LONG-TERM EFFECTS OF TWO ORGANIC AMENDMENTS ON BACTERIAL COMMUNITIES OF CALCAREOUS MEDITERRANEAN SOILS DEGRADED BY MINING

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Abstract

The application of organic amendments to improve the chemical and biological properties of degraded soils from calcareous quarries is necessary to accelerate restoration processes. The aim of this study is to assess the success of different restoration treatments in the long-term using two organic amendments (sewage sludge from urban waste water (SS) and compost from domestic solid waste (CW)). The chemical properties and bacterial communities of restored soils were compared with unamended soils (NA) and surrounding natural soils (NS) from a limestone quarry in a semi-arid ecosystem. After 10 years of the addition of organic amendments, the abundance of soil bacteria, diversity, and taxonomic composition at the phylum and genus level in each soil type was analysed by rRNA 16S amplification (PCR), sequencing using Illumina, and comparison with the

SILVA database using QIIME2 software. The relationships between soil bacterial taxa and chemical soil properties (pH, electrical conductivity (EC), total organic carbon (TOC), and total nitrogen content (TN)) were also studied, as well as the interrelations between soil bacterial taxa at the genus level or the next upper taxonomic level identified. The organic amendments changed the chemical properties of the restored soils, influencing the microbial communities of the restored soils. CW treatment was the organic amendment that most resembled NS, favouring in the long-term a greater diversity and proliferation of bacteria. Several bacterial communities, more abundant in NA and CW soils, were strongly correlated with each other (*Craurococcus*, *Phaselicystis, Crossiella,* etc.), forming a bacterial co-occurrence pattern (Co-occurrence pattern 1). Those bacteria showed high significant positive correlations with TOC, TN, and EC and negative correlations with the soil pH. In contrast, NA soils presented other groups of bacterial communities (Co-occurrence pattern 2) represented by *Sphingomonas, Rubellimicrobium, Noviherbaspirillum, Psychroglaciecola and Caenimonas,* which showed high significant positive correlations with soil pH and negative correlations with TOC, TN, and EC. Principal component analysis indicated that SS soils remained in an intermediate stage of chemical and biological quality between NS and NA soils. Our results demonstrate that soil chemical properties and soil bacterial communities significantly changed with organic amendments in calcareous Mediterranean soils degraded by mining.

Keywords: Soil restoration, metagenomics, bacterial diversity, soil degradation, soil bacterial co-occurrence patterns , semiarid.

1. Introduction

Soil is of vital importance in the biosphere, performing multiple functions necessary for life on the planet, such as maintaining plant and animal productivity, conserving water

and air quality, and improving human health (Singer and Sojka, 2002). Soil degradation caused by the indiscriminate exploitation of natural resources affects large areas of the world, leading to the loss of soil quality and the reduction of soil productivity through the loss of nutrients or unfavourable changes in physical, chemical, and biological soil properties. The problem of environmental degradation is aggravated in semi-arid climate and, in particular, in the Mediterranean ecosystems, due to scarce and torrential rainfall, high solar radiation, and low plant cover that favours the erosion processes (Bastida et al., 2007; Bruneel et al., 2019; Hueso et al., 2011; Miralles et al., 2009). In these ecosystems the practice of mining, especially the opencast type, causes the total loss of the soil and plant cover, which disturbs the soil–plant stability (Luna et al., 2016a), producing the leaching of nutrients by erosion and reducing the fertility of the soil (Li et al., 2018). Physical and chemical limitations of the soil properties and the low microbial activity in semi-arid zones, as well as the climatic characteristics, complicate the natural restoration of the soils, slowing down the vegetal regeneration in these regions (Juwarkar and Jambhulkar, 2008). Therefore, the restoration of the quarries is necessary to accelerate the recovery of the soil and cover plants, and one way of solving these problems could be the use of organic amendments such as sewage sludge or compost from domestic organic waste (Luna et al., 2016a, 2016b; Rodríguez-Berbel et al., 2019).

Several authors have shown that the application of organic amendments is a good method of restoration, because restoring absent soil microbiota that is vital for soil structural formation can contribute to plant establishment and transformation of organic matter (Diaz et al., 1994; Zink and Allen, 1998). Moreover, organic amendments improve soil properties such as pH, humidity, organic carbon, and plant cover, which are important factors influencing the microbial diversity in semi-arid soils (Bukar et al., 2019; Neilson et al., 2012; Peñuelas et al., 2013; Reynolds et al., 2007). Many authors have documented

the beneficial effects of the use of sewage sludge or compost on fertility and soil functionality, just as on the proliferation of microbial communities with different metabolic activities (Almendro-Candel et al., 2014; Bastida et al., 2015; Luna et al., 2016a; Rico Hernández et al., 2018; Yanardağ et al., 2017). Organic amendments such as sewage sludge, urban solid waste compost, and poultry manure also influence enzymatic activities involved in the cycles of C, N, and P and biochemical soil properties such as biomass-C and basal respiration (Bastida et al., 2007; Luna et al., 2016a; Tejada et al., 2006).

Soil microbial communities have a crucial role in the functioning of ecosystems because they have a direct relationship with biogeochemical cycles (Adak and Sachan, 2009), fundamental for plant growth and survival (Bender et al., 2016). Microbial enzymatic activity plays a key role in biochemical cycles by the recycling of nutrients, making them accessible to plants and other microorganisms (Ai et al., 2015). However, most studies focusing on soil microorganisms in restored soils are based on knowledge of the size and activity of microbial communities from biochemical techniques such as soil basal respiration and soil enzymatic activity, fatty acid profile, or molecular tools such as polymerase chain reaction (PCR) combined with denaturant gradient gel electrophoresis (DGGE) (Bastida et al., 2008; Garcia and Hernandez, 1994; Luna et al., 2016b; Schmalenberger et al., 2013; Trasar-Cepeda et al., 1998; Zornoza et al., 2007). There are many studies in which organic amendments have been used to restore degraded soils from semi-arid areas, but their influence on the structure of the microbial community in the soil is still poorly explored (Yanardağ et al., 2017). Moreover, at present, studies in which the microbial communities of natural soils are compared with environments degraded by human activities are also very scarce, and there is an important gap in our knowledge about the representative bacterial taxa necessary for the ecological restoration of degraded

soils to the natural state (Liddicoat et al., 2019). Recently, new methodologies based on massive sequencing have been used to amplify 16S RNA based on the Illumina MiSeq platform or shotgun metagenomic sequencing that allows the study of high-resolution soil microbial communities at the lowest classification level. Some studies have analysed microbial communities at the phylum level in restored soils with organic amendments by metaproteomic techniques. Bastida et al. (2015) deployed metaproteomics to study a restoration with organic amendments and concluded that these affected the functionality and structure of the microbial community at the phyla level in the short and long-term. However, the same phylum includes a great diversity of bacterial genera that could perform a wide variety of functions in soils. Few studies have investigated soil microbial communities at the lowest classification level (subgroup to genus) in semi-arid ecosystems (Miralles et al., 2020a; Sánchez-Marañón et al., 2017). The study of soil microbial communities can provide important information on the specific functions they perform in soils, as well as contrasting the most abundant bacterial taxa of degraded soils, restored soils with different organic treatments, and natural soils, in order to determine the optimal treatments that allow favouring bacterial proliferation similar to natural soils considered with the highest soil quality thresholds. Breed et al. (2019) refer to the potential of genomics techniques for ecological restoration, given the need to know and better understand soil microbial communities to be successful in restoration (Garris et al., 2016). Moreover, soil microbial communities are considered better soil quality indicators than physico-chemical soil properties (Deng et al., 2019; Van der Heijden et al., 2008). On the other hand, the ecological factors influencing these communities have also been poorly studied. Thus, the microclimate (humidity and soil temperature) and especially physical and chemical soil properties could drive the soil bacterial communities (Curiel Yuste et al., 2014; Garcia and Hernandez, 1994; Trasar-Cepeda et al., 1998). Some studies

on soil bacterial communities have focused on soils under different uses (Sánchez-Marañón et al., 2017), but they are of very limited relevance in restored soils with organic amendments, which can change soil microbial communities by incorporating new non-native communities to the soils (Bastida et al., 2013, 2008; Luna et al., 2016a). In this sense, the need to know the changes that occur in soil bacterial communities and the ecological factors influencing these changes are crucial for the assessment and monitoring of ecological restoration.

In view of all of the above, we hypothesized that restoration treatments consisting of the application of different organic amendments would modify the chemical and biological properties of the soils, helping the restored soils to present chemical and biological qualities similar to natural soils in the long-term. Therefore, the aim of this study was to analyse the effect of two organic amendments (compost from domestic waste and/or sewage sludge from urban waste water) in the long-term in chemical and microbiological quality of restored soils in a limestone quarry from a semi-arid ecosystem and to study the relation between chemical soil properties, diversity and composition of bacterial communities in the restored soils. To do this three task were undertaken: (a) study of the chemical properties and the relative abundance of bacterial taxa, diversity, and taxonomic composition at the phylum and bacterial taxa at the genus level or the next upper taxonomic level identified in restored soils with two different organic amendments applied 10 years ago in the quarry and in the surrounding natural soils considered as a quality threshold; (b) study of the relationships among soil bacterial taxa, diversity and chemical soil properties; and (c) study of soil bacterial co-occurrence patterns of soil bacterial taxa associated with chemical soil properties in the different soil types.

2. Material and methods

2.1. Study area and experimental design

The study was carried out in a homogeneous hillslope of a limestone quarry located between the intermountain basin formed by Tortonian (Tertiary Upper Miocene) marls (calcitic-gypsiferous mudstones and calcareous sandstones) and the Gádor range (Cenozoic dolomites and limestones), 15 km north of Almería (SE Spain, 36°55′20″N, 2°30′29″W). The lithology of the study area is fundamentally calcareous sandstones which overlay the marls and are partly quarried. Then, most of the restoration area rests on both types of rock. In undisturbed surrounding areas, soils are mainly Calcaric Regosols (FAO-IUSS-ISRIC, 2015) over (a) calcitic-gypsiferous mudstone (marl), (b) calcareous sandstone, and (c) slope deposits mostly fed by the shallow soils over limestone and dolomite from upper reliefs, which partly contain remains of pre-erosion terra-rossa. The climate is arid/semi-arid Mediterranean with a mean annual temperature of 17.6ºC and with a minimum absolute temperature of -2.6ºC and a maximum of 42.7ºC. The potential evapotranspiration is 1,225 mm $year^{-1}$, and the mean annual precipitation is 245 mm with precipitation mainly in winter and autumn. The area is predominantly grassland that is dominated by native vegetation including *Macrochloa tenacissima* (L.) Kunth and some dwarf perennial shrubs such as *Anthyllis terniflora* (Lag) Pau; other areas constitute a mosaic formed by patches of grassland alternating with patches of dwarf shrub scrubland, where *Anthyllis cytisoides* L. and other species are found. More information about the study area is found in Luna et al. (2016b).

The installation of the experimental plots was carried out in 2008 in an area completely degraded due to mining activity where all the limestone was extracted. The experimental plots were set up at 370 m.a.s.l. in 75 m^2 surfaces (15 m × 5 m) over a hillslope with an average slope of 19%. Two organic amendments for restoration were applied in the

173 experimental plots: (a) a filter-press dried sewage sludge from urban waste water (SS;
174 Total organic C = 351.5 g kg^{-1} and Total nitrogen = 54.3 g kg^{-1}; (Luna et al., 2016a,
175 2016b)) and (b) compost from domestic solid organic waste (CW; Total organic C = 196.5
176 g kg^{-1} and Total nitrogen = 20.7 g kg^{-1}; (Luna et al., 2016a, 2016b)). The crushed marly
177 substrate without organic amendments was used as a control (NA), and undisturbed
178 natural soils (NS) that have not suffered any anthropic alteration found surrounding the
179 experimental plots. These soils are shallow and stony calcaric regosols over marls and
180 calcaric sandsones and support mostly *Antyllis cytisoides* and *A. terninflora* among other
181 shrubs, and *Macrochloa tenacissima*. NS were considered as reference soils since they
182 follow the natural evolution determined by the climatic and ecological properties of study
183 area. The organic amendment treatments were laid on the soil surface layer and mixed
184 (0–20 cm) with the marly substrate with a mechanical backhoe, in NA soils the same
185 process was performed without adding any amendment. The quantity of organic
186 amendments used was determined according to their carbon content to increase the initial
187 organic matter content up to 2% in each plot. Additionally, one year shrubs and herbal
188 species were directly and manually planted from forestry pot seedlings at each
189 experimental plot the three most abundant species of native vegetation (*Macrochloa*
190 *tenacissima* (L.) Kunth, *Anthyllis terniflora* (Lag) Pau, and *Anthyllis cytisoides* L.) with
191 a separation of 1 m (75 per plot), alternating the species in the same proportion as they
192 are present in unaltered natural soils surrounding the quarry. A more detailed description
193 of the construction of experimental plots can be found in Luna et al. (2016a).

194 Ten years after the experimental plots were established, six composite soil samples (by
195 mixing 10 subsamples) were collected randomly to a depth of 10 cm throughout each
196 plot, as well as in soils without amendment and in reference soils. A total of 24 soil
197 samples were taken to the laboratory in isothermal bags. Samples were air-dried,

198 homogenized, and sieved through a 2-mm screen. Part of these dry soil samples was used
199 for chemical soil analysis and another part for DNA extraction and next-generation
200 sequencing (NGS) analysis.

201 *2.2. Chemical soil analysis*

202 Soil pH and electrical conductivity (EC) were analysed in an aqueous solution 1/1 (w/v)
203 (Thomas, 1996) by pH meter (Crison, Carpi, Italy) and by digital conductivity meter
204 (Crison, Carpi, Italy), respectively. Total organic carbon (TOC) was determined by
205 Walkley and Black's method (1934) (rectified by Mingorance et al., 2007), and total
206 nitrogen content (TN) was determined by total combustion (Vario Rapid N; Elementar,
207 Hanau, Germany).

208 2.3. *DNA extraction, high-throughput sequencing, and bioinformatics analysis*

209 Microbial DNA was extracted from 0.3 g of soil using the DNeasy PowerSoil Kit
210 (QIAGEN, Hilden, Germany) and quantified using an ND-2000 Nanodrop
211 spectrophotometer (Thermo Fisher Scientific, USA). The V4-V5 regions (400–500 pb)
212 of bacteria 16S ribosomal RNA gene were amplified *in vitro* by PCR using
213 515FB/8926Rr 16S rRNA gene primer pair (Walters et al., 2016) and paired-end
214 sequenced on an Illumina MiSeq platform using v3 chemistry (2x300bp), as described in
215 Comeau et al. (2017). Subsequently, sequences were processed with Quantitative Insights
216 Into Microbial Ecology version 2 (QIIME2 version 18.8) software (Bolyen et al., 2018)
217 following the protocol initially established in Comeau et al. (2017), more recently updated
218 on the Microbiome Helper website (Amplicon SOP v2 [qiime2 2018.8];
219 https://github.com/LangilleLab/microbiome_helper/wiki). Briefly, the bacterial raw
220 reads were trimmed of primers obtaining 643,774 sequences, quality controlled and
221 contaminant filtered, followed by the creation of Amplicon Sequence Variants (ASVs)
222 using the Deblur tool, resulting in a total of 531,060 sequences. The final taxonomic

identities of the ASVs were obtained using the QIIME2 feature-classifier plugin (sklearn method) against the SILVA database (version 132; trimmed to the V4V5 version of the 16S). The different statistical of diversity measures were calculated within QIIME2 using the ASV table normalized to 20000 sequences per sample. All these resources are available through the Microbiome Helper website (https://github.com/LangilleLab/microbiome_helper/wiki).

To check for no contamination during ADN extraction one blank control was done in one of the kit tubes. Besides PCR negative controls were done (1 for every 96-well plate = 4 per MiSeq run) which were verified to be clean (no bands present) and still were sequenced on the MiSeq in order to show no substantial reads coming through on this barcode combination.

2.4. Statistical analyses

Alpha-diversity (intragroup diversity) was determined with QIIME2 using the number of observed ASVs in each sample. Faith's Phylogenetic Diversity (Faith, 1992), Pielou (J'), and Shannon indices were evenly sampled at 20,000 reads per sample. Principal-coordinate analysis (PCoA) was obtained from QIIME2 to study β-diversity (intergroup diversity) by Bray-Curtis and Jaccard indices. The relative abundance of soil bacterial taxa was calculated based on the number of reads for the different phyla. The soil bacterial taxa (ASVs) were grouped in genera or the next upper taxonomic level identified in each of the 24 soil samples, and selected those bacterial taxa that had a relative abundance greater than 0.1% throughout all samples. One-way permutational multivariate analysis of variance (PerMANOVA with 9,999 perms, $p < 0.05$) was used to analyse the differences between the different soil types (restored soils - CW and SS, natural soils - NS, and non-treated soils - NA). A similarity matrix used by multivariate PerMANOVA was constructed using Bray–Curtis distance for obtaining significant differences between

248 soils according to the relative abundance of bacterial genera or the next upper taxonomic
249 level identified greater than 0.1% throughout all samples. To discern between which soils
250 there were significant differences, pairwise comparisons were made using a multivariate
251 analogue of the *t* statistic to find the probability levels by permutation (Eldridge et al.,
252 2016). Moreover, significant differences of each individual chemical soil property and
253 the diversity index between soils (organic amended soils - SS and CW -, NA, and NS)
254 were assessed using one-way permutational univariable analysis of variance (PerANOVA
255 with 9,999 perms, $p < 0.05$) and pairwise comparisons (Anderson et al., 2008). The
256 distance-based redundancy analysis (dbRDA) was calculated according to the relative
257 abundance of soil bacterial taxa and chemical soil properties. The statistical package
258 PRIMER + PERMANOVA (PRIMER-E Ltd., Plymouth Marine Laboratory, UK) for
259 Windows was used for PerMANOVA, PerANOVA and dbRDA analysis. A significance
260 level of 0.05 was used, unless otherwise indicated.

261 The R Project environment (R Core Team, 2018) with "stats" and "ggplot2" packages
262 was used to generate boxplot charts and to evaluate the influence of amendments on soil
263 bacterial communities. Generalized linear models (GLMs) were applied using the
264 "survival" library (Therneau, 2015). Pearson's correlations (r; $p < 0.05$) were applied to
265 determine the significant correlations between soil bacterial genera or the next upper
266 taxonomic level identified and chemical soil parameters. Soil bacterial taxa showing
267 highly significant correlations between them ($p < 0.05$) and with the highest correlation
268 values ($r > 0.7$) were selected as bacterial groups forming bacterial co-occurrence
269 patterns, as described in Xue et al. (2020) and Miralles et al. (2020b). This correlation
270 coefficient was selected because we could clearly identify two different co-occurrence
271 groups. Pearson's correlations were calculated using Statgraphics (version 16.2.04 for
272 Microsoft Windows). Network analysis was performed to graphically represent the

relationships between soil bacterial taxa that conform the co-occurrence patterns resulting from Pearson's correlations. Topological properties were calculated to describe the complex pattern of interrelations between nodes and to distinguish differences in taxon correlations. For this purpose, the method for obtaining modularity (Blondel et al., 2008) was used together with the Force Atlas 2 algorithm (Jacomy et al., 2014), based on the interactive platform Gephi 0.9.2 (Bastian et al., 2009) for visualizing networks.

3. Results

3.1. Chemical soil properties

The different restoration treatments applied generated changes in chemical soil properties. PerANOVA ($p < 0.05$) and pairwise tests showed that chemical soil properties were significantly different in SS, CW, NA, and NS soils 10 years after starting the restoration. Nevertheless, the treatment of compost (CW) and natural soils (NS) did not show significant differences between them, except in TN which was significatively higher in CW soils (Table 1 and S1). CW and NS soil showed significantly ($p < 0.05$) higher EC and TOC and significantly ($p < 0.05$) lower pH than SS soils, which values in such soil properties ranged between the CW and NS soils (Table 1). NS and CW soils presented also comparatively higher TOC values and EC values and lower soil pH values than NA soils (Table 1).

3.2. Bacterial richness and α- and β-diversity

Organic amendments added to restored soils produced changes in soil microbial properties. The restored soils (CW and SS soils) showed the significantly highest values ($p < 0.05$) of observed ASVs per soil type and Faith's phylogenetic index (Tables 2 and S2), while NA and NS soils showed the significantly lowest values in both indices, respectively. Shannon and Pielou indices presented higher values in CW and NS soils than in NA and SS soils, being NA soils those that presented the lowest values in Shannon

298 index and SS soils those that presented the lowest values in Pielou index (Table 2 and
299 S2). The PCoA graphs representing β-diversity showed that SS and CW soils were similar
300 to each other, but different from NA and NS soils in Bray-Curtis and Jaccard indices
301 (Figure 1).

302 *3.3. Bacterial community composition*

303 The number of sequences belonging to Bacteria domain was 127,348. Fourteen phyla plus
304 a phylum in which unclassified sequences were grouped showed relative frequencies
305 greater than 0.1% throughout all samples (Figure S1). The most abundant phyla in all
306 treatments were *Proteobacteria*, *Acidobacteria*, *Actinobacteria*, *Bacterioidetes* and
307 *Planctomycetes*, although in different proportions depending on each treatment (Figure
308 S1). The two most abundant phyla in SS and NS soils were *Proteobacteria* and
309 *Acidobacteria*, on the contrary, in CW and NA soils were *Proteobacteria* and
310 *Actinobacteria* (Figure S1).

311 In the studied soils 171 bacterial taxa were identified at the genus level or the next upper
312 taxonomic level available, with a relative abundance greater than 0.1% throughout all
313 samples. The PerMANOVA test revealed that all soil types (CW, SS, NA, and NS)
314 showed significant differences ($p < 0.05$) in bacterial communities at the genus level or
315 the next upper taxonomic level identified (Table S3).

316 Some of bacterial taxa were much more abundant or were associated almost exclusively
317 with each soil type, except in SS soils where no more abundant or exclusive bacterial taxa
318 were found associated with this type of soil (Figure S2). The bacterial taxa (e.g.,
319 *Caenimonas, Sphingomonas, Psychroglaciecola, Blastocatella, Noviherbaspirillum* and
320 *Rubellimicrobium*) were more abundant in NA soils (Figure S2a), whereas bacterial taxa
321 belonging to *Xanthomonadaceae* family*, Phaselicystis,* and *Amb-16S-1323* uncultured
322 bacteria were dominant in CW soils (Figure S2b). Other soil bacterial communities (e.g.,

323 *Craurococcus, Crossiella, uncultured* [family: Gemmataceae], and *uncultured* [order:
324 C0119]) were more abundant or almost exclusive in NS (Figure S1c). Several bacterial
325 taxa (*Pir4 lineage*, *Pedomicrobium, uncultured* [family: Micromonosporaceae],
326 *Terrimonas*, *uncultured* [family: Xanthobacteraceae], *uncultured* [family:
327 Solirubrobacterales 67-14*], Geodermatophilus*, *Singulisphaera*, *Chryseolinea* and
328 *Blastococcus*) were very abundant or almost exclusive in CW and NS soils and, in
329 contrast, present in low abundance in SS and NA soils (Figure S1d).

330 GLMs analyses showed that soil amendment with CW was the restoration treatment that
331 most significantly influenced ($p < 0.05$) for the largest number of soil bacterial taxa (95
332 of the 171 analysed), followed by NA soils with 44 bacterial taxa, 23 bacterial taxa were
333 influenced by NS and lastly soils amended with SS, which significantly influenced only
334 9 bacterial taxa (Table S4).

335 *3.4. Relationships between bacterial community taxa and soils' chemical properties*
336 According to the variations (out of the fitted model and out of the total variation)
337 explained by the axes of dbRDA, axis one (dbRDA1) applied to relative abundance of
338 bacterial taxa explained 63.7% of the fitted model and 35.9% of the total variation of the
339 variables, whereas the axis two (dbRDA2) explained 26.7% and 15% of the total variation
340 (Figure 2). The dbRDA analysis clearly clustered four different groups encompassing the
341 samples of each type of soil: CW, NS, NA and SS (Figure 2).

342 Chemical soil parameters were significantly correlated ($p < 0.05$) with several soil
343 bacterial taxa (Table S6). Moreover, numerous bacterial taxa were significantly correlated
344 between them and those with the highest correlation values ($r > 0.7$) were selected (Table
345 S7).

The relationships between the different bacterial rates and chemical soil properties allowed to differentiate two co-occurrence parts patterns (Figure 3, Table S7). The first co-occurrence pattern (Figure 3 – green, Table S7), represented by the genera *Craurococcus*, *Phaselicystis,* and *Crossiella*, showed high positive correlation with TOC (r ranged between 0.55 and 0.32), TN (r between 0.74 and 0.66), and EC (r between 0.60 and 0.41), but had negative correlations with pH (Table S6). The second co-occurrence pattern (Figure 3 – orange, Table S7), represented by the genera *Sphingomonas, Rubellimicrobium, Noviherbaspirillum, Psychroglaciecola,* and *Caenimonas*, showed positive correlation with pH (r ranged between 0.86 and 0.58) and negative correlations with TOC, TN, and EC (Table S6). The soil bacteria genera belonging to the first co-occurrence pattern were more abundant in NS and CW soils, whereas those bacteria taxa representative of the second co-occurrence pattern were more abundant in NA soils (Figure 3 and S2, Table S4). No clear tendency of soil bacterial groups was found in the SS-treated soils, since different soil bacterial taxa were shared with the other types of soils (Figure S2).

3. Discussion

The different soil types studied (restored soils -CW and SS-, soils without organic amendment -NA- and natural soils -NS-) present the same environmental conditions (climate, slope and geological material), therefore, the significant differences in soil properties are due to the impact of mining activity and restoration treatments carried out more than 10 years ago. Both types of organic amendments significantly modified the chemical soil properties, reducing pH and increasing nutrient availability (TOC, TN) and salinity in soils restored (Table 1 and S1). The compost treatment (CW) is the one that most contributed to increase TOC content through the long-term. This could be due to CW contains highly resilient carbon forms (González-Ubierna et al., 2012), acting as a

long-term nutrient reservoir (Table 1 and S1). However, lower TOC values in SS-treated soils (Table 1) than in CW soils suggested that SS initially presented a higher content of labile organic matter as has been also showed by González-Ubierna et al. (2012). Possibly a large part of this labile organic matter could have been quickly depleted after the application of the amendment through processes of biodegradation (Bernal et al., 1998; Cook and Allan, 1992; Jenkins et al., 2017; Ojeda et al., 2015). The pH in CW soils significantly decreased (Table 1), as was also observed by Tian et al. (2015). Possibly the high-acid organic compounds from TOC in CW soils could have contributed to the decrease in pH. Compost treatment increases soil salinity levels in the short-time but decreases progressively over time as has been observed by other authors (González-Ubierna et al., 2012; Luna et al., 2017). The highest TN content in CW soils could be due to inputs from plant residues (Ros et al., 2003), given that the plant cover showed the highest growth rates in those soils (Luna et al., 2017).

The identification of the greatest diversity and major soil bacterial taxa present in restored soils has clearly shown a change achieved by the restoration effort. The organic amendments contributed to increase the soil diversity and relative abundance of several bacterial taxa in the long-term (Figure 1, Table 2 and S2) like have been also shown by other authors (Bastida et al., 2008; Ros et al., 2003; Tejada et al., 2006). The dominant phyla presented different proportions depending on the soil type (Figure S1), although, in general, they were also common in other semi-arid ecosystems from North America, Kuwait desert, Brazil, China and Spain (Fernandes et al., 2018; Fierer et al., 2007; Lv et al., 2014; Miralles et al., 2020a, 2020b; Quoreshi et al., 2019).

The statistical analysis suggested that the distribution patterns of the soil microbial communities at the genus level or the next upper taxonomic level in the study soils are not random, but instead could be related to changes in soil properties generated by the

soil disturbance due to mining activity and the type of organic amendment used in the restoration treatment (Figure 2). Several bacterial taxa present in NA soils previously adapted to the harsh environmental conditions of semi-arid ecosystems (nutrient shortage, water scarcity, extreme environmental conditions during the summer, etc) could have proliferated in the restored soils favouring by the local soil conditions generated by the organic amendments in the long-term (Table S4 and S6). In soils restored with organic amendments, soil microbial communities suffer a dramatic change as the addition of organic amendments introduces new microbial communities into soils from the amendments themselves (Jurado et al., 2014; Kitamura et al., 2016). Our results lead us to think that some bacterial taxa could have been introduced with the compost and persisted in CW restored soils for more than ten years, since some bacterial genera were exclusive or practically exclusive in CW, but not in the rest soil types (Figure S2b and S4). The bacterial taxon *uncultured* [Family: Amb-16S 1323] was also found in waste deposit and in soil landfill cover (Freitas et al., 2008; Stralis-Pavese et al., 2006). However, the changes in the restored soil properties generated by organic amendments for more than 10 years have played a priority role in the diversity and composition of microbial communities in restored soils as it is supported by statistical analysis (Figure 2, Table S7). The chemical soil parameters studied are considered essential factors driving the composition of soil bacterial communities (Canfora et al., 2014; Goldfarb et al., 2011; Griffiths et al., 2011; Kielak et al., 2016; Lauber et al., 2009; Liu et al., 2014; Lucas-Borja et al., 2020; Miralles et al., 2020a; Sánchez-Marañón et al., 2017). The increase in TOC and TN from organic amendments and debris from vegetation cover colonizing the restored soils for 10 years could have favoured the presence and proliferation of different bacterial taxa (Table 1, 2 and S4, Figure S2b). Bastida et al. (2013) also observed that the nutrient availability (TOC and TN) and especially the soil organic carbon exerts deep

421 control over the dynamics of certain microbial groups in semi-arid soils. Moreover, the
422 different types of organic matter from compost and sewage sludge could have contributed
423 to select the soil bacterial communities, since some bacterial taxa present greater
424 preference for labile carbon sources, while others for recalcitrant carbon compounds such
425 as cellulose, lignin, or tannin-protein (Goldfarb et al., 2011). In turn, the organic
426 amendments generate changes in the vegetation cover which could have caused
427 modifications in diversity and composition of soil microbial communities essentially
428 through plant remains and deposition of phytochemicals within the rhizosphere (Barea et
429 al., 2002; Bastida et al., 2013; Kramer and Gleixner, 2008; Williams et al., 2013). Guo et
430 al. (2018) noted that revegetated soils had a high microbial diversity due to factors such
431 as the presence of roots, small aggregates, nutrients and seemingly governed pores that
432 improve the distribution of bacteria in microhabitats (Nunan et al., 2003), due to nutrient
433 accumulation at the root–soil interface (Kaplan et al., 2013; Nessner-Kavamura et al.,
434 2013). The changes in pH and salinity after the application of the organic amendments
435 could also play a key role in soil bacterial communities (Figure 2). Canfora et al. (2014)
436 observed that some soil bacterial taxa can adapt to changes in salinity, while others could
437 be highly sensitive to such changes. The significatively highest diversity and presence of
438 several bacterial communities more abundant in restored soils could be due to the high
439 salinity, especially in CW (Table 2). Such salinity levels could have contributed to the
440 selection of these microbial communities (Figure S1) by controlling the proliferation of
441 soil bacterial taxa more sensitive to salinity and offering in turn greater advantage to those
442 bacterial communities more adapted to EC values (Table 1 and S6). Likewise, the
443 microbial community in restored soils could have been influenced by soil pH, which can
444 induce significant stress on those soil bacterial taxa that are capable to survive in specific
445 pH ranges (Liu et al., 2014). Soil pH influence also other soil parameters such as nutrient

availability, organic carbon characteristics, soil moisture, cationic metal solubility and salinity, which could affect indirectly to soil microbial composition (Lauber et al., 2009).

Interestingly, the CW restored soils and NS soils presented several soil bacterial communities shared between them (Figure S2d), suggesting that those soil bacterial taxa could have the same preferences for soil local conditions (Figure 2 and S2, Table S6 and S7). In general, these soil bacterial taxa more abundant in CW and NS soils showed high significant positive correlations with TOC, EC and TN (Table S6). Therefore, these soil bacterial taxa could have benefited from a high content of nutrients (TOC, TN; Table 1), providing an advantage over other bacterial communities with survival strategies aimed at proliferating in more unfavourable conditions with low nutrient availability. Some of these bacteria showed lignocellulitic activity (Houfani et al., 2019), as for example Xanthomonadaceae and Beijerinckiaceae families were able to degrade lignin (Ceballos et al., 2017) or *Phaselycistis* that degraded different polysaccharides as cellulose demonstrating an important role in the soil carbon cycle (Sharma et al., 2016). On the other hand, the statistical analysis showed that SS-restored soils had an intermediate stage of development and soil functionality between NS, CW and NA soils. These soils shared different bacterial communities with the rest soil types (Figure 2 and S2, Table 2), being soils that can host a greater number of taxa (Figure S2, Table 2). The evolution of the restored soils properties for more than ten years, approaching natural soils could have also induced major changes in the functionality of bacterial community in N and C cycles. Therefore, new studies focused on exploring the taxonomy-function relationships in restored soils would be needed to clarify this knowledge gap.

Curiously, the results clearly showed two bacterial co-occurrence patterns, one dominant in CW and NS soils (defined as Co-occurrence pattern 1; Figure 3-green) and another dominant in NA soils (Co-occurrence pattern 2; Figure 3-orange). Relationships among

soil microbial taxa shape the distribution of microbial communities and therefore non-random patterns of occurrence and significant relationships among taxa in soils can be expected (Trivedi et al., 2013). As revealed by Barberán et al. (2012) finding general non-random associations forming different patterns of co-occurrence, which included common life strategies at broad taxonomic levels and relationships among soil bacterial community. In general, soil bacterial taxa with the highest correlations between them in the co-occurrence pattern 1 had also high significant positive correlations with TOC, TN, and EC and negative correlations with soil pH (Table S6 and S7). In contrast, the dominant bacterial taxa in co-occurrence pattern 2 showed high significant positive correlations with pH and negative correlations with TOC, TN, and EC (Table S6 and S7). These results suggested that different bacterial niches have been developed in the different soil types, largely depending on their chemical properties (TOC, TN, EC, and pH), where different groups of soil bacterial communities could have stabilized (Figure 3, Table S7). These soil bacterial communities that make up each of these co-occurrence patterns could not only find chemical conditions in the soil that are more favourable for their proliferation but also could establish synergistic relationships in their functions related to the biogeochemical cycles of the soils. A more in-depth study on the relationships between soil bacteria and their functions in biogeochemical cycles in restored soils is warranted.

4. Conclusions

Soil restoration treatments based on the use of organic amendments from residues have had an important effect on chemical properties and on diversity and composition of microbial communities of restored soils through the long-term. Domestic solid waste compost was the organic amendment that in the long-term favoured greater diversity and the proliferation of several more abundant bacterial taxa in those soils. This could be

mainly due to the organic amendment favoured greater improvement in soil quality, creating conditions of pH, salinity and nutrients that offer a greater advantage to certain microbial communities. On the other hand, soils with sewage sludge treatment did not regain their chemical and biological quality compared to natural soils, but rather they remained in an intermediate stage of chemical and biological quality between natural and no-amendment soils. These soils achieved a partial improvement but without reaching the development state of surrounding natural soils. Therefore, the compost amendment accelerated the soil restoration process, both at the level of bacterial communities and in chemical parameters, up to the natural soils in 10 years. On the contrary, soils without any type of organic amendment showed the lowest TOC, TN, and EC, and the highest soil pH, as well as other bacterial communities typical of degraded soils or extreme environments. Thus, the use of both organic amendments could be recommended to restore soils exploited by mining in semi-arid environments, although compost amendment proved to work better to speed up soil restoration to the reference soil state in the long-term. Likewise, the study of soil bacterial communities is key to understanding the evolution and naturalization of mining soils after restoration.

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822

FIGURE

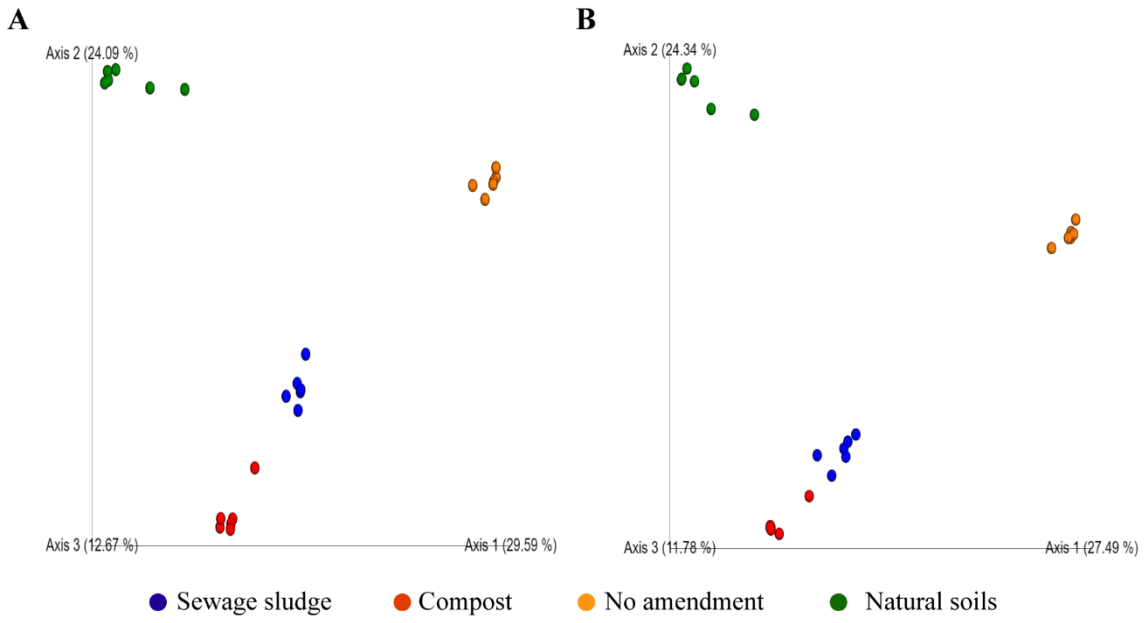
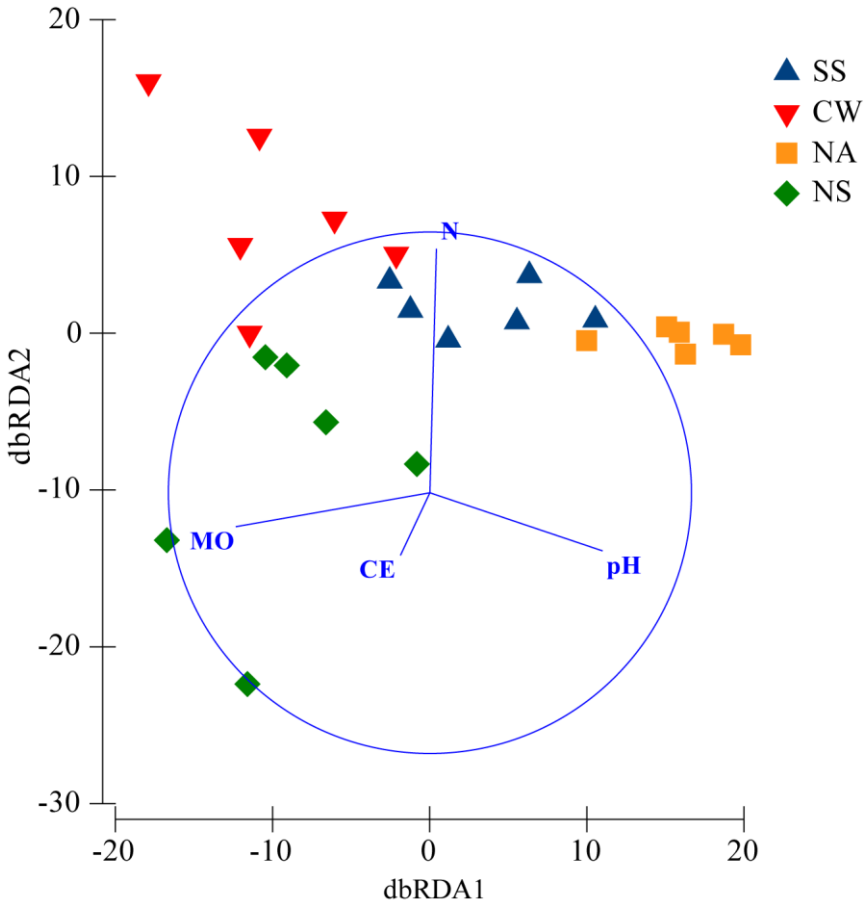
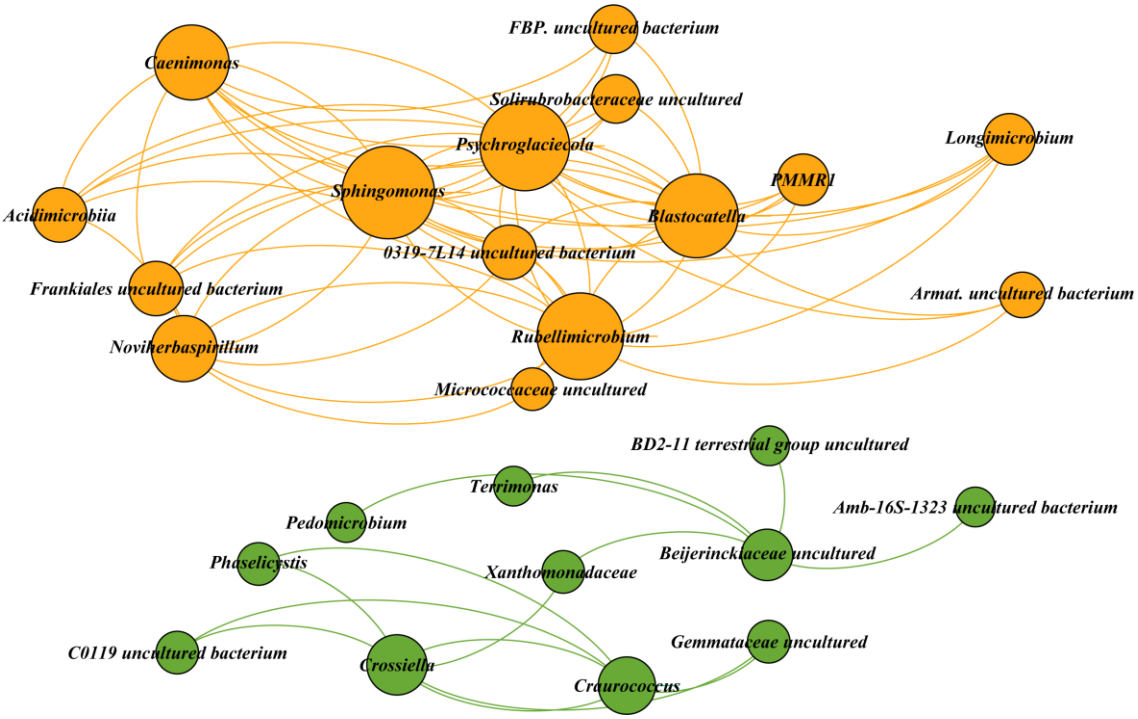


Figure 1. PCoA plots of microbial community analysis by (a) Bray–Curtis index and (b) Jaccard index from samples of all soil types analysed (natural soils, no-amendment soils, and restored soils).



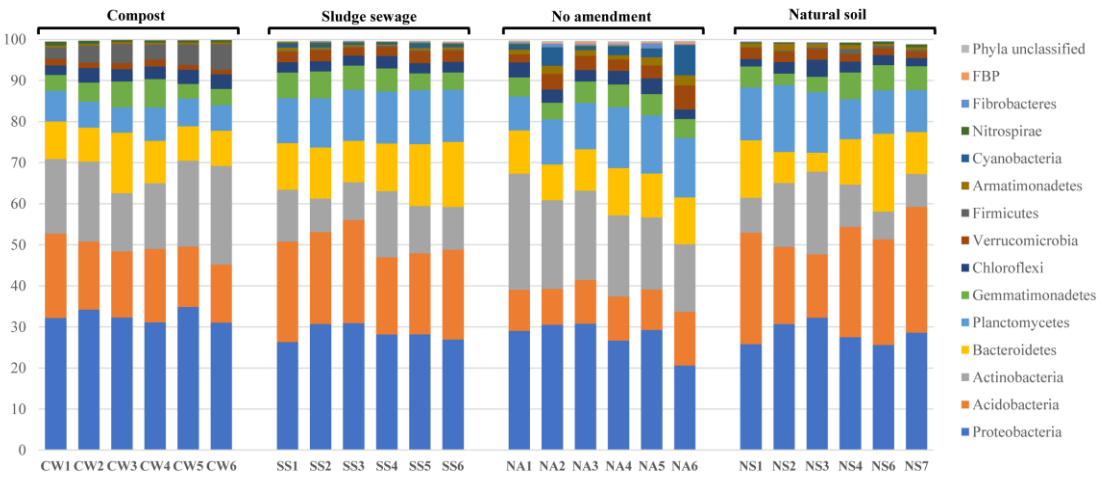
829

830 **Figure 2**. Redundancy analysis for the structure of the bacterial community and chemical
831 soil properties. Soil samples with sewage sludge (SS), compost (CW), no amendment
832 soils (NA) and natural soils (NS) are indicated by different colours and figures. Soil
833 properties are represented by continuous lines.



834

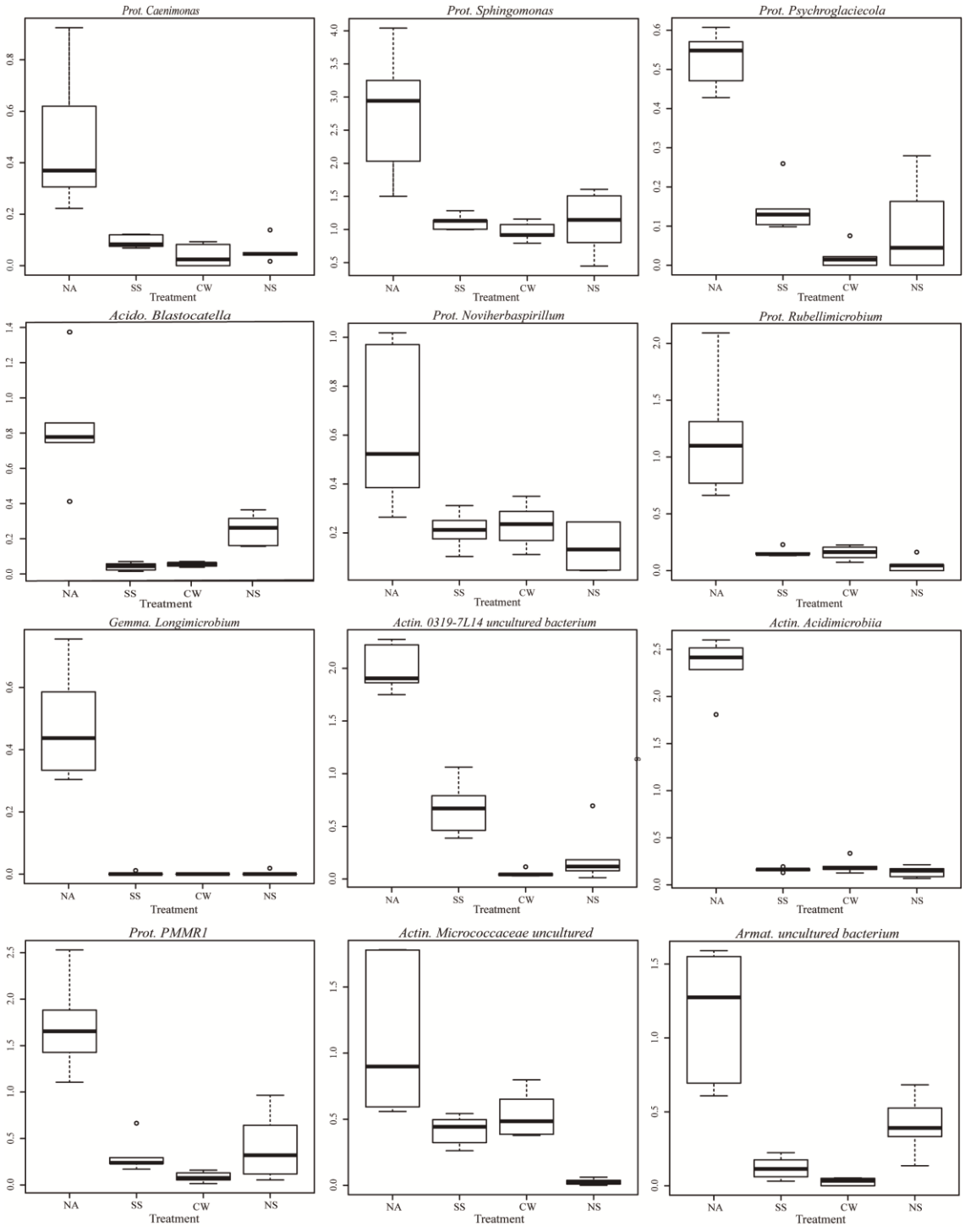
835 **Figure 3.** Co-occurring networks based on Pearson's correlation analysis ($r > 0.7$) for soil
836 bacterial taxa shown to be present in each soil type (CW, SS, NS and NA) with the highest
837 correlations detected. The size of each node is proportional to the number of connections
838 and density of the edges indicates intensity of the correlation. The resulting soil microbial
839 network is made up of 26 nodes and 83 edges (average degree or node connectivity =
840 3.19). The clustering coefficient (how the nodes are integrated into their neighborhood,
841 and therefore, the degree to which they tend to cluster) was 0.5 and modularity was 0.331.
842 Two consortia are shown: Co-occurrence pattern 1 (green) and Co-occurrence pattern 2
843 (orange).



844

845 **Supplementary Figure 1.** Bar chart analysis representing the relative abundance and
846 distribution of ASVs attributed to phylum taxonomic rank. The 15 most abundant phyla
847 are shown in the legend (relative abundance > 0.1%).

848 *Footnotes:* SS: soils restored with sewage sludge; CW: soils restored with urban solid
849 waste compost; NA: no-amendment soils; NS: natural soils.

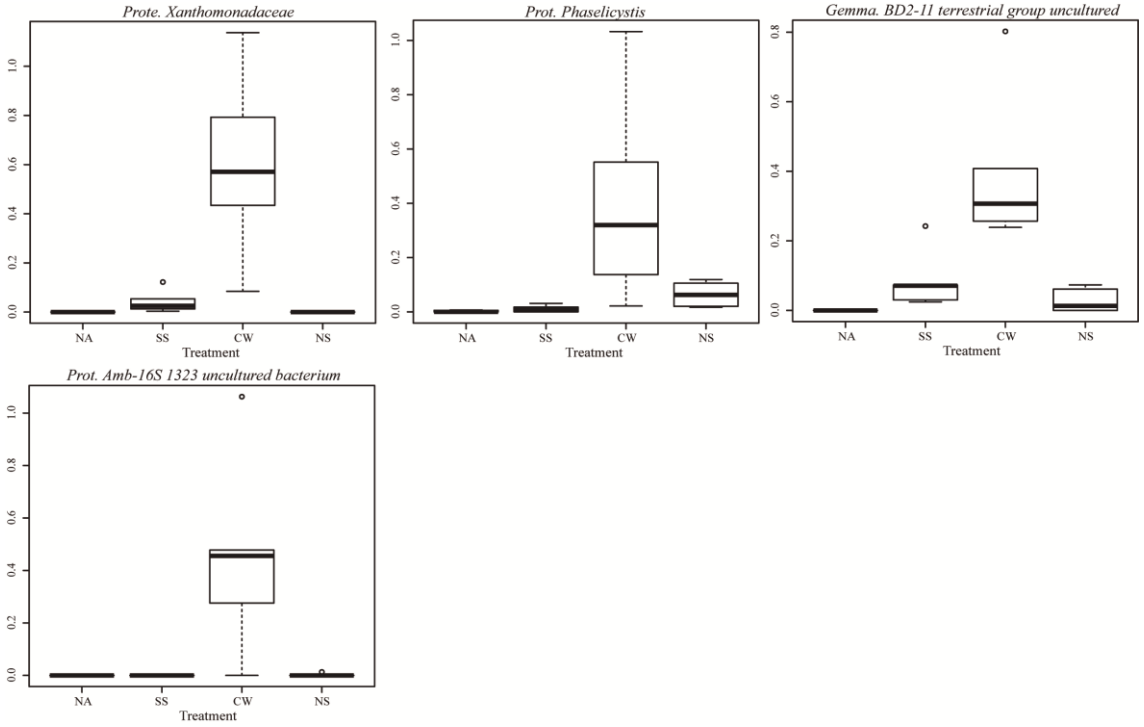

Prot. Caenimonas
Prot. Sphingomonas
Prot. Psychroglaciecola
Acido. Blastocatella
Prot. Noviherbaspirillum
Prot. Rubellimicrobium
Gemma. Longimicrobium
Actin. 0319-7L14 uncultured bacterium
Actin. Acidimicrobiia
Prot. PMMR1
Actin. Micrococcaceae uncultured
Armat. uncultured bacterium
Actin. Frankiales uncultured bacterium
FBP. uncultured bacterium
Actin. Solirubrobacteraceae uncultured
NA
SS
CW
NS
Treatment

850

851

Supplementary Figure 2a. Relative abundance of soil bacteria genera or the next upper taxonomic level exclusive or almost exclusive in soils without amendment.

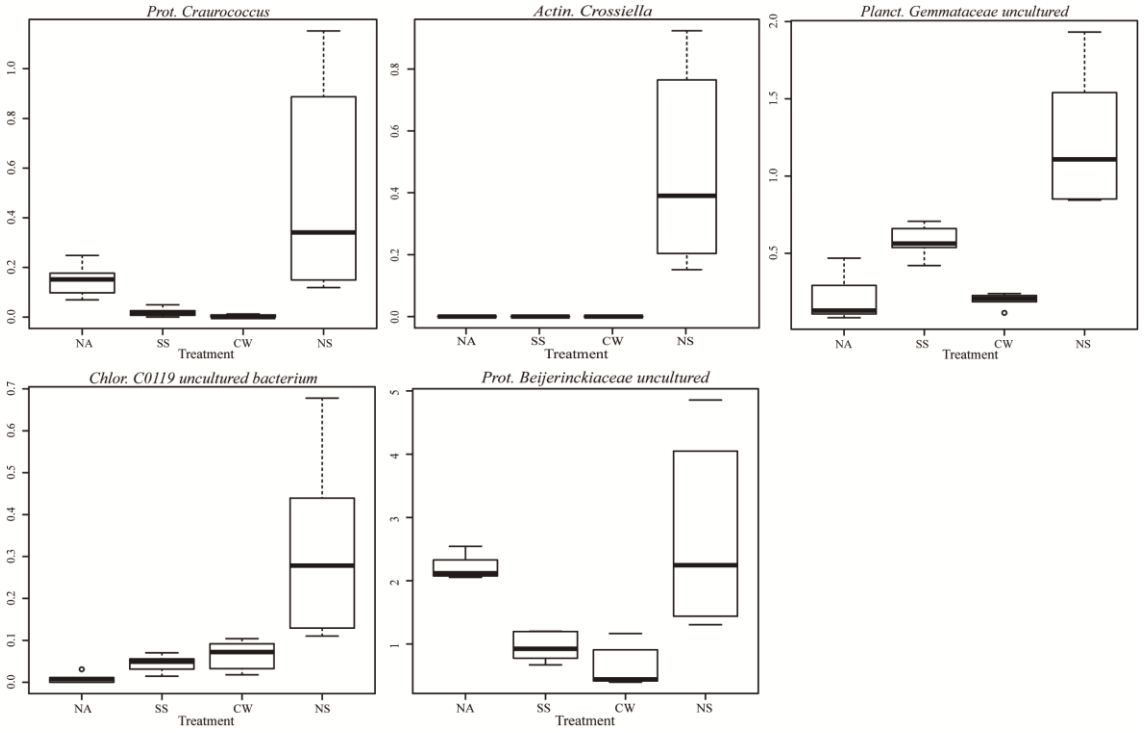
Footnotes: SS: soils restored with sewage sludge; CW: soils restored with urban solid waste compost; NA: no-amendment soils; NS: natural soils. The box represents the relative abundance of soil bacteria (%), with the upper and lower lines corresponding to the first and third quartiles (interquartile range) and the central line the median. Whiskers include those values that deviate up to a maximum distance of 1.5 times the interquartile range. Values with a deviation greater than 1.5 times are represented as circles.



Supplementary Figure 2b. Relative abundance of soil bacteria genera or the next upper taxonomic level exclusive or almost exclusive of CW-treated soils.

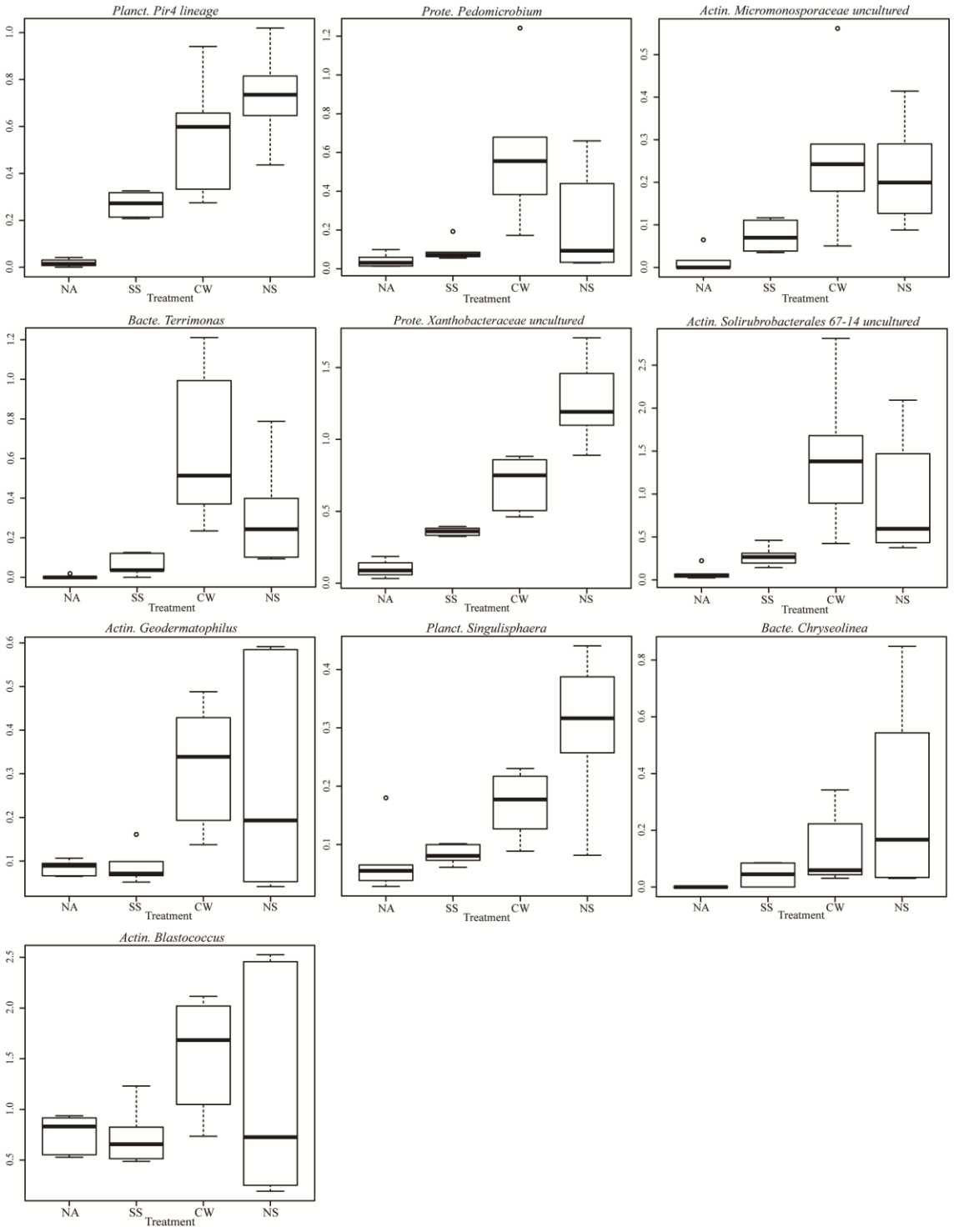
Footnotes: SS: soils restored with sewage sludge; CW: soils restored with urban solid waste compost; NA: no-amendment soils; NS: natural soils. The box represents the relative abundance of soil bacteria (%), with the upper and lower lines corresponding to the first and third quartiles (interquartile range) and the central line the median. Whiskers

include those values that deviate up to a maximum distance of 1.5 times the interquartile range. Values with a deviation greater than 1.5 times are represented as circles.



Supplementary Figure 2c. Relative abundance of soil bacteria genera or the next upper taxonomic level exclusive or almost exclusive of natural soils.

Footnotes: SS: soils restored with sewage sludge; CW: soils restored with urban solid waste compost; NA: no-amendment soils; NS: natural soils. The box represents the relative abundance of soil bacteria (%), with the upper and lower lines corresponding to the first and third quartiles (interquartile range) and the central line the median. Whiskers include those values that deviate up to a maximum distance of 1.5 times the interquartile range. Values with a deviation greater than 1.5 times are represented as circles.



878

879 **Supplementary Figure 2d.** Relative abundance of soil bacteria genera or the next upper

880 taxonomic level exclusive or almost exclusive of CW-treated and natural soils.

881 *Footnotes*: SS: soils restored with sewage sludge; CW: soils restored with urban solid

882 waste compost; NA: no-amendment soils; NS: natural soils. The box represents the

883 relative abundance of soil bacteria (%), with the upper and lower lines corresponding to

the first and third quartiles (interquartile range) and the central line the median. Whiskers include those values that deviate up to a maximum distance of 1.5 times the interquartile range. Values with a deviation greater than 1.5 times are represented as circles.

Tables

Table 1. Chemical soil properties of restored soils, no-amendment soils and natural soils (mean ± SD (n=6)).

	PH	EC	TOC	TN
SEWAGE SLUDGE	8.39 ± 0.13 a	0.21 ± 0.06 a	0.55 ± 0.14 a	0.08 ± 0.02 a
COMPOST	8.00 ± 0.15 b	0.39 ± 0.17 b	3.41 ± 1.52 b	0.53 ± 0.23 b
NO AMENDMENT	8.74 ± 0.09 c	0.11 ± 0.03 c	0.17 ± 0.03 c	0.03 ± 0.01 c
NATURAL SOIL	8.11 ± 0.14 b	0.39 ± 0.09 b	3.11 ± 0.54 b	0.26 ± 0.09 d

EC: Electrical Conductivity (mS cm^{-1}); TOC: Total Organic Carbon content (%); TN: Total Nitrogen content (%). Different letters indicate statistical differences for each treatment ($p < 0.05$).

Table 2. Results of diversity index expressed as media ± SD of each soil type (n=6). From univariate PerANOVA test ($p < 0.05$).

	Observed ASVs	***Faith PD***	***Shannon***	***Pielou e***
Sewage sludge	964 ± 20.81 a	41.44 ± 0.59 a	8.41 ± 0.12 ac	0.84 ± 0.01 ab
Compost	915 ± 65.03 a	38.70 ± 1.59 b	8.69 ± 0.16 b	0.88 ± 0.01 c
No amendment	798 ± 48.70 b	36.34 ± 1.24 c	8.36 ± 0.17 c	0.86 ± 0.01 a
Natural soil	802 ± 50.82 b	33.70 ± 1.30 d	8.57 ± 0.13 ab	0.88 ± 0.00 bc

Supplementary Table 1. PerANOVA analysis. Significant differences ($p < 0.05$) in chemical properties by soil types (SS: sewage sludge; CW: compost; NA: no amendment; NS: natural soil). Pairwise test comparing the different soil types.

PerANOVA

	Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
pH		3	69.28	23.093	37.864	0.0001	9950
	Residues	20	12.198	0.6099			
	Total	23	81.478				
	Pair-wise tests						
	Groups	t	P(perm)	Unique perms			
	SS, CW	4.8147	0.0038	336			
	SS, NA	5.2961	0.0048	245			
	SS, NS	3.613	0.0079	461			
	CW, NA	10.193	0.0031	336			
	CW, NS	1.3368	0.2088	336			
	NA, NS	9.2205	0.0019	336			
	Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
EC		3	11735	3911.8	16.159	0.0001	9948
	Residues	20	4841.5	242.08			
	Total	23	16577				
	Pair-wise tests						
	Groups	t	P(perm)	Unique perms			
	SS, CW	2.2743	0.0508	336			
	SS, NA	4.1192	0.0025	462			
	SS, NS	4.1018	0.005	462			
	CW, NA	4.6595	0.0021	336			
	CW, NS	0.56444	0.6094	179			
	NA, NS	7.7099	0.002	462			
	Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
TOC		3	39674	13225	55.777	0.0001	9944
	Residues	20	4742.1	237.1			
	Total	23	44416				
	Pair-wise tests						
	Groups	t	P(perm)	Unique perms			
	SS, CW	5.7574	0.0025	462			
	SS, NA	7.4077	0.0023	462			
	SS, NS	10.345	0.0015	462			
	CW, NA	7.9544	0.0014	462			
	CW, NS	0.57742	0.7683	461			
	NA, NS	15.86	0.0016	462			
	Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
TN		3	32693	10898	26.632	0.0001	9937
	Residues	20	8184	409.2			

	Total	23	40877				
	PAIR-WISE TESTS						
	Groups	t	P(perm)	Unique perms			
	SS, CW	5.921	0.0022	336			
	SS, NA	4.7714	0.0019	336			
	SS, NS	4.3935	0.0078	336			
	CW, NA	6.6156	0.0023	462			
	CW, NS	2.0721	0.0382	462			
	NA, NS	6.0502	0.0016	462			

Notes: df = degree of freedom; SS = Sum of squares; MS = Mean squares; Unique perms = Number of unique values of the test statistic obtained under permutation.

Supplementary Table 2. PerANOVA analysis. Significant differences ($p < 0.05$) in diversity indices by soil types (SS: sewage sludge; CW: compost; NA: no amendment; NS: natural soil). Pairwise test comparing the different soil types.

	Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Observed ASVs		3	1.24E+05	41167	17.121	0.0001	9467
	Residues	20	48089	2404.5			
	Total	23	1.72E+05				
	Pair-Wise test						
	Groups	t	P(perm)	Unique perms			
	SS, CW	1.7637	0.101	163			
	SS, NA	7.6619	0.0023	247			
	SS, NS	7.2175	0.002	275			
	CW, NA	3.5123	0.002	222			
	CW, NS	3.3436	0.0088	169			
	NA, NS	0.13339	0.909	136			
	Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Faith index		3	196.42	65.475	42.656	0.0001	9953
	Residues	20	30.699	1.5349			
	Total	23	227.12				
	Pair-Wise test						
	Groups	t	P(perm)	Unique perms			
	SS, CW	3.938	0.007	462			
	SS, NA	9.0473	0.0025	461			
	SS, NS	13.232	0.0033	461			
	CW, NA	2.8577	0.0174	462			
	CW, NS	5.9547	0.0022	462			
	NA, NS	3.5981	0.011	462			
	Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms

Shannon index		3	0.41666	0.13889	6.3005	0.0036	9959
	Residues	20	0.44088	2.20E-02			
	Total	23	0.85754				

Pair-Wise test

Groups	t	P(perm)	Unique perms
SS, CW	3.46	0.0113	461
SS, NA	0.52778	0.6048	462
SS, NS	2.171	0.06	462
CW, NA	3.4354	0.0071	461
CW, NS	1.4876	0.1673	461
NA, NS	2.3067	0.0348	462

	Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
Pielou eveness		3	5.97E-03	1.99E-03	18.495	0.0001	9960
	Residues	20	2.15E-03	1.08E-04			
	Total	23	8.12E-03				

Pair-Wise test

Groups	t	P(perm)	perms
SS, CW	3.46	0.0113	461
SS, NA	0.52778	0.6048	462
SS, NS	2.171	0.06	462
CW, NA	3.4354	0.0071	461
CW, NS	1.4876	0.1673	461
NA, NS	2.3067	0.0348	462

Notes: df = degree of freedom; SS = Sum of squares; MS = Mean squares; Unique perms = Number of unique values of the test statistic obtained under permutation.

Supplementary Table 3. PerMANOVA analysis. Significant differences ($p < 0.05$) in bacterial taxa by soil types (SS: sewage sludge; CW: compost; NA: no amendment; NS: natural soil). Pairwise test comparing all soil types.

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
	3	14023	4674.2	13.268	0.0001	9937
Residues	20	7046	352.3			
Total	23	21069				

Pair-wise tests

Groups	t	P(perm)	Unique perms
SS, CW	3.3574	0.0015	462
SS, NA	4.7902	0.0025	462
SS, NS	2.8334	0.0021	462

CW, NA	5.1973	0.0023	461
CW, NS	2.9939	0.0024	462
NA, NS	3.5004	0.0022	462

Notes: df = degree of freedom; SS = Sum of squares; MS = Mean squares; Unique perms = Number of unique values of the test statistic obtained under permutation.

911

912

913 **Supplementary Table 4.** Results of GLM analysis showing the influence of soil type (SS: sewage
914 sludge; CW: compost; NA: no amendment soil; NS: natural soil) on soil bacterial taxa. On the
915 right, the type of soil that most influenced each of the soil bacteria is shown.

		Compost	No amendment	Natural soils	Sewage sludge	Relationship soil type
Actin. Solirubrobacteraceae uncultured	Estimate	0.052	0.696	0.294	0.039	NA
	p-value	0.160	0.000***	0.007**	0.695	
Chlor. C0119 uncultured bacterium	Estimate	0.065	-0.055	0.254	-0.020	NS
	p-value	0.188	0.422	0.001**	0.769	
Gemma. BD2 11 terrestrial group uncultured bacterium	Estimate	0.387	-0.387	-0.360	-0.301	CW
	p-value	0.000***	0.000***	0.000***	0.000***	
Planc. Gemmataceae uncultured	Estimate	0.196	0.005	1.034	0.379	NS
	p-value	0.061.	0.973	0.000***	0.013*	
Prote. Craurococcus	Estimate	0.004	0.146	0.495	0.016	NS
	p-value	0.969	0.268	0.001***	0.905	
Prote. Amb 16S 1323 uncultured bacterium	Estimate	0.455	-0.455	-0.452	-0.455	CW
	p-value	0.000***	0.000***	0.000***	0.000***	
Prote. Psychroglaciecola	Estimate	0.021	0.508	0.068	0.123	NA
	p-value	0.485	0.000***	0.124	0.008**	
Prote. Beijerinckiaceae uncultured	Estimate	0.159	1.013	-0.107	0.000	NA
	p-value	0.064.	0.002**	0.000***	0.488	
Acido. Blastocatella	Estimate	0.056	0.768	0.198	-0.014	NA
	p-value	0.406	0.000***	0.0466*	0.883	
Prote. Rubellimicrobium	Estimate	0.159	1.013	-0.107	0.000	NA
	p-value	0.154	0.000***	0.487	1.000	
Prote. Sphingomonas	Estimate	0.960	1.825	0.150	0.154	NA
	p-value	0.000***	0.000***	0.621	0.610	
Prote. Phaselicystis	Estimate	0.397	-0.395	-0.333	-0.386	CW
	p-value	0.000***	0.001**	0.005**	0.002**	
Prote. Caenimonas	Estimate	0.037	0.431	0.019	0.055	NA
	p-value	0.504	0.000***	0.808	0.489	
Prote. Noviherbaspirillum	Estimate	0.232	0.382	-0.090	-0.021	NA
	p-value	0.004**	0.001**	0.376	0.384	
Prote. Xanthomonadaceae	Estimate	0.598	-0.598	-0.598	-0.558	CW
	p-value	0.000***	0.000***	0.000***	0.000***	
Actin. Crossiella	Estimate	0.000	0.000	0.471	0.000	NS

	p-value	0.000	0.000	0.000***	0.000	
Planc. WD2101 soil group	Estimate	0.404	1.698	1.039	1.451	NA, NS and SS
	p-value	0.032*	0.000***	0.000***	0.000***	
Prote. Psychroglaciecola	Estimate	0.021	0.508	0.068	0.123	NA
	p-value	0.486	0.000***	0.124	0.008**	
Actin. 319 7L14 uncultured bacterium	Estimate	0.053	1.934	0.148	0.621	NA
	p-value	0.531	0.000***	0.221	0.000***	
Actin. Acidimicrobiia	Estimate	0.196	2.144	-0.056	-0.034	NA
	p-value	0.004**	0.000***	0.525	0.698	
Actin. Frankiales uncultured bacterium	Estimate	0.030	0.526	0.013	-0.005	NA
	p-value	0.418	0.000***	0.804	0.927	
Actin. Micrococcaceae uncultured	Estimate	0.530	0.555	-0.503	-0.112	CW and NA
	p-value	0.000***	0.004**	0.008**	0.522	
Armat. uncultured bacterium	Estimate	0.028	1.137	0.381	0.092	NA
	p-value	0.778	0.000***	0.135*	0.523	
FBP. uncultured bacterium	Estimate	0.011	0.319	0.016	0.109	NA
	p-value	0.714	0.000***	0.699	0.014*	
Gemma. Longimicrobium	Estimate	0.000	0.476	0.003	0.002	NA
	p-value	1.000	0.000***	0.950	0.969	
Planc. WD2101 soil group uncultured	Estimate	0.774	2.121	0.645	1.984	NA and SS
	p-value	0.000***	0.000***	0.005**	0.000***	
Prote. PMMR1	Estimate	0.085	1.624	0.319	0.221	NA
	p-value	0.000	0.720	0.001	0.000	
Planc. Pir4 lineage	Estimate	0.567	-0.549	0.164	-0.299	CW
	p-value	0.000***	0.000***	0.010*	0.001***	
Prote. Pedomicrobium	Estimate	0.598	-0.556	-0.373	-0.508	CW
	p-value	0.504	0.000***	0.808	0.489	
Actin Micromonosporaceae uncultured	Estimate	0.261	-0.247	-0.041	-0.187	CW
	p-value	0.000***	0.048*	0.292	0.039*	
Bacte. Terrimonas	Estimate	0.640	-0.637	-0.328	-0.580	CW
	p-value	0.486	0.712	0.023*	0.583	
Prote. Xanthobacteraceae uncultured	Estimate	0.701	-0.602	0.554	-0.342	CW
	p-value	0.000***	0.033*	0.007**	0.059.	
Actin. Geodermatophilus	Estimate	0.321	-0.236	-0.045	-0.234	CW
	p-value	0.003**	0.778	0.000***	0.253	
Planc Singulisphaera	Estimate	0.170	-0.099	0.130	-0.087	CW
	p-value	0.294	0.706	0.000***	0.858	
Bacte Chryseolinea	Estimate	0.126	-0.126	0.172	-0.083	NS
	p-value	0.000***	0.003**	0.021*	0.003***	
Actin Blastococcus	Estimate	1.548	-0.782	-0.401	-0.820	CW
	p-value	0.000***	0.027*	0.003**	0.777	
Actin. Solirubrobacterales 67-14 uncultured bacterium	Estimate	1.428	-1.352	-0.501	-1.154	CW
	p-value	0.978	0.000***	0.006**	0.554	

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Supplementary Table 5. RDA results, explanatory variance and contribution of relative abundance of soil bacterial taxa and chemical properties.

Percentage of variation explained by individual axes (% explained variation out of total variation)

Axis	Individual	Cumulative
1	35,89	35,89
2	15,04	50,94

Weights
(Coefficients for linear combinations of X's in the formation of dbRDA coordinates)

Variable	dbRDA1	dbRDA2
pH	39,57	-12,14
CE	9,10	-23,85
MO	-2,30	-8,39
N	15,64	65,68

Supplementary Table 6. Significant correlations ($p < 0.05$) between soil parameters and soil bacteria (genus level or the next upper taxonomic level identified). Soil bacterial taxa are arranged in the same order as shown in Figure S1 (r). Negative correlations are in italics.

Soil bacterial taxa	pH	[1]EC	[2]TOC	[3]TN
Prote. Caenimonas	0,67	*-0,52*	*-0,55*	*-0,52*
Prote. Sphingomonas	0,67	*-0,49*	*-0,50*	*-0,43*
Prote. Psychroglaciecola	0,86	*-0,65*	*-0,72*	*-0,62*
Acido. Blastocatella	0,70	*-0,51*	*-0,42*	*-0,45*
Prote. Noviherbaspirillum	0,58	*-0,46*	*-0,41*	*-0,27*
Prote. Rubellimicrobium	0,72	*-0,57*	*-0,52*	*-0,42*
Gemma. Longimicrobium	0,77	*-0,60*	*-0,54*	*-0,47*
Actin. 0319-7L14 uncultured bacterium	0,85	*-0,69*	*-0,75*	*-0,65*
Actin. Acidimicrobiia	0,79	*-0,63*	*-0,56*	*-0,47*
Prote. PMMR1	0,79	*-0,58*	*-0,59*	*-0,53*
Actin. Micrococcaceae uncultured	0,55	*-0,49*	*-0,41*	*-0,20*
Armat. uncultured bacterium	0,73	*-0,52*	*-0,47*	*-0,50*
Actin. Frankiales uncultured bacterium	0,73	*-0,57*	*-0,54*	*-0,46*
FBP. uncultured bacterium	0,84	*-0,67*	*-0,68*	*-0,59*
Actin. Solirubrobacteraceae uncultured	0,63	*-0,39*	*-0,43*	*-0,45*
Prote. Xanthomonadaceae	*-0,59*	0,52	0,54	0,74
Prote. Phaselicystis	*-0,54*	0,60	0,55	0,66
Gemma. BD2-11 terrestrial group uncultured	*-0,55*	0,49	0,39	0,57
Prote. Amb-16S-1323 uncultured bacterium	*-0,55*	0,52	0,53	0,72

Prote. Craurococcus	*-0,59*	0,52	0,54	0,74
Actin. Crossiella	*-0,27*	0,41	0,32	0,09
Planct. Gemmataceae uncultured	*-0,28*	0,40	0,22	*-0,02*
Chlor. C0119 uncultured bacterium	*-0,38*	0,56	0,35	0,21
Prote. Beijerinckiaceae uncultured	*-0,52*	*0,38*	*0,19*	*0,28*
Planct. Pir4 lineage	*-0,76*	0,78	0,85	0,74
Prote. Pedomicrobium	*-0,62*	0,52	0,80	0,81
Actin. Micromonosporaceae uncultured	*-0,63*	0,65	0,78	0,72
Bacte. Terrimonas	*-0,73*	0,58	0,72	0,77
Prote. Xanthobacteraceae uncultured	*-0,64*	0,62	0,71	0,38
Actin. Solirubrobacterales 67-14 uncultured	*-0,65*	0,39	0,68	0,75
Actin. Geodermatophilus	*-0,50*	0,55	0,56	0,61
Planct. Singulisphaera	*-0,55*	0,49	0,55	0,35
Bacte. Chryseolinea	*-0,41*	0,38	0,45	0,12
Actin. Blastococcus	*-0,34*	0,30	0,30	0,42

926 [1]EC: Electrical conductivity; [2] TOC: Total organic carbon; [3]TN: Total nitrogen

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Supplementary Table 7. Soil bacterial co-occurrence patterns and significant correlations ($p < 0.05$) between the representative bacterial genera in each co-occurrence pattern.

	Prote. Sphingomonas	***Prote. Rubellimicrobium***	***Prote. Noviherbaspirillum***	***Acido. Blastocatella***	***Prote. Psychroglaciecola***	***Prote. Caenimonas***	***Prote. Craurococcus***	***Prote. Phaselicystis***	***Actin. Crossiella***
Prote. Caenimonas	0,91				0,74	1			
Prote. Sphingomonas	1	0,72	0,72	0,74	0,81	0,91			
Prote. Psychroglaciecola	0,81	0,77		0,82	1	0,74			
Acido. Blastocatella	0,74	0,75		1	0,82				
Prote. Noviherbaspirillum	0,72	0,89	1						
Prote. Rubellimicrobium	0,72	1	0,89	0,75	0,77				
Gemma. Longimicrobium	0,73	0,75		0,88	0,90	0,72			
Actin. 0319-7L14 uncultured bacterium	0,85	0,83	0,75	0,76	0,94	0,84			
Actin. Acidimicrobiia	0,82	0,87	0,76	0,82	0,91	0,79			
Prote. PMMR1	0,80	0,76		0,93	0,94	0,71			
Actin. Micrococcaceae uncultured		0,85	0,93						
Armat. uncultured bacterium		0,79		0,92	0,83				
Actin. Frankiales uncultured bacterium	0,82	0,96	0,86	0,82	0,87	0,76			
FBP. uncultured bacterium	0,78			0,76	0,84	0,80			
Actin. Solirubrobacteraceae uncultured	0,81			0,77	0,80	0,74			
Prote. Xanthomonadaceae								0,88	0,70
Gemma. BD2-11 terrestrial group uncultured								0,87	
Prote. Amb-16S-1323 uncultured bacterium								0,91	
Prote. Craurococcus							1		0,95
Actin. Crossiella							0,95		1
Planct. Gemmataceae uncultured							0,82		0,91
Chlor. C0119 uncultured bacterium							0,92		0,95
Prote. Beijerinckiaceae uncultured							0,92		0,80
Prote. Pedomicrobium								0,73	
Bacte. Terrimonas								0,83	

Soil bacterial consortia were selected from significant positive correlations ($p < 0.05$) between soil bacterial genera with $r > 0.7$. The soil bacterial genera with the highest correlations of the co-occurrence patterns, selected as the bacteria genus representative of each co-occurrence pattern, are shown at the top of the table. The soil bacteria marked present co-occurrence pattern with positive correlations with TOC, EC and TN and negative correlations with pH, associated with no amendment soils (NA). The rest of unmarked soil bacterial had showed a co-occurrence pattern with positive correlations with pH and a negative correlation with TOC, EC and TN.

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