017/CBO9780511809026
703 Wiegand, S., Jogler, M., Jogler, C., 2018. On the maverick Planctomycetes. FEMS Microbiol.
704 Rev. 42, 739–760. https://doi.org/10.1093/femsre/fuy029
705 Wong, M.H., 2003. Ecological restoration of mine degraded soils, with emphasis on metal
706 contaminated soils. Chemosphere 50, 775–780. https://doi.org/10.1016/S0045-
707 6535(02)00232-1
708 You, F., Dalal, R., Huang, L., 2018. Biochar and biomass organic amendments shaped different
709 dominance of lithoautotrophs and organoheterotrophs in microbial communities
710 colonizing neutral copper(Cu)-molybdenum(Mo)-gold(Au) tailings. Geoderma 309, 100–
711 110. https://doi.org/10.1016/j.geoderma.2017.09.010
712 Zeng, X.Y., Li, S.W., Leng, Y., Kang, X.H., 2020. Structural and functional responses of
713 bacterial and fungal communities to multiple heavy metal exposure in arid loess. Sci. Total
714 Environ. 723, 138081. https://doi.org/10.1016/j.scitotenv.2020.138081
715 Zornoza, R., Acosta, J.A., Martínez-Martínez, S., Faz, A., Bååth, E., 2015. Main factors
716 controlling microbial community structure and function after reclamation of a tailing pond
717 with aided phytostabilization. Geoderma 245–246, 1–10.

https://doi.org/10.1016/j.geoderma.2015.01.007

Figure 1

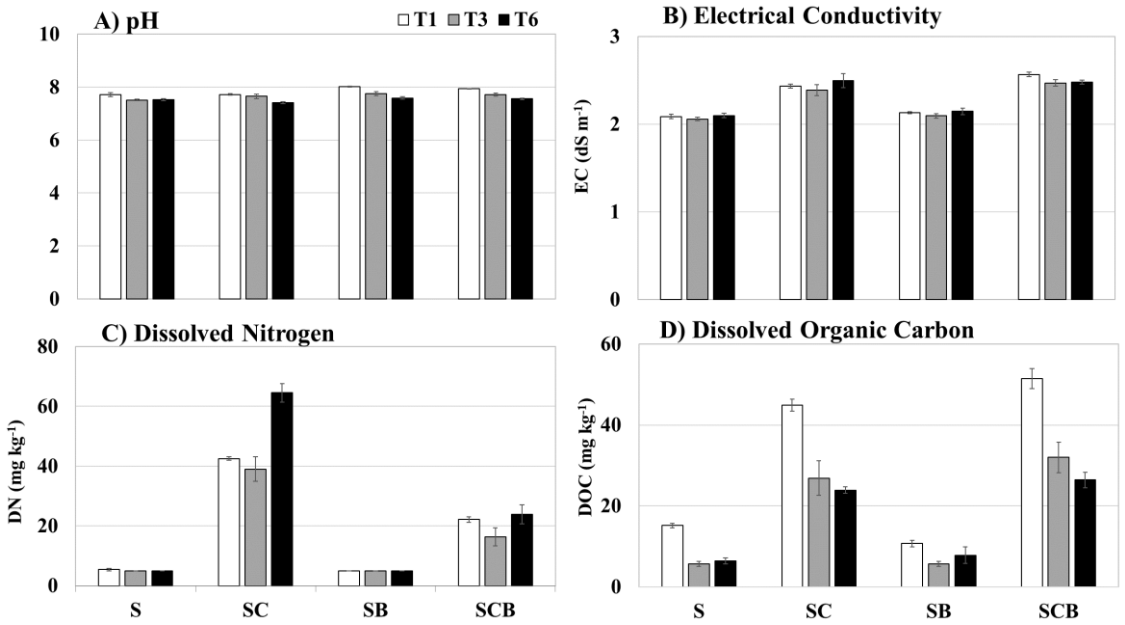

Figure 1: Results of the edaphic parameters analysed in the 1:2.5 soil water extract in each treatment (S, SC, SB and SCB) at each sampling time (T1, T3 and T6): pH, electrical conductivity, dissolved nitrogen and dissolved organic carbon. Bars on columns indicate standard error (n=3). The treatments are: S, bulk tailings; SC, tailings + 4% compost; SB, tailings + 4% biochar; SCB, tailings + 4% compost + 4% biochar.

Figure 2

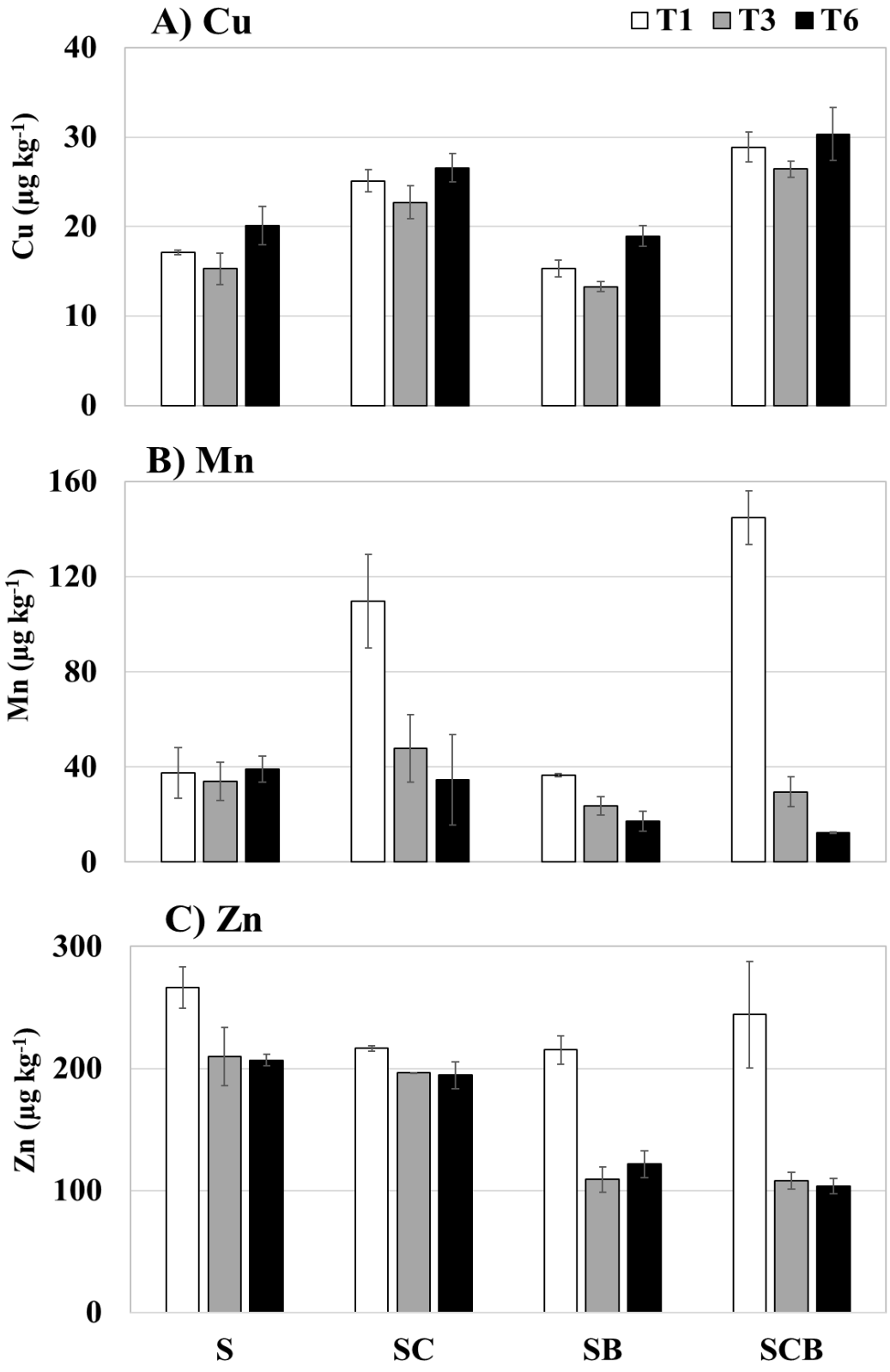

Figure 2: Metal concentrations (Cu, Mn and Zn) analysed in the 1:2.5 soil water extract in each treatment (S, SC, SB and SCB) at each sampling time (T1, T3 and T6). Bars on columns indicate standard error (n=3). The treatments are: S, bulk tailings; SC, tailings + 4% compost; SB, tailings + 4% biochar; SCB, tailings + 4% compost + 4% biochar.

Figure 3

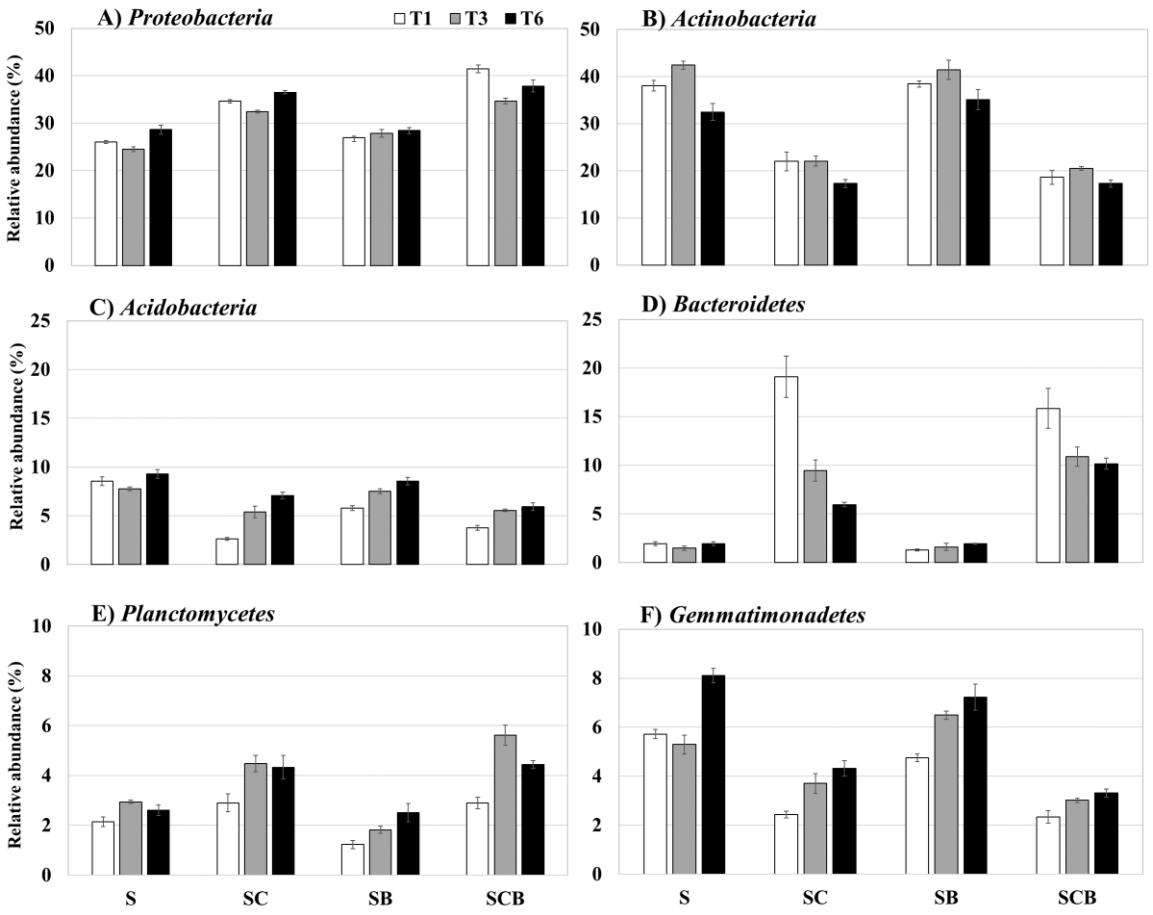

Figure 3: Relative abundance percentages of the six main phyla of bacteria (A-F) detected in each treatment (S, SC, SB and SCB) at each sampling time (T1, T3 and T6). Bars on columns indicate standard error (n=3). The treatments are: S, bulk tailings; SC, tailings + 4% compost; SB, tailings + 4% biochar; SCB, tailings + 4% compost + 4% biochar.

Figure 4

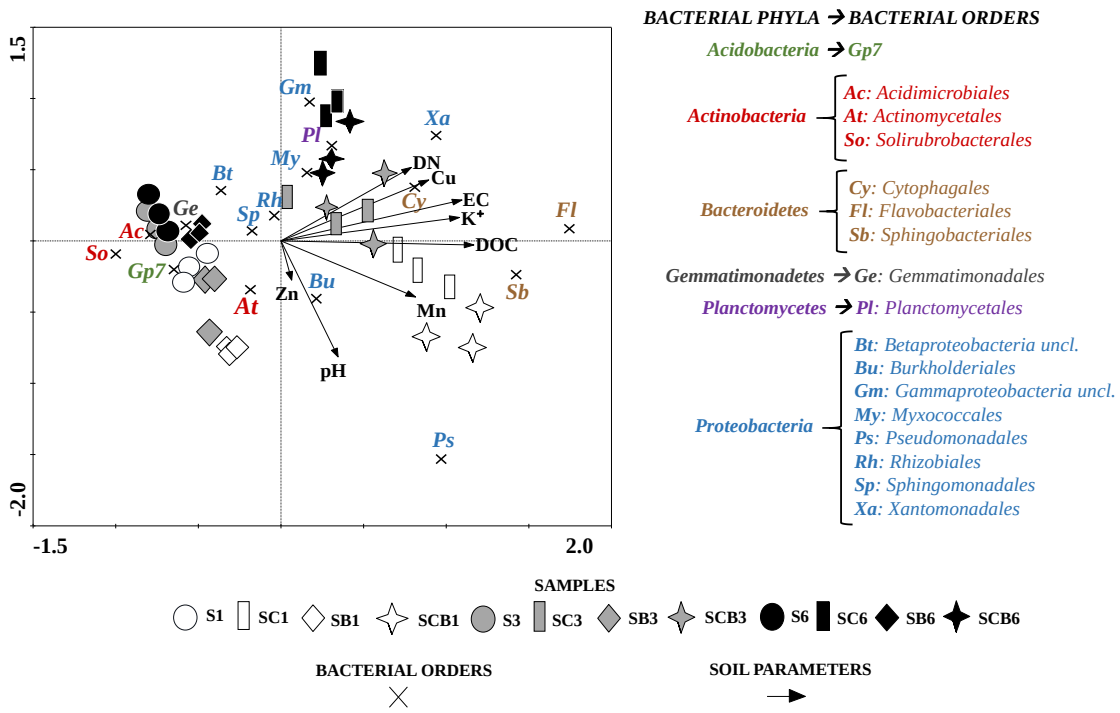

Figure 4: Ordination diagram obtained for the Canonical Correspondence Analysis (CCA) of the relative abundance percentages for bacterial orders and selected soil properties in each treatment (S, SC, SB and SCB) at each sampling time (T1, T3 and T6). Cu, Mn, Zn and K^+ represent water extractable concentrations; "DOC" is Dissolved Organic Carbon concentration; "DN" is Dissolved Nitrogen concentration; "EC" and "pH" are Electrical Conductivity and pH, respectively. All soil parameters were analysed in the 1:2.5 soil:water extract (n=3). Samples from the T1 are represented in white colour; samples from the T3 are represented in grey colour; samples from the T6 are represented in black colour. The treatments are: S, bulk tailings (circles); SC, tailings + 4% compost (rectangles); SB, tailings + 4% biochar (diamonds); SCB, tailings + 4% compost + 4% biochar (stars).

Figure 5

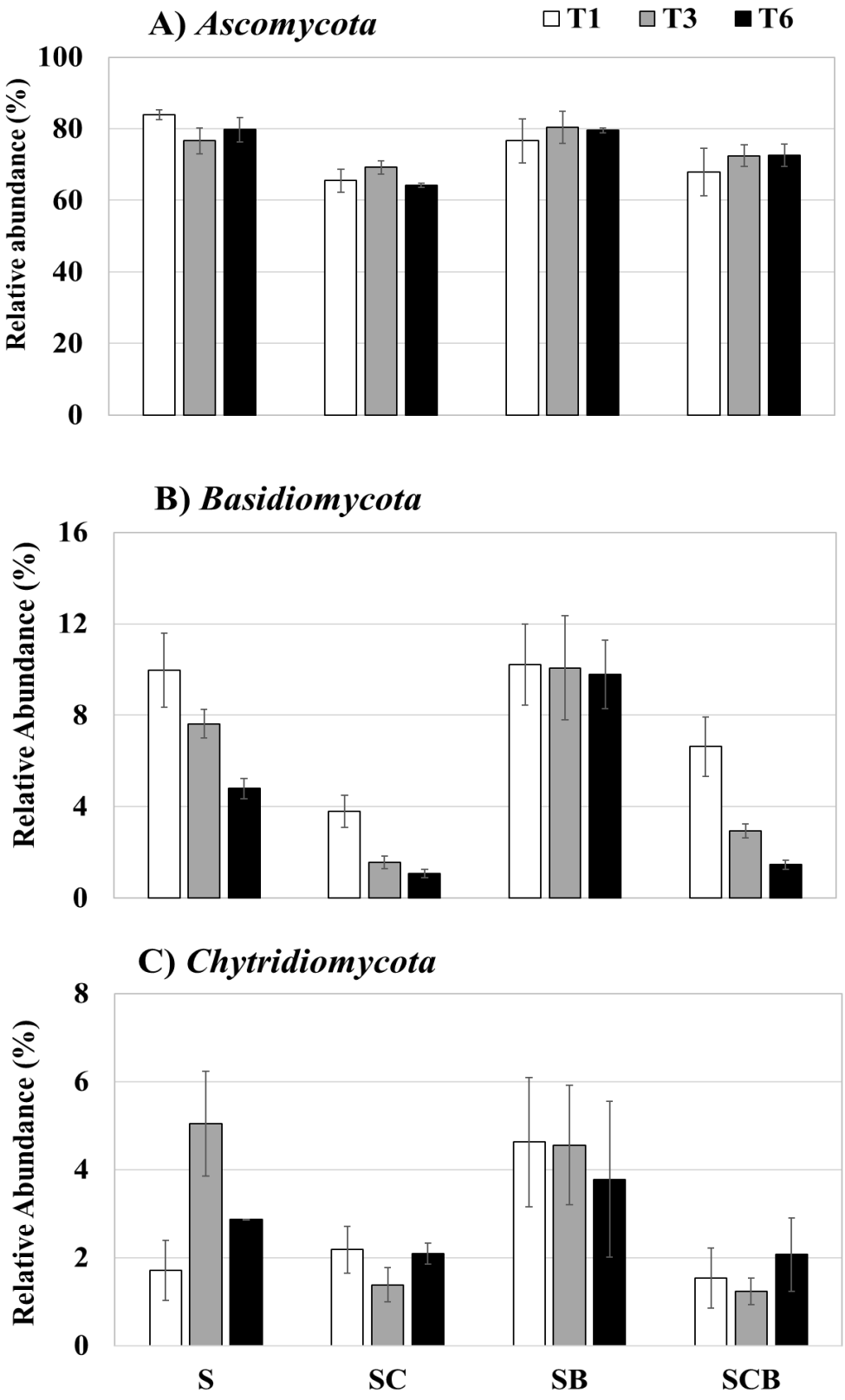

Figure 5: Relative abundance of the three main phyla of fungi (A, B and C) detected in each treatment (S, SC, SB and SCB) at each sampling time (T1, T3 and T6). Bars on columns indicate standard error (n=3). The treatments are: S, bulk tailings; SC, tailings + 4% compost; SB, tailings + 4% biochar; SCB, tailings + 4% compost + 4% biochar.

Figure 6

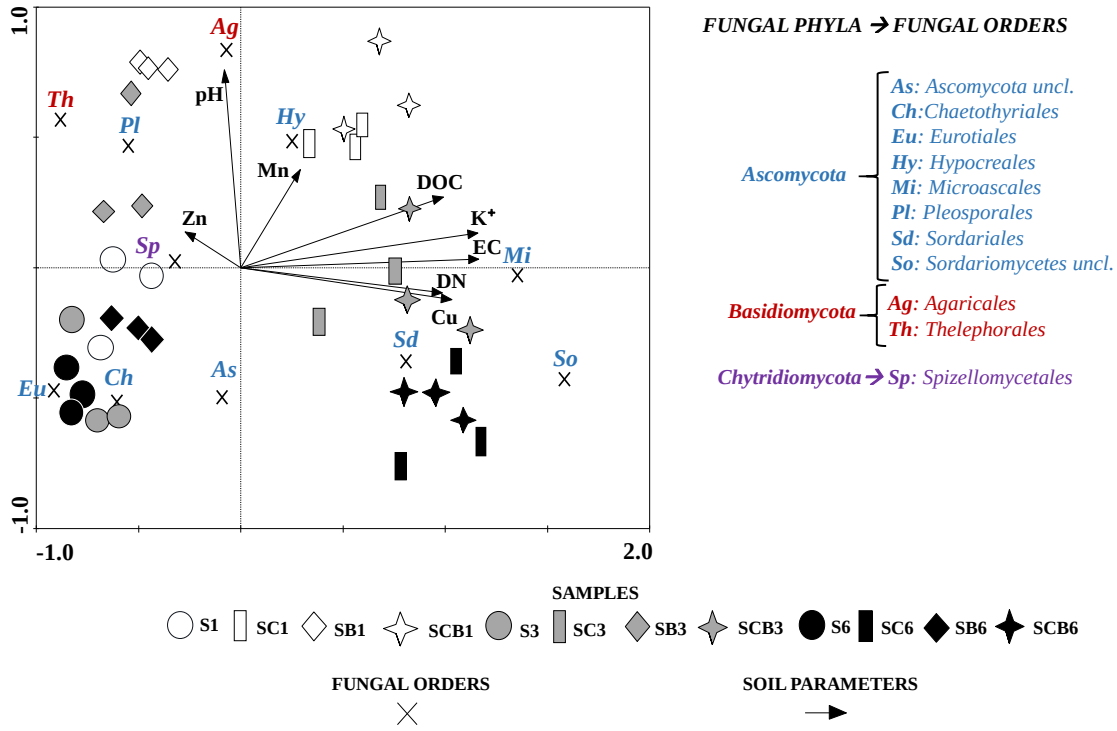

Figure 6: Ordination diagram obtained for the Canonical Correspondence Analysis (CCA) of the relative abundance percentages for fungal orders and selected soil properties in each treatment (S, SC, SB and SCB) at each sampling time (T1, T3 and T6). Cu, Mn, Zn and K+ represent water extractable concentrations; "DOC" is Dissolved Organic Carbon concentration; "DN" is Dissolved Nitrogen concentration; "EC" and "pH" are Electrical Conductivity and pH, respectively. All soil parameters were analysed in the 1:2.5 soil:water extract (n=3). Samples from the T1 are represented in white colour; samples from the T3 are represented in grey colour; samples from the T6 are represented in black colour. The treatments are: S, bulk tailings (circles); SC, tailings + 4% compost (rectangles); SB, tailings + 4% biochar (diamonds); SCB, tailings + 4% compost + 4% biochar (stars).

Supplementary Material for:

A critical assessment on the short-term response of microbial relative composition in a mine tailings soil amended with biochar and manure compost

Yolanda Risueño[1*], *César Petri*[2], *Héctor M. Conesa*[1]

[1] Universidad Politécnica de Cartagena, Escuela Técnica Superior de Ingeniería Agronómica, Departamento de Ingeniería Agronómica. Paseo Alfonso XIII, 48. 30203 Cartagena (Spain)

[2]IHSM-UMA-CSIC La Mayora, Departamento de Fruticultura Subtropical y Mediterránea. Avenida Dr. Wienberg, s/n. 29750 Algarrobo-Costa, Málaga (Spain)

[1]* (corresponding author)

Telephone: +34968327034; Fax: +34 968 327046

e-mail: yolanda.risueno@upct.es

This Supplementary Material includes, extended methods protocols, 6 Figures and 4 Tables

Extended protocols for DNA extraction, PCR amplification and sequencing

Microbial (bacteria and fungi) DNA was extracted from 0.25g soil using the PowerSoil DNA Isolation Kit (MOBIO), according to the manufacturer's instructions. The isolated DNA was quantified using a NanoDrop 2000 spectrophotometer. Library preparation and Illumina sequencing were carried out at the IPBLN Genomics Facility (CSIC, Granada, Spain). Amplicon libraries targeting the 16S rRNA gene and ITS2 region were generated by a two-steps PCR strategy. Gene-specific amplification was performed in triplicate with 15ng of soil-extracted DNA in a final volume of 10 µl. Gene specific primers, V3V4fw (5′ CCTACGGGNGGCWGCAG 3′), V3V4rev (5′ GACTACHVGGGTATCTAATCC 3'), ITS3_KYO2-Fw (5′ GATGAAGAACGYAGYRAA 3') and ITS4-Rev (5′ TCCTCCGCTTATTGATATGC 3'), were designed with Nextera overhang adapters. Primers were used at a final concentration of 0.2 µM. Reaction was performed with 1x KAPA HiFi Hot Start Ready Mix DNA polymerase (Roche Diagnostics, West Sussex, United Kingdom). Cycling conditions were 95°C for 3 min; 25 x (95°C for 30 s, 55°C for 30 s, 72°C for 30 s) and then 72°C for 5 min for 16S amplification and 95°C for 3 min; 27 x (95°C for 30 s, 47°C for 30 s, 72°C for 30 s) and then 72°C for 5 min for ITS2 amplification. Triplicates were pooled together and validated through visualization on a 1.8% (w/v) agarose gel. Amplicons were then purified using NucleoMag® NGS Clean-up and Size Select Kit (Macherey-Nagel, Düren, Germany). A second PCR step attached dual combinatorial indices and Illumina sequencing adapters using Nextera XT v2 index kit. Cycling conditions were 95°C for 3 min, 8 x (95°C for 30 s, 55°C for 30 s, 72°C for 30 s) and then 72°C for 5 min. Amplicon generation was validated again through visualization on a 1.8% (w/v) agarose gel and cleaned with NucleoMag® NGS Clean-up and Size Select Kit (Macherey-Nagel). Concentration was measured on the Qubit® fluorometer (Thermo). Amplicons were pooled in an equimolecular manner and final library mix was run on a Bioanalyzer HS DNA chip to verify quality and size distribution. The library pool was then diluted and denatured as recommended by Illumina MiSeq library preparation guide. The 300x2nt paired-end sequencing was conducted on a MiSeq sequencer.

Bioinformatics and statistical analysis

Raw sequence data in FASTQ format (16S and ITS2) were subjected to quality control analysis with FastQC software and prepared for taxonomic classification using the Mothur software (version 1.43.0) (Schloss et al., 2009) and following the standard operating protocol proposed by (Kozich et al., 2013). Overlapping pairs of sequence reads were merged into contigs. In addition, reads with ambiguous bases, duplicated contigs and homopolymers longer than 13 bp were removed. The VSEARCH algorithm (embedded in the Mothur framework) was used to remove chimeras and these were subsequently omitted. The resulting sequences were classified according to the taxonomy into the corresponding Operational Taxonomic Units at 97% similarity, besides using the reference trainset 16_022016.pds from https://mothur.org/wiki/RDP_reference_files for Bacteria and ITS sequences provided by the UNITE ITS database (version 7.2) at https://unite.ut.ee/repository.php for Fungi. Undesired lineages such as Plantae, Animalia, Protista, "unknown" and other were removed. The final sequences were then grouped into taxonomic groups (phylum, order, etc), using the phylotype command in Mothur, which relies upon reference taxonomic outlines to classify sequences to taxonomic bins (Schloss and Westcott, 2011). Relative abundances of different taxonomic levels of each bacterial and fungal group were calculated as the percentage from the total count of reads in each sample using the get.relabund command in Mothur. Taxa relative abundances for each study site were calculated by means of the three replicates of each treatment. Phyla (both bacteria and fungi) that showed >5% abundance in at least one sampling site and orders that showed >3% in at least two sampling sites, were considered.

References

Kozich, J.J., Westcott, S.L., Baxter, N.T., Highlander, S.K., Schloss, P.D., 2013. Development of a Dual-Index Sequencing Strategy and Curation Pipeline for Analyzing Amplicon Sequence Data on the MiSeq Illumina Sequencing Platform. Appl. Environ. Microbiol. 79, 5112–5120. https://doi.org/10.1128/aem.01043-13

Schloss, P.D., Westcott, S.L., 2011. Assessing and improving methods used in operational taxonomic unit-based approaches for 16S rRNA gene sequence analysis. Appl. Environ. Microbiol. 77, 3219–3226. https://doi.org/10.1128/AEM.02810-10

Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., Sahl, J.W., Stres, B., Thallinger, G.G., Van Horn, D.J., Weber, C.F., 2009. Introducing mothur: Open-Source, Platform-Independent, Community-Supported Software for Describing and Comparing Microbial Communities. Appl. Environ. Microbiol. 75, 7537–7541. https://doi.org/10.1128/AEM.01541-09

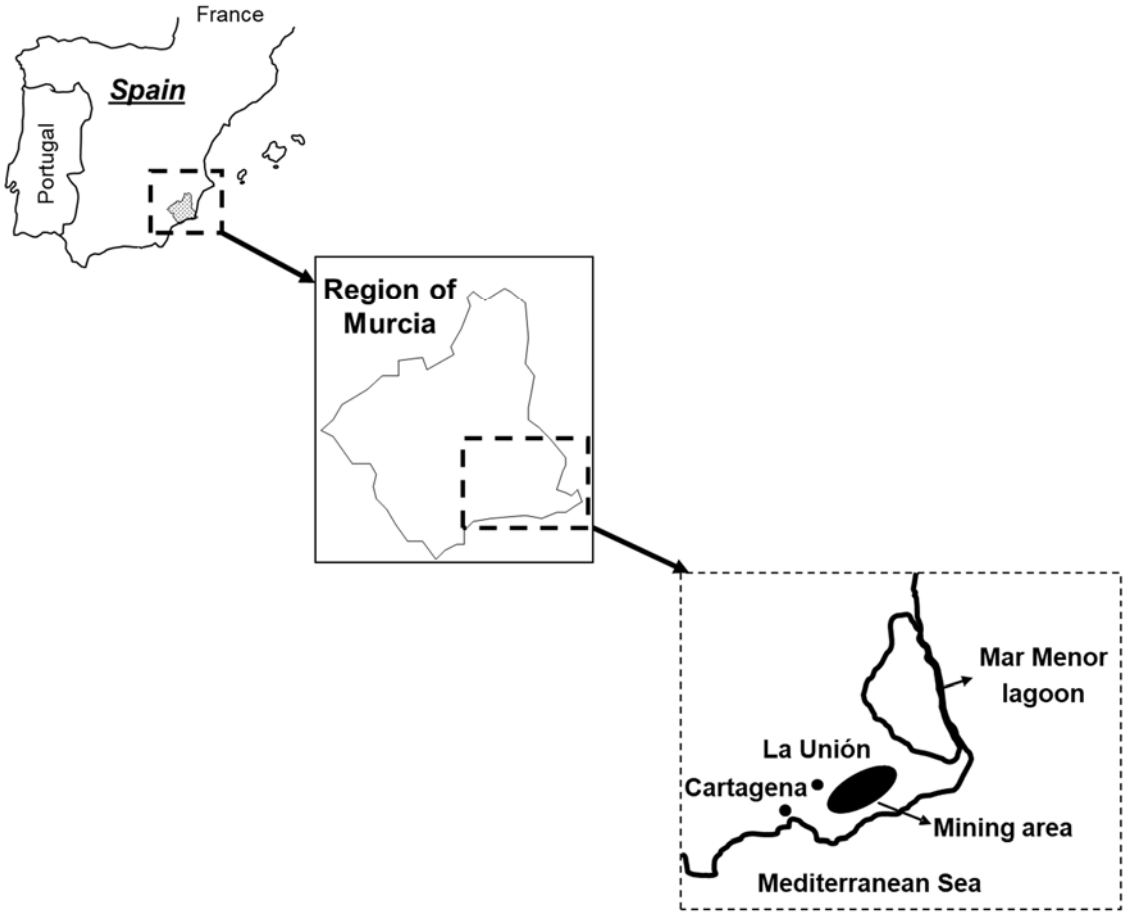

Figure SM1- Location of the studied area.

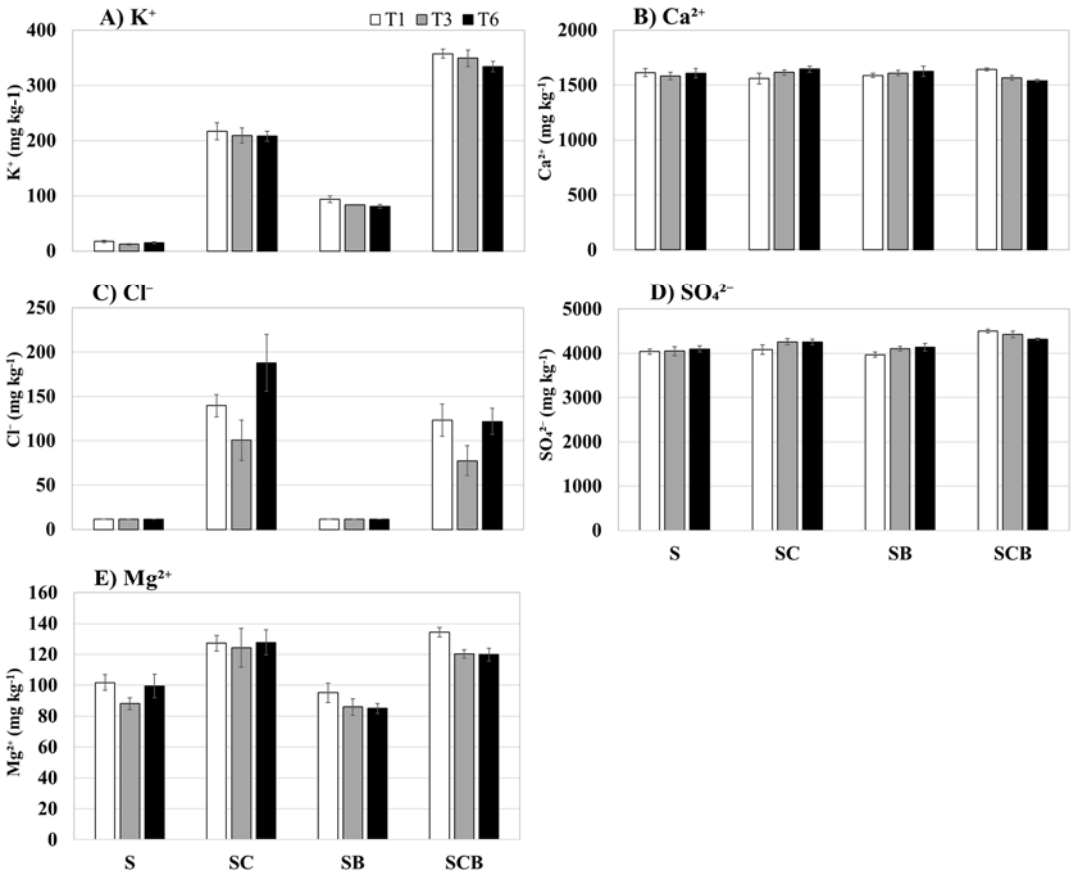

Figure SM2- Ion concentrations (K^+, Ca^{2+}, Cl^-, SO_4^{2-} and Mg^{2+}) measured of the 1:2.5 soil:water extract at each treatment (S, SC, SB and SCB) and sampling time (T1, T3 and T6). Bars on columns indicate standard error (n=3). The treatments are: S, bulk tailings; SC, tailings + 4% compost; SB, tailings + 4% biochar; SCB, tailings + 4% compost + 4% biochar.

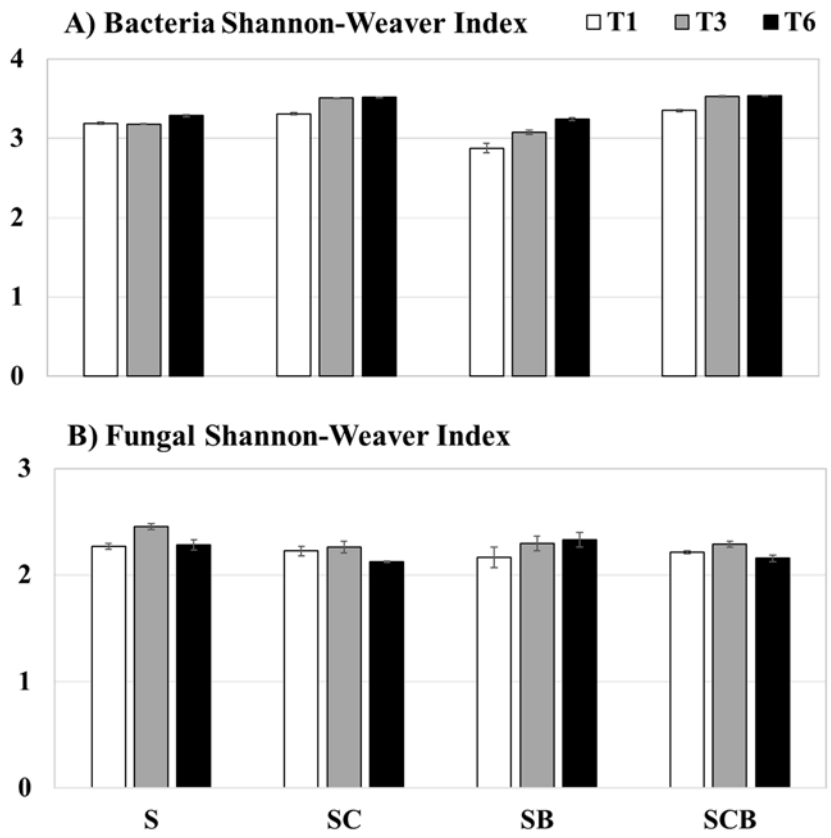

Figure SM3- Shannon-Weaver index for bacteria (A) and fungi (B) in each treatment (S, SC, SB and SCB) at each sampling time (T1, T3 and T6). Bars on columns indicate standard error (n=3). The treatments are: S, bulk tailings; SC, tailings + 4% compost; SB, tailings + 4% biochar; SCB, tailings + 4% compost + 4% biochar.

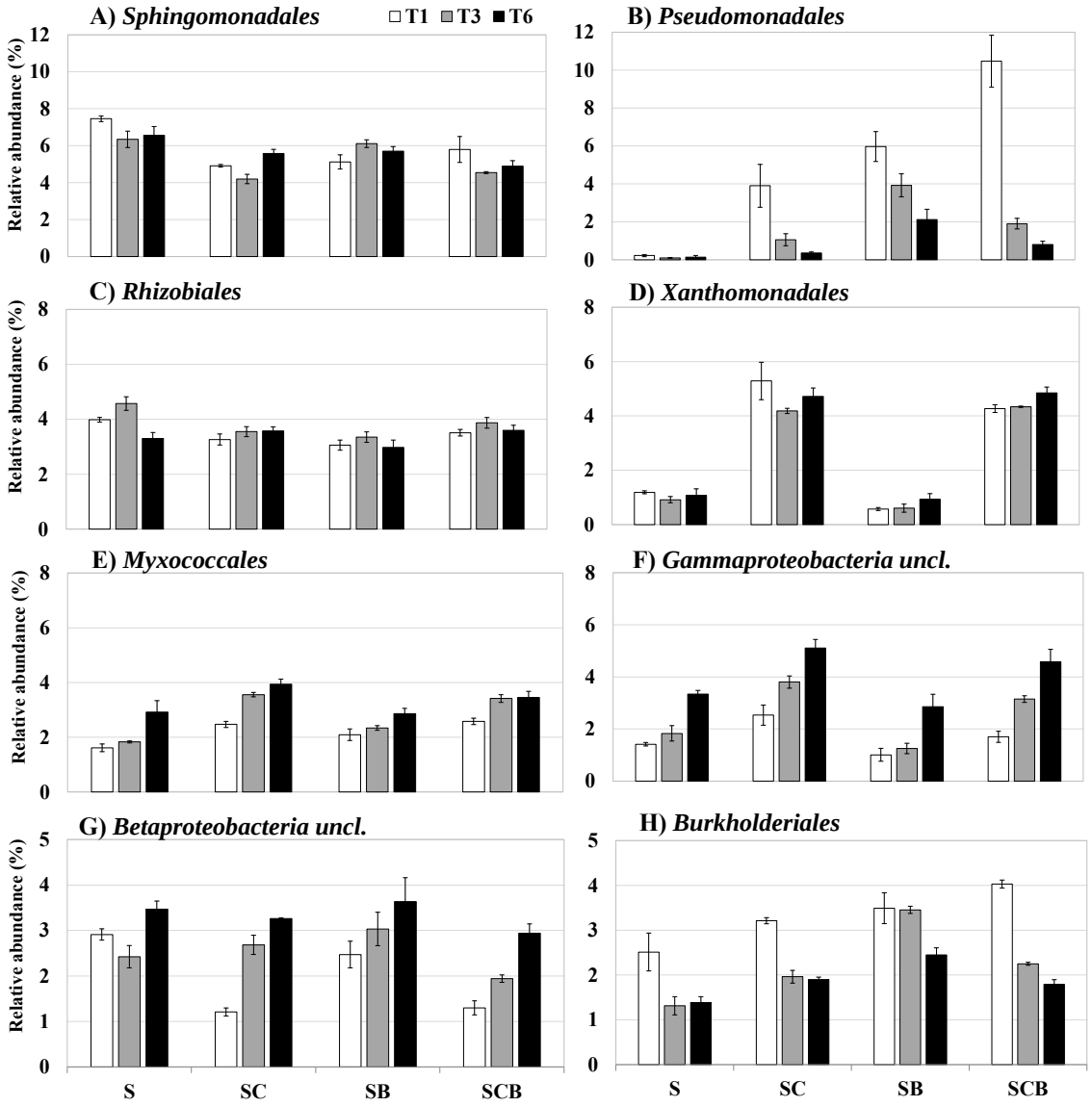

Figure SM4- Relative abundance of the main bacterial orders of *Proteobacteria* phylum (A-H) in each treatment (S, SC, SB and SCB) at each sampling time (T1, T3 and T6). Bars on columns indicate standard error (n=3). The treatments are: S, bulk tailings; SC, tailings + 4% compost; SB, tailings + 4% biochar; SCB, tailings + 4% compost + 4% biochar.

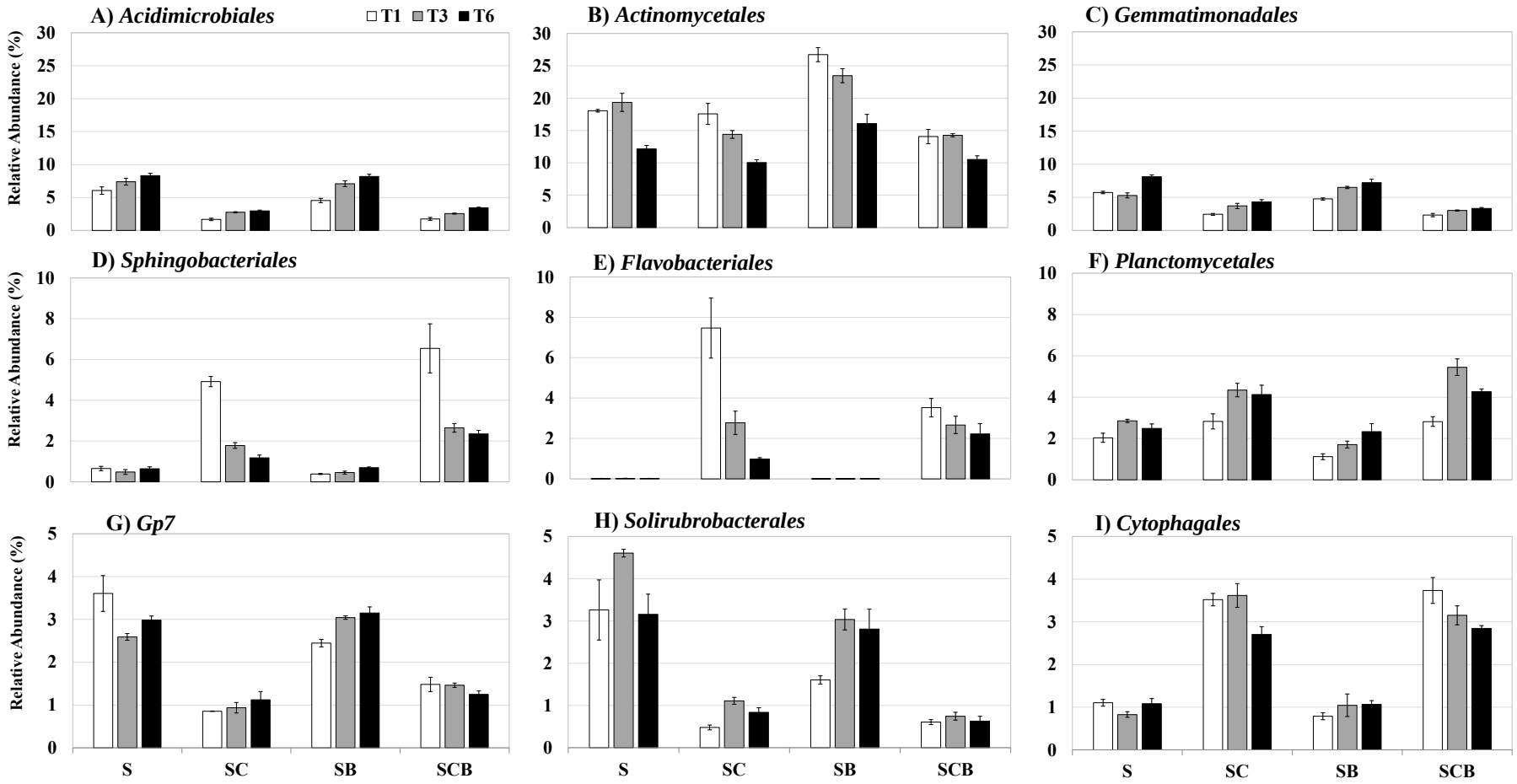

Figure SM5- Relative abundance of the main bacterial orders of *Actinobacteria* (A,B,H), *Acidobacteria* (G), *Bacteroidetes* (D,E,I), *Planctomycetes* (F) and *Gemmatimonadetes* (C) phyla in each treatment (S, SC, SB and SCB) at each sampling time (T1, T3 and T6). Bars on columns indicate standard error (n=3). The treatments are: S, bulk tailings; SC, tailings + 4% compost; SB, tailings + 4% biochar; SCB, tailings + 4% compost + 4% biochar.

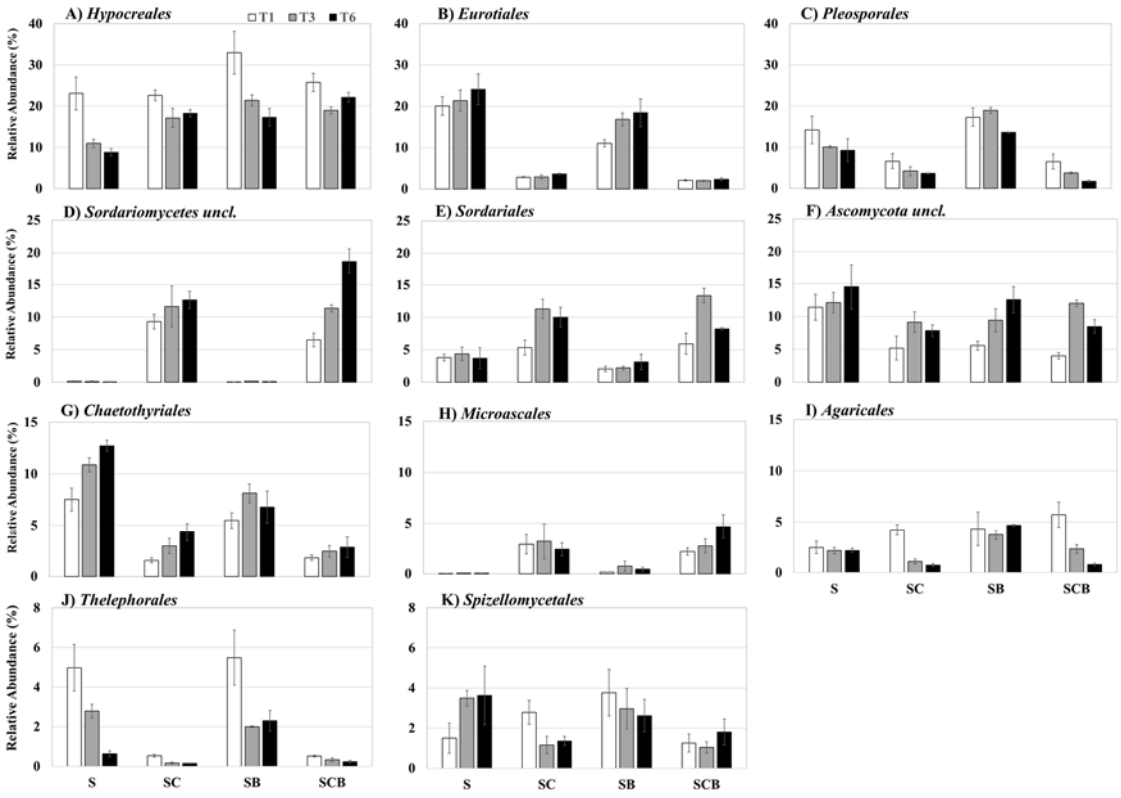

Figure SM6- Relative abundance percentages of the main fungal orders of *Ascomycota* (A-H), *Basidiomycota* (I,J) *and Chytridiomycota* (K) phyla in each treatment (S, SC, SB and SCB) at each sampling time (T1, T3 and T6). Bars on columns indicate standard error (n=3). The treatments are: S, bulk tailings; SC, tailings + 4% compost; SB, tailings + 4% biochar; SCB, tailings + 4% compost + 4% biochar.

Table SM1- Results of initial characterization of the mine tailings soil and the two amendments used in this study (compost and biochar). pH, EC, DOC, DN and ions were determined in the 1:2.5 soil:water extract. Water extractable ions concentrations were determined by using an Ion Chromatographer (Metrohm), DOC and DN by using a TOC-automatic analyser (TOC-VCSH Shimadzu) and TOC and TN were determined in solid samples by a CHN 628 Leco analyser. Total metal(loid) concentrations were determined by X-ray fluorescence (Bruker S4 Pioneer). Data are average ± standard error of 3 pseudo-replicates.

Soil parameters		Units	Mine Tailings			Compost			Biochar		
pH		-	7.38	±	<0.1	8.17	±	<0.1	9.90	±	<0.1
Electrical Conductivity (EC)		dS m^{-1}	2.07	±	<0.1	9.75	±	0.11	2.65	±	<0.1
Total Organic Carbon (TOC)		%	0.45	±	0.01	25.80	±	0.20	82.90	±	0.04
Disolved Organic Carbon (DOC)		mg kg^{-1}	10.8	±	0.5	4490	±	153	790	±	55
Total Nitrogen (TN)		%	0.24		0.01	2.50	±	0.01	0.70	±	0.01
Dissolved Nitrogen (DN)		mg kg^{-1}	<2.5			1244	±	28	8.7	±	0.2
Ions water extract	Cl^-	mg kg^{-1}	14	±	1	4537	±	250	130	±	10
	SO_4^{2-}		4130	±	40	3711	±	194	110	±	6
	Ca^{2+}		1613	±	11	387	±	38	190	±	26
	K^+		15	±	2	6011	±	169	4720	±	270
	Mg^{2+}		87	±	5	175	±	21	70	±	17
	Na^+		10	±	<1	1889	±	50	230	±	14
Total elemental concentrations	Ca	%	7.246	±	0.132	22.538	±	0.138	7.077	±	0.074
	K		0.604	±	0.011	4.360	±	0.035	1.871	±	0.018
	Mg		1.283	±	0.043	1.333	±	0.013	0.533	±	0.003
	Na		0.102	±	0.015	0.689	±	0.013	0.071	±	0.003
	P		0.037	±	0.001	2.428	±	0.015	0.143	±	<0.001
Total metal(loid) concentrations	Cu	mg kg^{-1}	163	±	6	331	±	5	67	±	1
	Mn		11973	±	202	1295	±	14	960	±	13
	Pb		10041	±	386	77	±	6	<10		
	Zn		14913	±	409	1255	±	16	100	±	1

Table SM2- Elemental composition of the four treatments tested in the experiment. Total concentrations were determined by X-ray fluorescence (Bruker S4 Pioneer). Data are average ± standard error of 3 repetitions. The treatments are: S, bulk tailings; SC, tailings + 4% compost; SB, tailings + 4% biochar; SCB, tailings + 4% compost + 4% biochar.

Parameter		Units	Treatment			
			S	SC	SB	SCB
Total elemental concentrations	Ca	%	7.246 ± 0.132	7.652 ± 0.075	6.822 ± 0.093	7.028 ± 0.095
	K		0.604 ± 0.011	0.615 ± 0.007	0.576 ± 0.008	0.628 ± 0.009
	Mg		1.283 ± 0.043	1.276 ± 0.031	1.359 ± 0.037	1.085 ± 0.009
	Na		0.102 ± 0.015	0.135 ± 0.011	0.112 ± 0.012	0.091 ± 0.005
	P		0.037 ± 0.001	0.119 ± 0.002	0.042 ± 0.001	0.139 ± 0.002
Total metal(loid) concentrations	Cu	$mg\ kg^{-1}$	163 ± 6	164 ± 7	130 ± 5	145 ± 6
	Mn		11973 ± 202	11375 ± 102	10280 ± 121	10530 ± 145
	Pb		10041 ± 386	8998 ± 248	7861 ± 265	8229 ± 209
	Zn		14913 ± 409	13653 ± 269	11980 ± 296	12078 ± 271

Table SM3- Data of the Canonical Correspondence Analysis (CCA) including eigenvalues of the two first axis and cumulative percentage of variance of bacteria-soil and fungal-soil data interaction. The significance of the first canonical axis (Monte-Carlo test, p-value) is also provided.

Parameter evaluated	Axis	Eigenvalue	% cumulative variance	Significance
Bacterial orders	I	0.141	63.1	0.002
	II	0.060	89.7	
Fungal orders	I	0.261	78.3	0.002
	II	0.053	94.1	

Table SM4- Weighted correlation matrix for the first two species axes and environmental variables for bacterial and fungal orders.

		Bacterial orders		Fungal orders	
Soil parameters		**Species Ax1**	**Species Ax2**	**Species Ax1**	**Species Ax2**
pH		0.28	-0.76	-0.06	0.78
Electrical Conductivity (EC)		0.88	0.27	0.90	0.03
Dissolved Organic Carbon (DOC)		0.94	-0.03	0.77	0.28
Dissolved Nitrogen (DN)		0.64	0.48	0.76	-0.10
Water extractable K^+		0.87	0.15	0.90	0.14
Water extractable metals	**Cu**	0.72	0.40	0.80	-0.13
	Mn	0.66	-0.37	0.23	0.39
	Zn	0.05	-0.26	-0.21	0.14

Credit Author Statement

Yolanda Risueño: Methodology, Investigation, Visualization, Writing-Original Draft, Writing-Review and Editing

César Petri: Methodology, Writing-Original Draft, Writing- Review and Editing, Funding acquisition

Héctor M. Conesa: Conceptualization, Investigation, Writing-Original Draft, Writing- Review and Editing, Project administration, Funding acquisition

Declaration of interests

X The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: