Journal of Environmental Management 241 (2019) 284–292

Contents lists available at ScienceDirect

Journal of Environmental Management

journal homepage: www.elsevier.com/locate/jenvman

Research article

Successional trajectories of soil bacterial communities in mine tailings: The role of plant functional traits

Yannick Colin[a,*], Marta Goberna[a,b], Miguel Verdú[a], Jose A. Navarro-Cano[a]

[a] Centro de Investigaciones sobre Desertificación (CSIC-UVEG-GV), Carretera Moncada – Náquera, km 4.5, Moncada, Valencia, 46113, Spain
[b] Department of Environment and Agronomy, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), Ctra. de la Coruña, km 7.5, E-28040, Madrid, Spain

ARTICLE INFO

Keywords:
Bacterial community assembly
Ecological succession
Heavy metal
Leaf decomposability
Root type
Stress tolerance

ABSTRACT

Plant species identity is assumed to be a major driver of belowground microbial diversity and composition. However, diagnosing which plant functional traits are responsible for shaping microbial communities remains elusive. Primary succession on barren metalliferous mining substrates was selected as the framework to study above-belowground interactions, and plant functional traits that lead the successional trajectories of soil bacterial communities were identified. The impact of the plant functional group (i.e. trees, shrubs, dwarf shrubs, perennial grasses), a trait integrating the life span and morphological structure, on the bacterial primary succession was monitored. Bacterial diversity and composition was estimated along plant size gradients including over 90 scattered patches ranging from seedlings to mature multispecific patches. Soil bacterial diversity was affected by heavy metals levels and increased towards higher resource availability underneath mature patches, with stress-tolerant heterotrophs and phototrophs being replaced by competitive heterotrophs. The plant functional group modulated these general patterns and shrubs had the greatest impact belowground by inducing the largest increase in soil fertility. Functional traits related to leaf decomposability and root architecture further determined the composition and structure of bacterial communities. These results underline the importance of plant functional traits in the assembly of soil bacterial communities, and can help guiding restoration of degraded lands.

1. Introduction

The diversity and community structure of soil microbes undoubtedly determines ecosystem performance, as microbes are key in controlling biogeochemical cycling, soil nutrient storage, gas interchange with the atmosphere and the structure of aboveground communities (Bardgett and van der Putten, 2014). Understanding how the wealth of abiotic and biotic ecosystem components interplay to structure soil microbial communities is thus a fundamental question in ecology (see Fierer, 2017 for a review). Among all abiotic and biotic factors, plant species identity is recognized as a main determinant of the composition of soil bacterial communities (Fierer, 2017; Wardle et al., 2004). The effect of plant identity on soil microbes is necessarily a reflection of the plant's functional traits, which determine where a species can live and how it interacts with the surrounding environment (Cadotte et al., 2011). Plant traits alter the amount, composition and bioavailability of carbon and other nutrient sources in soil and therefore can strongly affect soil microbial communities and ecosystem functioning (De Deyn et al., 2008). Specifically, traits related to leaves (de Vries et al., 2012; Grigulis et al., 2013; Laughlin, 2011), roots (Klumpp et al., 2009; Legay et al., 2014; Valé et al., 2005) and nutrient uptake efficiency (Moreau et al., 2015) impact soil microbial activity, abundance and community composition. However, unravelling the contribution of these different drivers remains complex, as many of the processes are interwoven and their importance may vary depending on environmental conditions.

Primary ecological succession, i.e. the process by which ecological communities assemble over time following the new exposure of barren substrates that leaves little or no biological legacy, can be an ideal setting to study how plants and soil microbes interact (Walker and del Moral, 2011). Indeed, shifts in soil microbial communities undergoing primary succession have been consistently found to vary along with soil age and were suggested to mirror changes in aboveground vegetation (Banning et al., 2011; Edwards et al., 2006; Knelman et al., 2012; Li et al., 2013; Miniaci et al., 2007). Several natural or human-induced disturbances can lead to the formation of barren lands such as volcanic

* Corresponding author.
E-mail address: colin.yannick@gmail.com (Y. Colin).

https://doi.org/10.1016/j.jenvman.2019.04.023
Received 2 October 2018; Received in revised form 20 February 2019; Accepted 7 April 2019
Available online 19 April 2019

lava flow, ash fields, mined sites, deserts or forelands of receding glaciers (Walker and del Moral, 2003). As a general trend, these newly exposed or deposited substrates are characterized by harsh environmental conditions and are rapidly colonized by microorganisms that contribute to critical processes for soil formation and ecological succession (i.e. weathering of parent material, stabilization of soil aggregates and nutrient cycling) (Ciccazzo et al., 2016; Nemergut et al., 2007; Schmidt et al., 2008; Schulz et al., 2013; Velbel, 1988). Bacteria have a main role as early colonizers probably due to their high dispersion rates and large panel of metabolisms, which allow a wealth of taxa to cope with environmental conditions (e.g. nutrients limitation, desiccation, UV radiation and temperature fluctuations) (Schmidt et al., 2008).

On barren mine lands, high metal loads can affect soil microorganisms by limiting their biomass and activity, or altering their diversity and community structure (Borymski et al., 2018; Kandeler et al., 2000; Stefanowicz et al., 2008; Zhang et al., 2016). Microbial taxa tolerating high metal concentrations are selected (Mohamad et al., 2017), some of which alter metal bioavailability in soil through acidification, chelation, complexation, precipitation, and redox reactions (Gadd, 2000; Piotrowska-Seget et al., 2005; Roane, 1999). On the other hand, the natural colonization of mine tailings by plants induce significant changes in soil microbial communities (Borymski et al., 2018; Kozdroj and Elsas, 2000; Wood et al., 2016). In particular, nurse plants as early-colonizers are likely to impact soil microbes thriving in the vicinity of their root system (Edwards et al., 2006; Miniaci et al., 2007). Different nurse species show distinct levels of metal bioaccumulation in leaves (Navarro-Cano et al., 2018), which would indicate a species-specific contribution to the metal concentrations in soils beneath their canopies through litter deposition. As they grow, nurse plants modify the environmental and soil fertility conditions underneath their canopy and facilitate the growth of less stress-tolerant plants (Navarro-Cano et al., 2018, 2014). Such plant-plant facilitation process leads to the formation of vegetated patches and initiates a cascade of belowground processes, including the promotion of ecosystem functions related to soil microbial productivity, decomposition and C, N and P cycling (Navarro-Cano et al., 2014). The identity and stage of plant development can impact specific microbial groups, such as rhizobacteria (Chaparro et al., 2014; Melo et al., 2011; Wang et al., 2008). Moreover, the ability of a plant to promote microbially-mediated processes in mine tailings depends on a set of functional traits associated with the plant life span and morphological structure, photosynthetic metabolism, leaves and roots (Grigulis et al., 2013; Legay et al., 2014; Navarro-Cano et al., 2018). One could therefore expect these plant functional traits to be key determinants of the shifts in bacterial diversity and community composition throughout the restoration of mining sites.

To test this hypothesis, the responses of soil bacterial community to the establishment of nurse plants species belonging to four different plant functional groups (i.e. trees, shrubs, dwarf shrubs or perennial grasses) was monitored in metal-polluted mine tailings in drylands. Here, plant functional group was used as an integrative trait of the plant life span and morphological structure. The successional trajectories of soil bacterial communities were studied in bare soil and throughout plant size gradients, encompassing from seedlings to 30-year-old full-grown vegetation patches either assembled by trees, shrubs, dwarf shrubs or perennial grasses (Navarro-Cano et al., 2018; Figs. S1 and S2). This study specifically tested whether the increase in soil bacterial diversity and shifts in community composition associated with plant development depends on the plant functional group. It was further assessed whether such an increase in bacterial diversity can be explained by the plant's ability to increase soil fertility (i.e. organic substances, mineral nutrients and moisture) and/or reduce abiotic stress (i.e. pH, salinity and concentration of heavy metals). In addition, the importance of plant functional traits, including general morphological and physiological traits, as well as leaf and root traits, in determining the changes in soil bacterial composition was evaluated. Identifying key plant traits that trigger microbial succession in barren ecosystems can be fundamental to provide new tools for land rehabilitation and ecological restoration of microbially-mediated ecosystem services through a better selection of target plant species.

2. Material and methods

2.1. Site description

The Cartagena-La Unión Mining District (Murcia, Southeastern Spain; 30 S 689151 E, 4164433 N) is located in a semiarid Mediterranean area, where metalliferous ores have been extracted over centuries. During the 2nd half of the twentieth century and up to 1991, mining activities generated waste tailings often characterized by low pH values, high heavy metal concentrations (iron, lead, zinc, copper, arsenic, and cadmium among others), high electrical conductivity and poor nutrient and water contents that limit natural restoration (Conesa et al., 2008). Despite these harsh environmental conditions, scattered vegetation patches are naturally colonizing the abandoned barren tailings. In this system, plant patches can be triggered by several nurse-plant species belonging to different plant functional groups including trees, shrubs, perennial grasses and dwarf shrubs (Figs. S1 and S2). Moreover, different ecological succession stages can occur within the same mining deposit, ranging from nurse plants at early stages of development to mature nurse plants hosting complex biological communities below their canopies (Navarro-Cano et al., 2018) (Figs. S1 and S2).

2.2. Soil sampling

In May 2015, 106 soil samples were collected across seven mining deposits (see Navarro-Cano et al. (2018) for a complete description of the sampling site and the methodology). Briefly, 93 composite (5 subsamples each) surface soil samples (0–5 cm) were collected below nurse plants belonging to four plant functional groups. For each plant functional groups, 3 to 4 replicates were analyzed (i.e. nurse plant species): trees (*Pinus halepensis*, *Tamarix canariensis* and *Osyris lanceolata*), shrubs (*Atriplex halimus*, *Salsola oppositifolia* and *Dorycnium pentaphyllum*), dwarf shrubs (*Helichrysum stoechas*, *Paronychia suffruticosa* and *Limonium carthaginense*), and perennial grasses (*Lygeum spartum*, *Stipa tenacissima*, *Piptatherum miliaceum* and *Hyparrhenia synaica*) (Table S1; Fig. S1). Soil samples were collected below each nurse-plant species following a plant size gradient ranging from seedlings to mature plants that represent the different steps of natural tailings restoration. The size of each nurse plant was measured as the mean canopy diameter and was used as a surrogate of the plant age (Navarro-Cano et al., 2015). The number of soil samples taken to describe the plant size gradients of each plant species varied depending on the functional groups (trees: 10; shrubs: 10; perennial grasses: 8 and dwarf shrubs: 6) so as to balance the sampling effort across the gradients of plants that can differ up to two decades in their lifespan (Fig. S1). In a previous study, it has been shown that these unbalanced sampling did not significantly impact the soil parameters under study across plant species (Navarro-Cano et al., 2018). Additionally, 13 composite soil samples were taken to characterize the gaps, that is, the open spaces adjacent to plant patches. Biological crusts were not detected to develop significantly in the study area. Soils were transported to the laboratory on ice, sieved through a 2-mm mesh and stored at 4 °C during analyses. Gap samples were characterized by neutral pH values (7.3 ± 0.9), low contents of total organic carbon (3.4 ± 0.9 g kg^{-1}) and total nitrogen (0.4 ± 0.2 g kg^{-1}) and high concentrations of heavy metals and metalloids such as zinc (7533 ± 3952 mg kg^{-1}), lead (5832 ± 6113 mg kg^{-1}), copper (56.9 ± 32.5 mg kg^{-1}), cadmium (18.1 ± 11.8 mg.kg-1) and arsenic (235 ± 153 mg.kg-1) (Table S2). Detailed characterization of soil samples is available on the Dryad Digital Repository (https://datadryad.org/resource/doi:10.5061/dryad.

j70qf).

2.3. Nucleic acid extraction and amplification of microbial 16S rRNA genes

Genomic DNA was extracted from ca. 10 g of homogenized soil using the PowerMax® Soil DNA Isolation Kit (MO BIO Laboratories Inc., CA, USA). The amplicon sequencing protocol targets the V3 and V4 regions of the 16S rRNA genes with the universal prokaryotic primers (Pro341F/Pro805R) designed surrounding conserved regions (Takahashi et al., 2014). Following the Illumina protocol, DNA amplicon libraries were generated using a limited cycle PCR: initial denaturation at 95 °C for 5 min, followed by 28 cycles of annealing (95 °C 30 s, 55 °C 30 s, 72 °C 30 s) extension at 72 °C for 5 min, using a KAPA HiFi HotStart ReadyMix (KK2602). Then Illumina sequencing adaptors and dual-index barcodes (Nextera XT index kit v2, FC-131-2001) were added to the amplicon. Libraries were then normalized and pooled prior to sequencing. The pool containing indexed amplicons was then loaded onto the MiSeq reagent cartridge v3 (MS-102-3003) spiked with 25% PhiX control to improve base calling during sequencing, as recommended by Illumina for amplicon sequencing. Sequencing was conducted on a MiSeq Illumina platform using a 2 × 300 bp paired-end reads protocol at the Fisabio sequencing service (http://fisabio.san.gva.es).

2.4. Processing of sequencing data

Of the 106 soil samples collected in the mine tailings, 13 samples (mainly taken from gaps or underneath seedlings) had too low DNA concentrations to allow 16S rRNA gene high-throughput sequencing although 10 g of soil was used for each extraction, which is 40 times greater than the amount usually employed for soil DNA extraction (Table S1). This reflects that metal-polluted mine deposits barely have any biological legacy, and thus constitute an appropriate scenario to study primary succession. A total of 5,219,471 paired-end raw reads were obtained across 93 soil samples. Raw sequences were quality filtered with the *prinseq-lite* program (Schmieder and Edwards, 2011). Sequences were trimmed from the 3′-end sequences having a mean quality score lower than 20 using a sliding window of 20 nucleotides and removed those shorter than 50 bp. Demultiplexed paired reads were joined with the *fastq-join* program from the ea-tools suite (Aronesty, 2011). Joined sequences shorter than 200 bp or including ambiguous base calls were removed, and chimeras identified with USEARCH 6.1 (Edgar, 2010) using QIIME 1.7 (Caporaso et al., 2011). After quality filtering, the dataset contained 5,159,426 reads with an average length of 450 bp. Despite the use of universal primers (Pro341F/Pro805R) for the simultaneous detection of bacterial and archaeal communities (Takahashi et al., 2014), only few archaeal sequences were detected in the samples. Thus, sequences belonging to the archaeal domain (1035 sequences) or with no taxonomic affiliation at the domain level (41,277 sequences) were excluded from the analysis. Moreover, one sample (osqu7) with poor sequencing yield (< 1000 reads), as well as singleton OTUs were discarded from the dataset. For all downstream analyses, the library size was rarefied to 17,808 randomly selected reads per sample to correct for differences in sequencing depth. Subsampling procedure resulted in the analysis of 1,638,336 bacterial sequences among the 92 soil samples. At this depth of coverage, Operational Taxonomic Units (OTUs) were clustered with UCLUST (Edgar, 2010) at a 97% sequence similarity, and OTU taxonomy was determined using the GreenGenes database (version 13_5) (McDonald et al., 2012). In total, 30,146 OTUs were defined, with an average of 2716 OTUs per sample. Of these bacterial OTUs, 99.81% were classifiable to the phylum level and 24.37% were classifiable to the genus level.

Several alpha-diversity estimates were calculated (Table S3). Richness (S.obs) was calculated as the number of observed OTUs in each sample. The diversity within each individual sample was estimated using the non-parametric Shannon index. Evenness of the bacterial community was estimated using Pielou's evenness index (J = D/log(S)), where S is rarefied OTU richness and D is Shannon's diversity index.

2.5. Accession numbers

The sequencing dataset generated for this study was deposited in the European Nucleotide Archive (www.ebi.ac.uk/ena/data/view/PRJEB23564).

2.6. Plant functional traits

For each plant nurse species, morphological and physiological traits are presented in Table S4 (Navarro-Cano et al., 2018). Briefly, the plant functional groups (trees, shrubs, perennial grasses and dwarf shrubs) were assigned based on (Paula et al., 2009) and authors' criterion. Other trait values were either obtained from the literature (photosynthetic metabolism and halophytism) (Cornelissen et al., 2003; Mateo and Crespo, 2014; Pyankov et al., 2010) or from five adult plants sampled in the mine tailings (root intensivity and leaf C/N ratio). Root intensivity is indicative of a plant's effort to explore the soil volume with its fine root system and was estimated as the ratio between the fresh root length (maximum length of stretched roots) and the dry root weight (cleaned and 65 °C oven-dried roots) after digging up the whole root system. The leaf C/N ratio is a good predictor of decomposability (Gallardo and Merino, 1993; Poca et al., 2014) and was estimated from total organic carbon and nitrogen concentrations contained in ca. 10 g of fresh ground leaves per plant using standard procedures as in (Navarro-Cano et al., 2015).

2.7. Statistical analysis

First, it was evaluated whether soil bacterial alpha-diversity increases with plant size depending on the plant functional group. Three to four nurse species were used within each functional group as independent replicates. Specifically, a generalized linear mixed model (GLMM) was performed to test the effect of the plant functional group, which was used as an integrative trait, on bacterial Shannon's diversity index including the plant diameter as a covariable to account for the different size of species belonging to distinct plant functional groups. Plant diameter was log-transformed to better approximate a normal distribution. The ‘mining deposit’ was also included as a random factor in the model, so as to account for site effects (Table S5). Similar GLMMs were performed to test the effect of the plant development on the diversity of major bacterial phyla. The p-values in the GLMMs were adjusted for multiple comparisons with the Benjamini-Hochberg correction to control for the false discovery rate. GLMMs were performed with the ‘lme’ function in the ‘nlme’ package for R (Pinheiro et al., 2017).

It was then tested whether the increase in soil bacterial diversity was related to an increase in soil fertility and/or a reduction in abiotic stress. A principal component analyses (PCA) was carried out for soil fertility (total organic carbon, total nitrogen, phosphorus, potassium, soil moisture) and abiotic stress parameters (soil pH, electrical conductivity, and concentrations of arsenic, copper, cadmium, lead, zinc, aluminum, lithium) using the ‘FactoMineR’ package (Lê et al., 2008). The first and second principal components (PC1 and PC2) were used as predictors of soil bacterial diversity including plant diameter as a covariable in a GLMM as above (Table S5). The function ‘dimdesc’ from the FactominR package was used to identify the variables with the highest correlations with the principal components. It was further tested whether the shifts in PC1 and PC2 were related to the plant size or depended on the plant functional group. A log-transformation was applied to PC2 to reach a normal distribution.

The evolution of the relative abundance of bacterial taxa along with plant size was statistically assessed by using the relative abundance of each taxon as a dependent variable in separate GLMMs (Table S5),

including false discovery rate correction, as above. In addition, in order to investigate the relationships between the bacterial community structure, soil fertility and abiotic stress, as well as plant functional traits, a non-metric multidimensional scaling was performed using the 'metaMDS' function in the 'Vegan' package for R (Oksanen, 2017). Dissimilarity in community structure was calculated using the Bray–-Curtis index with the 'vegdist' function. 'Envfit' was run with 999 permutations to plot significantly correlated variables on the ordination. To determine whether bacterial community structure changes along with plant development, GLMMs were performed using the principal coordinates from the multivariate analysis (NMDS1 and NMDS2) in separate models, in which the effect of soil fertility and abiotic stress predictors was assessed. The 'plant diameter' was included as covariable, to account for differences in plant size across functional groups, and both 'mining deposit' and 'plant functional group' as random factors, to account for site and functional group effects (Table S5). Furthermore, it was tested whether plant functional traits indicative of the plant physiology (carbon fixation metabolism and halophytism), leaf decomposability (leaf C/N ratio) and the root exploration volume (root intensivity) determined bacterial community structure (NMDS1 and NMDS2) in two GLMMs as above (Table S5). All statistical analyses were carried out in R (v.3.3.2).

3. Results

3.1. Soil bacterial alpha-diversity

Plant functional group impacted significantly the increase in soil bacterial diversity towards more mature plant patches ($F_{3,7} = 3.5$; $P < 0.05$) and no significant interaction was observed between functional groups and plant size. In particular, a significant positive relationship between soil bacterial diversity and plant diameter was revealed underneath trees ($F_{1,22} = 25.2$; $P < 0.001$), shrubs ($F_{1,20} = 9.5$; $P < 0.01$) and perennial grasses ($F_{1,21} = 18.5$; $P < 0.001$) but not below dwarf shrubs ($F_{1,13} = 0.8$; $P = 0.39$) (Fig. 1). Interestingly, bacterial diversity appeared to increase more rapidly with size below shrubs (slope = 0.32 ± 0.10) than below trees (slope = 0.23 ± 0.05) and perennial grasses (slope = 0.24 ± 0.06) (Fig. 1). Overall, this

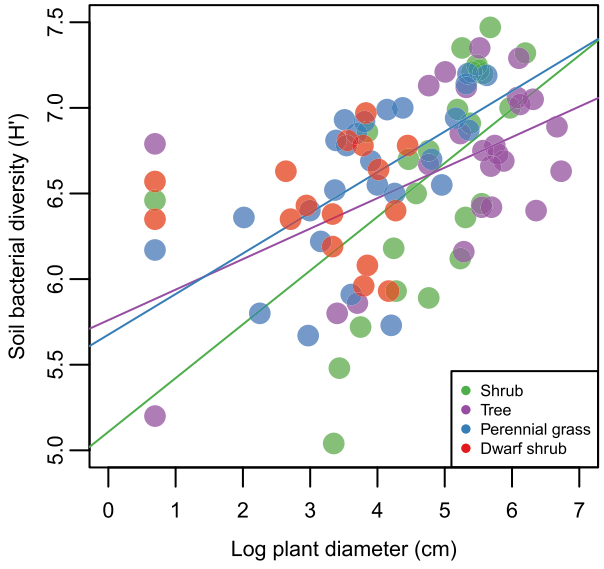

Fig. 1. Relationship between soil bacterial diversity and plant development. The impact of plant size on soil bacterial diversity (Shannon index) was tested independently for each plant life form in GLMMs and regression lines were drawn for significant correlations. The canopy diameter (in cm) was used as a surrogate of plant age and was log-transformed to better approximate a normal distribution.

increasing pattern in soil bacterial diversity held for all dominant phyla, i.e. those accounting for more than 97% of the total reads, except for Cyanobacteria (Fig. S3). That is to say, mature plant patches host more diverse bacterial lineages of Proteobacteria, Actinobacteria, Acidobacteria, Bacteroidetes, Planctomycetes, Chloroflexi, Verrucomicrobia, Gemmatimonadetes and the candidate division TM7 (Fig. S3).

To further analyze the causes of the shifts in soil bacterial diversity, soil physicochemical parameters were reduced to a set of orthogonal dimensions in a principal component analysis (Fig. S4). The first principal component (hereafter PC1.ab.stress) explained 31.5% of the total variance. PC1.ab.stress was positively correlated with electrical conductivity and the concentration of some heavy metals (lead, cadmium and zinc) and negatively correlated with aluminum, lithium and phosphorus levels. The second principal component (hereafter PC2.fert; 21.4%) was mostly explained by total organic carbon, total nitrogen and potassium. Using these two principal components as explanatory variables in GLMMs revealed that Shannon index was inversely correlated with abiotic stress in soil (PC1.ab.stress: $F_{1,80} = 9.9$; $P < 0.01$) and positively correlated with soil fertility (PC2.fert.log: $F_{1,83} = 17.8$; $P < 0.001$). In both cases, significant correlations were observed below plant patches facilitated by trees (PC1.ab.stress: $F_{1,22} = 4.4$; $P < 0.05$ and PC2.fert.log: $F_{1,22} = 7.8$; $P = 0.01$) and shrubs (PC1.ab.stress: $F_{1,20} = 6.5$; $P < 0.05$ and PC2.fert.log: $F_{1,20} = 4.6$; $P < 0.05$), while no significant correlation could be reported below perennial grasses and dwarf shrubs ($P > 0.05$) (Fig. 2). Change in abiotic stress could not be correlated to the biological succession, since no significant correlation was observed between plant size and PC1.ab.stress, whatever the plant functional group considered. In contrast, soil fertility (PC2.fert.log) was shown to increase below mature plant patches triggered by trees ($F_{1,22} = 5.2$; $P < 0.05$) shrubs ($F_{1,20} = 5.0$; $P < 0.05$) and perennial grasses ($F_{1,21} = 4.9$; $P < 0.05$) but not below dwarf shrubs ($P > 0.05$) (Fig. S5).

3.2. Soil bacterial community structure

Major bacterial phyla displayed opposing abundance patterns along the plant size gradients. Specifically, early successional bacterial communities were enriched in Actinobacteria ($F_{1,83} = 33.9$, $P < 0.001$), Chloroflexi ($F_{1,83} = 21.1$, $P < 0.001$), Gemmatimonadetes ($F_{1,83} = 6.7$, $P < 0.1$) and Cyanobacteria ($F_{1,83} = 31.1$, $P < 0.001$), whose relative abundance decreased significantly below mature plants (Fig. 3). Actinobacteria displayed the most drastic drop in abundance that was mainly due to that of the *Acidimicrobiales*, *Solirubrobacterales* and *Rubrobacterales* orders (Fig. S6). Inversely, late successional bacterial communities were significantly enriched in Proteobacteria ($F_{1,83} = 19.4$, $P < 0.001$), Planctomycetes ($F_{1,83} = 15.4$, $P < 0.01$) and Acidobacteria ($F_{1,83} = 11.5$, $P < 0.01$), and a similar but not significant trend was observed for the Verrucomicrobia ($F_{1,83} = 2.4$, $P = 0.24$) and Bacteroidetes ($F_{1,83} = 2.5$, $P = 0.24$) (Fig. 3). At lower taxonomic levels, the relative abundance of Alpha, Beta, and Delta-Proteobacteria were marginally or significantly increased below mature plants (Fig. 3). This shift in abundance was partly driven by the *Rhizobiales, Rhodospirillales* and *Caulobacterales* (Fig. S7), uncharacterized order Ellin6067 (Fig. S8), *Xanthomonadales* and *Legionellales* (Fig. S9) and *Myxococcales* orders (Fig. S10).

Based on the relative abundance of bacterial phyla, NMDS ordination analysis demonstrated that the structure of soil bacterial community was determined by abiotic stress and plant ability to increase soil fertility during biological succession (Fig. 4). In particular, both NMDS axes significantly responded to PC1.ab.stress (NMDS1: $F_{1,81} = 2.3$, $P = 0.13$; NMDS2: $F_{1,75} = 9.0$, $P < 0.01$) and PC2.fert.log (NMDS1: $F_{1,81} = 15.4$, $P < 0.001$; NMDS2: $F_{1,81} = 4.0$; $P < 0.05$). Plant functional traits also modulated the response of the soil bacterial community structure to plant size. Specifically, NMDS1 was significantly determined by leaf C/N ($F_{1,80} = 18.9$; $P < 0.001$) and root intensivity ($F_{1,80} = 4.0$; $P < 0.05$), while NMDS2 responded mainly to root

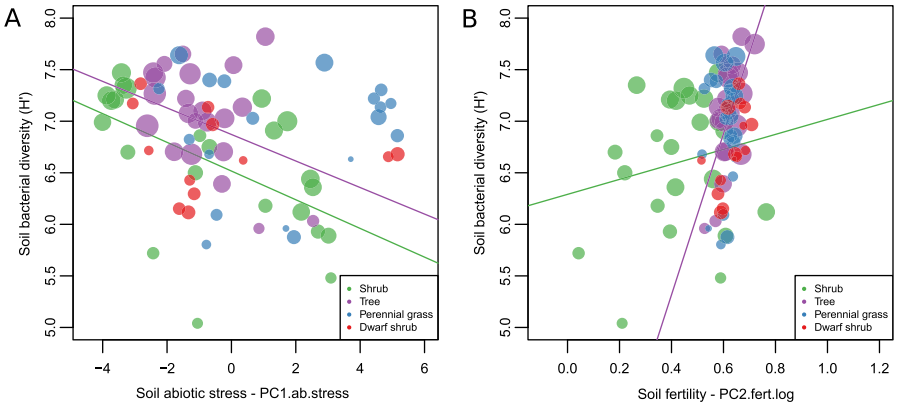

Fig. 2. Effect of abiotic stress (A) and soil fertility (B) on soil bacterial diversity. Principal components were used as explanatory variables to untangle the effect of the relieved abiotic stress (PC1.ab.stress) and increased soil fertility (PC2.fert.log) on soil bacterial diversity (Shannon index). The impact of soil fertility and abiotic stress on soil bacterial diversity was tested independently for each plant life form in GLMMs and regression lines were drawn for significant correlations. Bubble size is proportional to plant diameter.

intensity ($F_{1,80} = 6.3$; $P < 0.05$) (Fig. 4). A detailed analysis further revealed that the increase in relative abundance of Acidobacteria, Planctomycetes, Verrucomicrobia and Deltaproteobacteria could be attributed to the increase in resource availability (PC2.fert.log) mainly ensured by nurse plants with low root intensivity and low leaf C/N ratio such as trees and shrubs (Fig. 4). Moreover, higher relative abundance of Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria and Bacteroidetes rather responded to higher abiotic stresses (PC1.ab.stress).

4. Discussion

The contribution of plant-microbe interactions to ecosystem functioning is getting more and more attention based on trait-based approaches focused on some vegetation types such as grasslands or forests (Kembel et al., 2014; Legay et al., 2014; Moreau et al., 2015; Orwin et al., 2010). This responds to the fact that functional traits underlie the ecological differences between species, and are thus the basis to understand the mechanisms of community assembly, maintenance of biodiversity and performance of ecosystems (De Bello et al., 2017). The present work describes how the growth of functionally different plant species in human-induced barren lands drives soil bacterial succession towards different trajectories. The results show that plant functional traits - particularly those related to plant life span, morphological structure, leaf decomposability and root architecture - exert permeating effects that condition the diversity and community structure of soil bacteria along primary succession in abandoned metal mining deposits. The feedbacks between plants and soil microbes promote multiple ecosystem services that need to be reestablished in degraded lands.

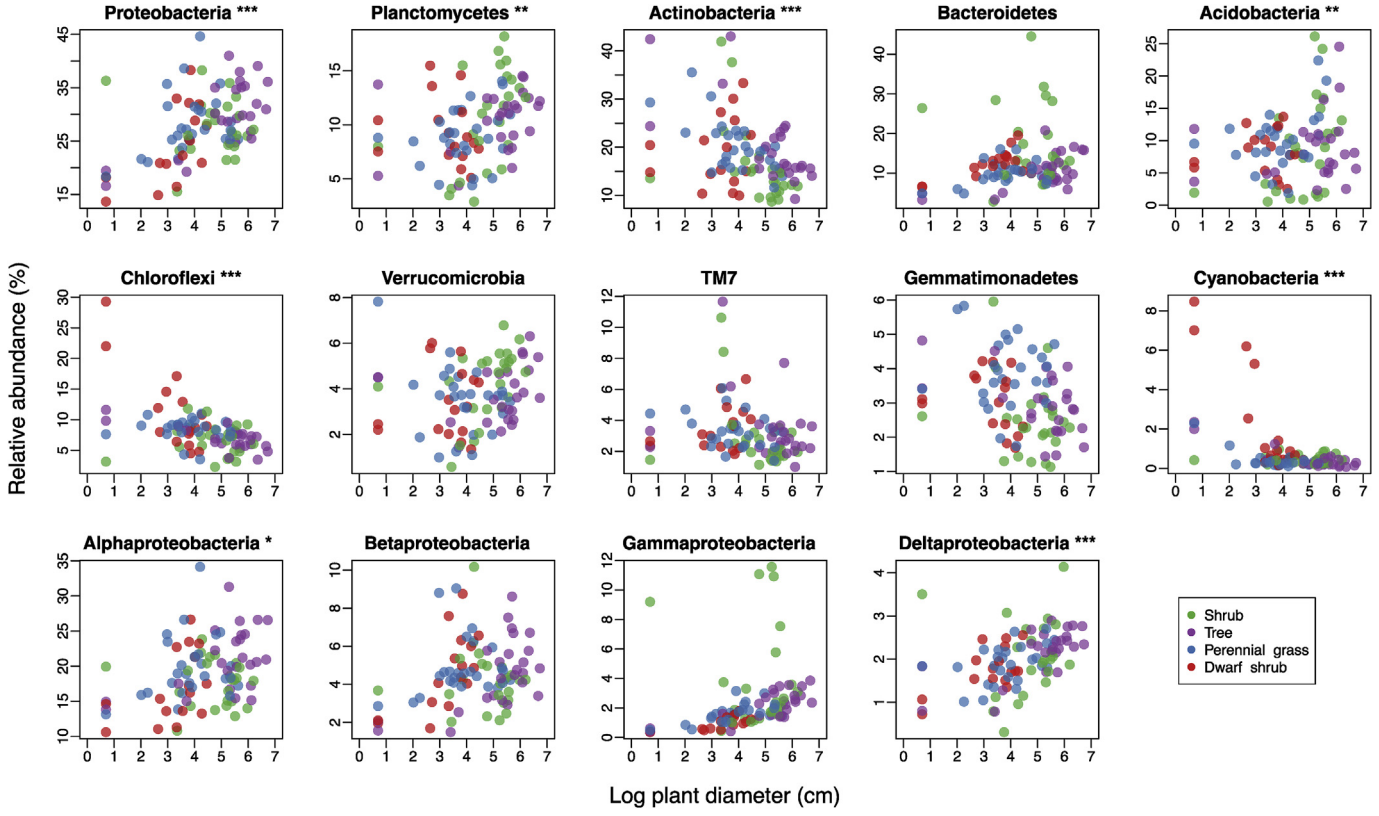

Fig. 3. Impact of plant development on the relative abundance of bacterial phyla. Shifts in relative abundance of the main bacterial phyla were statistically tested along the plant size gradient using GLMMs. The p-values were adjusted for multiple comparisons with the Benjamini-Hochberg correction to control for the false discovery rate and significant relationships are indicated on the plots by the following superscripts: *($p < 0.05$); **($p < 0.01$) or ***($p < 0.001$). The canopy diameter (in cm) was used as a surrogate of plant age and was log-transformed to better approximate a normal distribution.

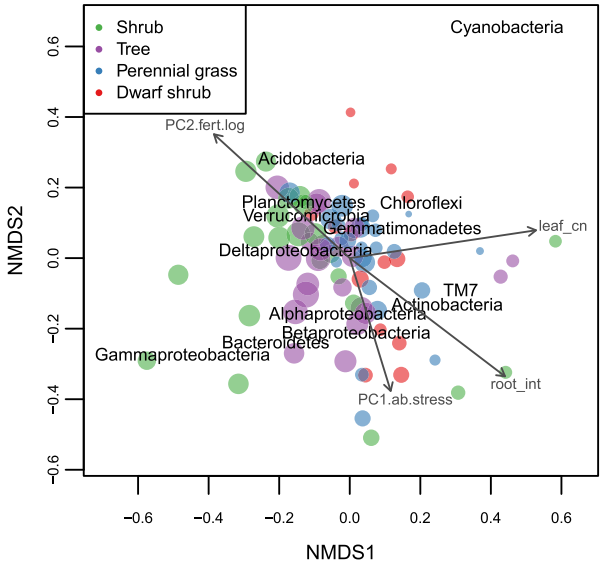

Fig. 4. NMDS plot of bacterial community composition at the phylum level. Environmental parameters that are significantly correlated ($P < 0.05$) are indicated as arrows. The length of an arrow indicates the relative importance of that parameter to the ordination and the angle between arrows indicates the approximate degree to which explanatory variables are correlated. Bubble size is proportional to plant diameter. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

4.1. Impact of plant functional group on soil bacterial alpha-diversity throughout primary succession

Soil bacterial diversity increased significantly along primary succession depending on the plant functional group. The overall bacterial enrichment during ecological succession is in line with previous studies performed on soil chronosequences in which microbial diversity, activity and biomass increased along soil age and revegetation processes (Nemergut et al., 2007; Nicol et al., 2005; Ohtonen et al., 1999; Tscherko et al., 2003). This work further revealed that this increase in diversity holds for all major phyla, except Cyanobacteria, suggesting that natural colonization of barren soils by plants creates heterogeneous niches and resources enhancing bacterial coexistence. Despite this global pattern in bacterial diversity, significant differences were reported depending on plant functional group. In particular, trees, shrubs and perennial grasses development led to a significant increase in soil bacterial diversity while dwarf shrubs did not. Shrubs increased soil bacterial diversity faster than the rest of plant functional groups, but similar bacterial diversities were finally reached at late successional stages of trees, shrubs and perennial grasses despite remarkable differences in plant size and age across plant functional groups. In the same study system, shrubs were previously reported to be efficient at restoring microbially-mediated ecosystem functions, such as organic matter decomposition and nutrient cycling, (Navarro-Cano et al., 2018). Here, the rise in soil bacterial diversity along the primary succession was mainly related to an increase of soil fertility (i.e. total organic carbon, total nitrogen and potassium) below plant patches. Plant nutrient inputs to soil mainly derive from root exudates and leaf litter decomposition (Aneja et al., 2006; Bray et al., 2012; Philippot et al., 2013; Pugnaire et al., 2011). These processes are known to stimulate soil biological activity and shape microbial communities (Hortal et al., 2013; Kuzyakov, 2002; Navarro-Cano et al., 2014; Serna-Chavez et al., 2013). Specifically, plants with larger plant functional groups and longer life spans (i.e. trees and shrubs) were the ones that provided the highest inputs in organic substances and mineral nutrients to soil, and below which a significant positive correlation between the soil fertility, size of the vegetated patch and soil bacterial diversity was reported. Heavy metal loads (Zn, Cd, Pb) and electrical conductivity were also demonstrated to affect soil bacterial diversity. In line with this results, metal contaminated soils were formerly showed to harbor lower microbial diversity and to select metal resistant microorganisms (Chodak et al., 2013; Desai et al., 2009; Gans et al., 2005; Moffett et al., 2003). However, in the present study, heavy metal concentrations were not found to vary according to the stage of development of vegetated patches or the plant functional group but were rather found to differ across the different mining deposits investigated.

4.2. Changes in soil bacterial community structure throughout primary succession

Along with the development of plant patches, soil bacterial composition shifted from communities dominated by stress-tolerant hetero- and autotrophs to mainly heterotrophic communities with high competitive abilities under high resource availability. Within Actinobacteria, the bacterial orders that dominated open spaces or early successional stages (i.e. Acidimicrobiales, Solirubrobacterales, Rubrobacterales and Gaiellales) feature sporulation ability, wide metabolic capacity, secondary metabolite and multiple UV repair mechanisms (McCarthy and Williams, 1992; Ventura et al., 2007; Zenova et al., 2011) or metabolisms based on chemolithotrophic processes (Norris, 2015), making them good candidates for the colonization of oligotrophic barren lands. Although less abundant, Chloroflexi, Cyanobacteria and Gemmatimonadetes exhibited also higher relative abundance in early-stages. These taxa, previously reported in deglaciated soils (Nemergut et al., 2007; Schmidt et al., 2008) and deserts (Makhalanyane et al., 2015), were described to correlate with low soil moisture content and to harbor the photosynthetic machinery (Hanada and Pierson, 2006; Makhalanyane et al., 2015; Zeng et al., 2016), which is an advantageous feature in oligotrophic habitats. Below mature patches, bacterial communities were significantly enriched in Proteobacteria, Acidobacteria and Planctomycetes. Specifically, taxa related to the *Rhizobiales, Xanthomonadales, Legionellales* and *Myxococcales* were notably promoted below shrubs and trees and are likely to fulfill important functions during ecological succession (i.e. nitrogen fixation, phytopathogen biocontrol, phosphate solubilization, production of phytohormones and enzymes) (Busti et al., 2006; Lagos et al., 2015). The composition of rhizobacteria communities in metal-contaminated soils is critical as soil bacteria may enhance indirectly the effectiveness of phytoremediation processes by facilitating metal translocation towards plants or reducing the mobility and therefore the availability of metal contaminants (phytostabilization) (Hao et al., 2014; Teng et al., 2015). In addition, rhizobacteria may confer plant metal tolerance and enhance the plant biomass production that may promote the removal and stabilization of pollutants (Gadd, 2000; Kidd et al., 2009; Rajkumar et al., 2010; Wenzel, 2009). However, the general successional trend was modulated by the plant functional group, suggesting that traits related to plant morphology or physiology drive belowground primary succession in mine tailings.

4.3. Importance of plant functional traits

Plant traits related to leaf decomposability and the root-system architecture were identified as two main determinants of soil bacterial community structure during primary succession. Litter and rhizodeposits represent crucial sources of energy and nutrients for soil microorganisms (De Deyn and Van der Putten, 2005; Personeni and Loiseau, 2004). Here, nurse plants characterized by low leaf C/N ratios or low root intensivity contributed to increase soil fertility during their life span and thus contributed to the replacement of stress-tolerant heterotrophic and phototrophic bacterial taxa by competitive heterotrophic microorganisms. Accordingly, leaf decomposability was formerly demonstrated to increase with decreasing leaf C/N ratios and to promote bacterial abundance and activity (Gallardo and Merino, 1993;

Poca et al., 2014). In addition, the root system architecture determines the capacity of a plant to explore the surrounding soil and as consequence to acquire nutrients for growth and functional metabolism (Dunbabin et al., 2004). Higher root intensivity could indicate a larger nutrient uptake rate by plants, which might adversely affect bacterial communities in the rhizosphere based on competition for resources (Moreau et al., 2015). Moreover, nurse plants with lower root intensivity might favour the establishment of other plants species in their vicinity (De Baets et al., 2007) and ultimately increase the amount and the diversity of root exudates (Eisenhauer et al., 2017; Zak et al., 2003). These results add upon evidence highlighting the importance of incorporating leaf and root traits to explain soil microbial activity, abundance and community structure (de Vries et al., 2012; Grigulis et al., 2013; Legay et al., 2014; Orwin et al., 2010).

5. Conclusions

We show that the plant functional group, which captures the plant's life span and morphological structure, is a key determinant of the increase in soil bacterial diversity and shifts in community structure during primary succession. Specific functional traits related to root architecture and leaf decomposability were identified as drivers of the successional trajectory of soil bacterial communities, stressing the importance of taking plant traits into account to understand the mechanisms of community assembly. The selection of plant species to be used in restoration programs based on their functional traits can promote belowground microbial community assembly, and thus eventually increase soil microbially-mediated ecosystem services in mining sites (Li et al., 2016).

Acknowledgements

The authors thank S. Donat and D. Rodríguez for field and laboratory assistance. This work was supported by the I Convocatoria de ayudas de la Fundación BBVA a proyectos de investigación, the Spanish Ministerio de Economía y Competitividad (projects CGL2014-58333-P; CGL2017-89751-R) and the Generalitat Valenciana (project SEJI/2017/030). MG acknowledges support by the Ramón y Cajal programme (Ministerio de Economía y Competitividad).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2019.04.023.

Conflict of interest

The authors declare no conflict of interest.

References

Aneja, M.K., Sharma, S., Fleischmann, F., Stich, S., Heller, W., Bahnweg, G., Munch, J.C., Schloter, M., 2006. Microbial colonization of beech and spruce litter—influence of decomposition site and plant litter species on the diversity of microbial community. Microb. Ecol. 52, 127–135. https://doi.org/10.1007/s00248-006-9006-3.

Aronesty, E., 2011. ea-utils : "Command-Line Tools for Processing Biological Sequencing Data".

Banning, N.C., Gleeson, D.B., Grigg, A.H., Grant, C.D., Andersen, G.L., Brodie, E.L., Murphy, D.V., 2011. Soil microbial community successional patterns during forest ecosystem restoration. Appl. Environ. Microbiol. 77, 6158–6164. https://doi.org/10.1128/AEM.00764-11.

Bardgett, R.D., van der Putten, W.H., 2014. Belowground biodiversity and ecosystem functioning. Nature 515, 505–511. https://doi.org/10.1038/nature13855.

Borymski, S., Cycoń, M., Beckmann, M., Mur, L.A.J., Piotrowska-Seget, Z., 2018. Plant species and heavy metals affect biodiversity of microbial communities associated with metal-tolerant plants in metalliferous soils. Front. Microbiol. 9. https://doi.org/10.3389/fmicb.2018.01425.

Bray, S.R., Kitajima, K., Mack, M.C., 2012. Temporal dynamics of microbial communities on decomposing leaf litter of 10 plant species in relation to decomposition rate. Soil Biol. Biochem. 49, 30–37. https://doi.org/10.1016/j.soilbio.2012.02.009.

Busti, E., Monciardini, P., Cavaletti, L., Bamonte, R., Lazzarini, A., Sosio, M., Donadio, S., 2006. Antibiotic-producing ability by representatives of a newly discovered lineage of actinomycetes. Microbiology 152, 675–683. https://doi.org/10.1099/mic.0.28335-0.

Cadotte, M.W., Carscadden, K., Mirotchnick, N., 2011. Beyond species: functional diversity and the maintenance of ecological processes and services. J. Appl. Ecol. 48, 1079–1087.

Caporaso, J.G., Lauber, C.L., Costello, E.K., Berg-Lyons, D., Gonzalez, A., Stombaugh, J., Knights, D., Gajer, P., Ravel, J., Fierer, N., Gordon, J.I., Knight, R., 2011. Moving pictures of the human microbiome. Genome Biol. 12, 1–8. https://doi.org/10.1186/gb-2011-12-5-r50.

Chaparro, J.M., Badri, D.V., Vivanco, J.M., 2014. Rhizosphere microbiome assemblage is affected by plant development. ISME J. 8, 790–803. https://doi.org/10.1038/ismej.2013.196.

Chodak, M., Go\lębiewski, M., Morawska-P\loskonka, J., Kuduk, K., Niklińska, M., 2013. Diversity of microorganisms from forest soils differently polluted with heavy metals. Appl. Soil Ecol. 64, 7–14.

Ciccazzo, S., Esposito, A., Borruso, L., Brusetti, L., 2016. Microbial communities and primary succession in high altitude mountain environments. Ann. Microbiol. 66, 43–60.

Conesa, H.M., Schulin, R., Nowack, B., 2008. Mining landscape: a cultural tourist opportunity or an environmental problem?: the study case of the Cartagena–La Unión Mining District (SE Spain). Ecol. Econ. 64, 690–700.

Cornelissen, J.H.C., Lavorel, S., Garnier, E., Diaz, S., Buchmann, N., Gurvich, D.E., Reich, P.B., Ter Steege, H., Morgan, H.D., Van Der Heijden, M.G.A., 2003. A handbook of protocols for standardised and easy measurement of plant functional traits worldwide. Aust. J. Bot. 51, 335–380.

De Baets, S., Poesen, J., Knapen, A., Barberá, G.G., Navarro, J.A., 2007. Root characteristics of representative Mediterranean plant species and their erosion-reducing potential during concentrated runoff. Plant Soil 294, 169–183.

De Bello, F., Šmilauer, P., Diniz-Filho, J.A.F., Carmona, C.P., Lososová, Z., Herben, T., Götzenberger, L., 2017. Decoupling phylogenetic and functional diversity to reveal hidden signals in community assembly. Methods Ecol. Evol. 8, 1200–1211.

De Deyn, G.B., Cornelissen, J.H.C., Bardgett, R.D., 2008. Plant functional traits and soil carbon sequestration in contrasting biomes. Ecol. Lett. 11, 516–531. https://doi.org/10.1111/j.1461-0248.2008.01164.x.

De Deyn, G.B., Van der Putten, W.H., 2005. Linking aboveground and belowground diversity. Trends Ecol. Evol. 20, 625–633. https://doi.org/10.1016/j.tree.2005.08.009.

de Vries, F.T., Manning, P., Tallowin, J.R.B., Mortimer, S.R., Pilgrim, E.S., Harrison, K.A., Hobbs, P.J., Quirk, H., Shipley, B., Cornelissen, J.H.C., Kattge, J., Bardgett, R.D., 2012. Abiotic drivers and plant traits explain landscape-scale patterns in soil microbial communities. Ecol. Lett. 15, 1230–1239. https://doi.org/10.1111/j.1461-0248.2012.01844.x.

Desai, C., Parikh, R.Y., Vaishnav, T., Shouche, Y.S., Madamwar, D., 2009. Tracking the influence of long-term chromium pollution on soil bacterial community structures by comparative analyses of 16S rRNA gene phylotypes. Res. Microbiol. 160, 1–9. https://doi.org/10.1016/j.resmic.2008.10.003.

Dunbabin, V., Rengel, Z., Diggle, A.J., 2004. Simulating form and function of root systems: efficiency of nitrate uptake is dependent on root system architecture and the spatial and temporal variability of nitrate supply. Funct. Ecol. 18, 204–211. https://doi.org/10.1111/j.0269-8463.2004.00827.x.

Edgar, R.C., 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26, 2460–2461. https://doi.org/10.1093/bioinformatics/btq461.

Edwards, I.P., Bürgmann, H., Miniaci, C., Zeyer, J., 2006. Variation in microbial community composition and culturability in the rhizosphere of leucanthemopsis alpina (L.) heywood and adjacent bare soil along an alpine chronosequence. Microb. Ecol. 52, 679–692. https://doi.org/10.1007/s00248-006-9097-x.

Eisenhauer, N., Lanoue, A., Strecker, T., Scheu, S., Steinauer, K., Thakur, M.P., Mommer, L., 2017. Root biomass and exudates link plant diversity with soil bacterial and fungal biomass. Sci. Rep. 7. https://doi.org/10.1038/srep44641.

Fierer, N., 2017. Embracing the unknown: disentangling the complexities of the soil microbiome. Nat. Rev. Microbiol. 15, 579–590.

Gadd, G.M., 2000. Bioremedial potential of microbial mechanisms of metal mobilization and immobilization. Curr. Opin. Biotechnol. 11, 271–279.

Gallardo, A., Merino, J., 1993. Leaf decomposition in two mediterranean ecosystems of southwest Spain: influence of substrate quality. Ecology 74, 152–161. https://doi.org/10.2307/1939510.

Gans, J., Wolinsky, M., Dunbar, J., 2005. Computational improvements reveal great bacterial diversity and high metal toxicity in soil. Science 309, 1387–1390. https://doi.org/10.1126/science.1112665.

Grigulis, K., Lavorel, S., Krainer, U., Legay, N., Baxendale, C., Dumont, M., Kastl, E., Arnoldi, C., Bardgett, R.D., Poly, F., Pommier, T., Schloter, M., Tappeiner, U., Bahn, M., Clément, J.-C., 2013. Relative contributions of plant traits and soil microbial properties to mountain grassland ecosystem services. J. Ecol. 101, 47–57. https://doi.org/10.1111/1365-2745.12014.

Hanada, S., Pierson, B.K., 2006. The family chloroflexaceae. In: The Prokaryotes, pp. 815–842. https://doi.org/10.1007/0-387-30747-8_33.

Hao, X., Taghavi, S., Xie, P., Orbach, M.J., Alwathnani, H.A., Rensing, C., Wei, G., 2014. Phytoremediation of heavy and transition metals aided by legume-rhizobia symbiosis. Int. J. Phytoremediation 16, 179–202. https://doi.org/10.1080/15226514.2013.773273.

Hortal, S., Bastida, F., Armas, C., Lozano, Y.M., Moreno, J.L., García, C., Pugnaire, F.I., 2013. Soil microbial community under a nurse-plant species changes in composition, biomass and activity as the nurse grows. Soil Biol. Biochem. 64, 139–146. https://doi.org/10.1016/j.soilbio.2013.04.018.

Kandeler, E., Tscherko, D., Bruce, K.D., Stemmer, M., Hobbs, P.J., Bardgett, R.D.,

Amelung, W., 2000. Structure and function of the soil microbial community in microhabitats of a heavy metal polluted soil. Biol. Fertil. Soils 32, 390–400. https://doi.org/10.1007/s003740000268.
Kembel, S.W., O'Connor, T.K., Arnold, H.K., Hubbell, S.P., Wright, S.J., Green, J.L., 2014. Relationships between phyllosphere bacterial communities and plant functional traits in a neotropical forest. Proc. Natl. Acad. Sci. Unit. States Am. 111, 13715–13720.
Kidd, P., Barceló, J., Bernal, M.P., Navari-Izzo, F., Poschenrieder, C., Shilev, S., Clemente, R., Monterroso, C., 2009. Trace element behaviour at the root-soil interface: implications in phytoremediation. Environ. Exp. Bot. 67, 243–259.
Klumpp, K., Fontaine, S., Attard, E., Le Roux, X., Gleixner, G., Soussana, J.-F., 2009. Grazing triggers soil carbon loss by altering plant roots and their control on soil microbial community. J. Ecol. 97, 876–885. https://doi.org/10.1111/j.1365-2745.2009.01549.x.
Knelman, J.E., Legg, T.M., O'Neill, S.P., Washenberger, C.L., González, A., Cleveland, C.C., Nemergut, D.R., 2012. Bacterial community structure and function change in association with colonizer plants during early primary succession in a glacier forefield. Soil Biol. Biochem. 46, 172–180.
Kozdroj, J., Van Elsas, J.D., 2000. Response of the Bacterial Community to Root Exudates in Soil Polluted with Heavy Metals Assessed by Molecular and Cultural Approaches.
Kuzyakov, Y., 2002. Review: factors affecting rhizosphere priming effects. J. Plant Nutr. Soil Sci. 165, 382–396. https://doi.org/10.1002/1522-2624(200208)165:4<382::AID-JPLN382>3.0.CO;2-#.
Lagos, L., Maruyama, F., Nannipieri, P., Mora, M.L., Ogram, A., Jorquera, M.A., 2015. Current overview on the study of bacteria in the rhizosphere by modern molecular techniques: a mini–review. J. Soil Sci. Plant Nutr. 15, 504–523. https://doi.org/10.4067/S0718-95162015005000042.
Laughlin, D.C., 2011. Nitrification is linked to dominant leaf traits rather than functional diversity. J. Ecol. 99, 1091–1099. https://doi.org/10.1111/j.1365-2745.2011.01856.x.
Lê, S., Josse, J., Husson, F., 2008. FactoMineR: an R package for multivariate analysis. J. Stat. Softw. 25, 1–18.
Legay, N., Baxendale, C., Grigulis, K., Krainer, U., Kastl, E., Schloter, M., Bardgett, R.D., Arnoldi, C., Bahn, M., Dumont, M., Poly, F., Pommier, T., Clément, J.C., Lavorel, S., 2014. Contribution of above- and below-ground plant traits to the structure and function of grassland soil microbial communities. Ann. Bot. 114, 1011–1021. https://doi.org/10.1093/aob/mcu169.
Li, J.-J., Zheng, Y.-M., Yan, J.-X., Li, H.-J., He, J.-Z., 2013. Succession of plant and soil microbial communities with restoration of abandoned land in the Loess Plateau, China. J. Soils Sediments 13, 760–769. https://doi.org/10.1007/s11368-013-0652-z.
Li, Y., Jia, Z., Sun, Q., Zhan, J., Yang, Y., Wang, D., 2016. Ecological restoration alters microbial communities in mine tailings profiles. Sci. Rep. 6.
Makhalanyane, T.P., Valverde, A., Gunnigle, E., Frossard, A., Ramond, J.-B., Cowan, D.A., 2015. Microbial ecology of hot desert edaphic systems. FEMS Microbiol. Rev. 39, 203–221.
Mateo, G., Crespo, M.B., 2014. Claves ilustradas para la flora valenciana. Monogr. Flora Montiberica 6.
McCarthy, A.J., Williams, S.T., 1992. Actinomycetes as agents of biodegradation in the environment — a review. Gene 115, 189–192. https://doi.org/10.1016/0378-1119(92)90558-7.
McDonald, D., Price, M.N., Goodrich, J., Nawrocki, E.P., DeSantis, T.Z., Probst, A., Andersen, G.L., Knight, R., Hugenholtz, P., 2012. An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. ISME J. 6, 610–618.
Melo, M.R., Flores, N.R., Murrieta, S.V., Tovar, A.R., Zúñiga, A.G., Hernández, O.F., Mendoza, A.P., Pérez, N.O., Dorantes, A.R., 2011. Comparative plant growth promoting traits and distribution of rhizobacteria associated with heavy metals in contaminated soils. Int. J. Environ. Sci. Technol. 8, 807–816. https://doi.org/10.1007/BF03326264.
Miniaci, C., Bunge, M., Duc, L., Edwards, I., Bürgmann, H., Zeyer, J., 2007. Effects of pioneering plants on microbial structures and functions in a glacier forefield. Biol. Fertil. Soils 44, 289–297. https://doi.org/10.1007/s00374-007-0203-0.
Moffett, B.F., Nicholson, F.A., Uwakwe, N.C., Chambers, B.J., Harris, J.A., Hill, T.C.J., 2003. Zinc contamination decreases the bacterial diversity of agricultural soil. FEMS Microbiol. Ecol. 43, 13–19. https://doi.org/10.1111/j.1574-6941.2003.tb01041.x.
Mohamad, R., Maynaud, G., Quéré, A.L., Vidal, C., Klonowska, A., Yashiro, E., Cleyet-Marel, J.-C., Brunel, B., 2017. Ancient heavy metal contamination in soils as a driver of tolerant anthyllis vulneraria rhizobial communities. Appl. Environ. Microbiol. 83 e01735-16. https://doi.org/10.1128/AEM.01735-16.
Moreau, D., Pivato, B., Bru, D., Busset, H., Deau, F., Faivre, C., Matejicek, A., Strbik, F., Philippot, L., Mougel, C., 2015. Plant traits related to nitrogen uptake influence plant-microbe competition. Ecology 96, 2300–2310.
Navarro-Cano, J.A., Goberna, M., Valiente-Banuet, A., Montesinos-Navarro, A., García, C., Verdú, M., 2014. Plant phylodiversity enhances soil microbial productivity in facilitation-driven communities. Oecologia 174, 909–920. https://doi.org/10.1007/s00442-013-2822-5.
Navarro-Cano, J.A., Verdú, M., García, C., Goberna, M., 2015. What nurse shrubs can do for barren soils: rapid productivity shifts associated with a 40 years ontogenetic gradient. Plant Soil 388, 197–209. https://doi.org/10.1007/s11104-014-2323-2.
Navarro-Cano, J.A., Verdú, M., Goberna, M., 2018. Trait-based selection of nurse plants to restore ecosystem functions in mine tailings. J. Appl. Ecol. 55, 1195–1206.
Nemergut, D.R., Anderson, S.P., Cleveland, C.C., Martin, A.P., Miller, A.E., Seimon, A., Schmidt, S.K., 2007. Microbial community succession in an unvegetated, recently deglaciated soil. Microb. Ecol. 53, 110–122. https://doi.org/10.1007/s00248-006-9144-7.
Nicol, G.W., Tscherko, D., Embley, T.M., Prosser, J.I., 2005. Primary succession of soil Crenarchaeota across a receding glacier foreland. Environ. Microbiol. 7, 337–347.
Norris, P.R., 2015. Acidimicrobiales. In: Bergey's Manual of Systematics of Archaea and Bacteria. American Cancer Society, pp. 1–2. https://doi.org/10.1002/9781118960608.obm00004.
Ohtonen, R., Fritze, H., Pennanen, T., Jumpponen, A., Trappe, J., 1999. Ecosystem properties and microbial community changes in primary succession on a glacier forefront. Oecologia 119, 239–246. https://doi.org/10.1007/s004420050782.
Oksanen, J., 2017. Vegan: an Introduction to Ordination.
Orwin, K.H., Buckland, S.M., Johnson, D., Turner, B.L., Smart, S., Oakley, S., Bardgett, R.D., 2010. Linkages of plant traits to soil properties and the functioning of temperate grassland. J. Ecol. 98, 1074–1083. https://doi.org/10.1111/j.1365-2745.2010.01679.x.
Paula, S., Arianoutsou, M., Kazanis, D., Tavsanoglu, Ç., Lloret, F., Buhk, C., Ojeda, F., Luna, B., Moreno, J.M., Rodrigo, A., 2009. Fire-related traits for plant species of the Mediterranean Basin. Ecology 90 1420–1420.
Personeni, E., Loiseau, P., 2004. How does the nature of living and dead roots affect the residence time of carbon in the root litter continuum? Plant Soil 267, 129–141. https://doi.org/10.1007/s11104-005-4656-3.
Philippot, L., Raaijmakers, J.M., Lemanceau, P., van der Putten, W.H., 2013. Going back to the roots: the microbial ecology of the rhizosphere. Nat. Rev. Microbiol. 11, 789–799. https://doi.org/10.1038/nrmicro3109.
Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., Heisterkamp, S., Van Willigen, B., Maintainer, R., 2017. Package 'nlme.' Linear Nonlinear Mix. Eff. Models Version 3–1. .
Piotrowska-Seget, Z., Cycoń, M., Kozdrój, J., 2005. Metal-tolerant bacteria occurring in heavily polluted soil and mine spoil. Appl. Soil Ecol. 3, 237–246. https://doi.org/10.1016/j.apsoil.2004.08.001.
Poca, M., Pérez-Harguindeguy, N., Vaieretti, M.V., Cingolani, A.M., 2014. Descomposición y calidad físico-química foliar de 24 especies dominantes de los pastizales de altura de las sierras de Córdoba, Argentina. Ecol. Austral 24, 249–257.
Pugnaire, F.I., Armas, C., Maestre, F.T., 2011. Positive plant interactions in the Iberian Southeast: mechanisms, environmental gradients, and ecosystem function. J. Arid Environ., Deserts of the World Part IV: Iberian Southeast 75, 1310–1320. https://doi.org/10.1016/j.jaridenv.2011.01.016.
Pyankov, V.I., Ziegler, H., Akhani, H., Deigele, C., Luettge, U., 2010. European plants with C4 photosynthesis: geographical and taxonomic distribution and relations to climate parameters. Bot. J. Linn. Soc. 163, 283–304.
Rajkumar, M., Ae, N., Prasad, M.N.V., Freitas, H., 2010. Potential of siderophore-producing bacteria for improving heavy metal phytoextraction. Trends Biotechnol. 28, 142–149. https://doi.org/10.1016/j.tibtech.2009.12.002.
Roane, null, 1999. Lead resistance in two bacterial isolates from heavy metal-contaminated soils. Microb. Ecol. 37, 218–224.
Schmidt, S.K., Reed, S.C., Nemergut, D.R., Grandy, A.S., Cleveland, C.C., Weintraub, M.N., Hill, A.W., Costello, E.K., Meyer, A.F., Neff, J.C., Martin, A.M., 2008. The earliest stages of ecosystem succession in high-elevation (5000 metres above sea level), recently deglaciated soils. Proc. R. Soc. Lond. B Biol. Sci. 275, 2793–2802. https://doi.org/10.1098/rspb.2008.0808.
Schmieder, R., Edwards, R., 2011. Quality control and preprocessing of metagenomic datasets. Bioinformatics 27, 863–864. https://doi.org/10.1093/bioinformatics/btr026.
Schulz, S., Brankatschk, R., Dümig, A., Kögel-Knabner, I., Schloter, M., Zeyer, J., 2013. The role of microorganisms at different stages of ecosystem development for soil formation. Biogeosciences 10, 3983–3996. https://doi.org/10.5194/bg-10-3983-2013.
Serna-Chavez, H.M., Fierer, N., van Bodegom, P.M., 2013. Global drivers and patterns of microbial abundance in soil. Glob. Ecol. Biogeogr. 22, 1162–1172. https://doi.org/10.1111/geb.12070.
Stefanowicz, A.M., Niklińska, M., Laskowski, R., 2008. Metals affect soil bacterial and fungal functional diversity differently. Environ. Toxicol. Chem. 27, 591–598. https://doi.org/10.1897/07-288.
Takahashi, S., Tomita, J., Nishioka, K., Hisada, T., Nishijima, M., 2014. Development of a prokaryotic universal primer for simultaneous analysis of bacteria and archaea using next-generation sequencing. PLoS One 9, e105592. https://doi.org/10.1371/journal.pone.0105592.
Teng, Y., Wang, X., Li, L., Li, Z., Luo, Y., 2015. Rhizobia and their bio-partners as novel drivers for functional remediation in contaminated soils. Front. Plant Sci. 6, 32. https://doi.org/10.3389/fpls.2015.00032.
Tscherko, D., Rustemeier, J., Richter, A., Wanek, W., Kandeler, E., 2003. Functional diversity of the soil microflora in primary succession across two glacier forelands in the Central Alps. Eur. J. Soil Sci. 54, 685–696. https://doi.org/10.1046/j.1351-0754.2003.0570.x.
Valé, M., Nguyen, C., Dambrine, E., Dupouey, J.L., 2005. Microbial activity in the rhizosphere soil of six herbaceous species cultivated in a greenhouse is correlated with shoot biomass and root C concentrations. Soil Biol. Biochem. 37, 2329–2333. https://doi.org/10.1016/j.soilbio.2005.04.014.
Velbel, M.A., 1988. Weathering and soil-forming processes. Ecol. Stud. Anal. Synth. USA 66, 93–102.
Ventura, M., Canchaya, C., Tauch, A., Chandra, G., Fitzgerald, G.F., Chater, K.F., van Sinderen, D., 2007. Genomics of Actinobacteria: tracing the evolutionary history of an ancient phylum. Microbiol. Mol. Biol. Rev. MMBR 71, 495–548. https://doi.org/10.1128/MMBR.00005-07.
Walker, L.R., del Moral, R., 2011. Primary succession. In: ELS. John Wiley & Sons, Ltd. https://doi.org/10.1002/9780470015902.a0003181.pub2.
Walker, L.R., del Moral, R., 2003. Primary Succession and Ecosystem Rehabilitation. Cambridge University Press.
Wang, Y.P., Li, Q.B., Shi, J.Y., Chen, X.C., Wu, W., Chen, Y.X., 2008. Assessment of microbial activity and bacterial community composition in the rhizosphere of a copper

accumulator and a non-accumulator. Soil Biol. Biochem. 40, 1167–1177.
Wardle, D.A., Bardgett, R.D., Klironomos, J.N., Setälä, H., Van Der Putten, W.H., Wall, D.H., 2004. Ecological linkages between aboveground and belowground biota. Science 304, 1629–1633.
Wenzel, W.W., 2009. Rhizosphere processes and management in plant-assisted bioremediation (phytoremediation) of soils. Plant Soil 321, 385–408. https://doi.org/10.1007/s11104-008-9686-1.
Wood, J.L., Zhang, C., Mathews, E.R., Tang, C., Franks, A.E., 2016. Microbial community dynamics in the rhizosphere of a cadmium hyper-accumulator. Sci. Rep. 6, 36067. https://doi.org/10.1038/srep36067.
Zak, D.R., Holmes, W.E., White, D.C., Peacock, A.D., Tilman, D., 2003. Plant diversity, soil microbial communities, and ecosystem function: are there any links? Ecology 84, 2042–2050.
Zeng, Y., Baumbach, J., Barbosa, E.G.V., Azevedo, V., Zhang, C., Koblížek, M., 2016. Metagenomic evidence for the presence of phototrophic Gemmatimonadetes bacteria in diverse environments. Environ. Microbiol. Rep. 8, 139–149. https://doi.org/10.1111/1758-2229.12363.
Zenova, G.M., Manucharova, N.A., Zvyagintsev, D.G., 2011. Extremophilic and extremotolerant actinomycetes in different soil types. Eurasian Soil Sci. 44, 417–436. https://doi.org/10.1134/S1064229311040132.
Zhang, C., Nie, S., Liang, J., Zeng, G., Wu, H., Hua, S., Liu, J., Yuan, Y., Xiao, H., Deng, L., Xiang, H., 2016. Effects of heavy metals and soil physicochemical properties on wetland soil microbial biomass and bacterial community structure. Sci. Total Environ. 557–558, 785–790. https://doi.org/10.1016/j.scitotenv.2016.01.170.