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ARTICLE

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# High phylogenetic turnover magnifies evolutionary relatedness along bacterial primary succession

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## Abstract

Current models of microbial primary succession postulate that stochasticity dominates the early stages of soil community assembly, generating phylogenetically random patterns that are lost as abiotic and biotic filters gain relevance. We hypothesized that, under severe environmental stress, abiotic filters may override stochasticity from early successional stages, inducing phylogenetic clustering due to the overrepresentation of stress-tolerant lineages. Clustering may be magnified towards more carbon-rich stages due to intensified biotic interactions based on the overrepresentation of competitive clades. We also hypothesized that different plant life forms, with their contrasting effects on soil conditions, might modulate the phylogenetic patterns across microbial succession. To test these hypotheses, we collected 106 soil samples along plant size gradients of 13 species belonging to 4 life forms (trees, shrubs, perennial grasses, and dwarf shrubs) in seven sites in Mediterranean drylands in SE Spain. Samples in each gradient were taken in open areas (antecedent situation), underneath seedlings, juveniles, and mature plant individuals. In all cases, we detected large levels of phylogenetic clustering across the succession indicating a negligible role of stochasticity. Plant and soil variables drove different processes along the succession: Clustering of shallow-divergent lineages intensified along with plant growth and enrichment in soil organics, whereas clustering of deep-divergent lineages varied across plant life forms based on their effect on soil moisture. During primary succession, phylogenetic turnover increased due to the progressive enrichment in large lineages that were composed of closely related organisms and phylogenetically distant to the initial communities. Our data help fine-tune current models of bacterial primary succession indicating that (1) environmental filters can override stochasticity from the early stages of primary succession and (2) large levels of phylogenetic turnover can concur with increased evolutionary relatedness across gradients of microbial community assembly. Shifts in phylogenetic patterns may critically influence key ecosystem functions, such as decomposition and biogeochemical cycling, driven by soil microbiota.

**KEYWORDS**
abiotic stress, biotic interactions, community assembly, ecological succession, environmental filter, Mediterranean ecosystems, phylogenetic clustering, phylogenetic turnover, soil bacteria

## INTRODUCTION

The assembly of biological communities is governed by stochastic processes (e.g., dispersal, ecological drift) and niche-based processes (HilleRisLambers et al., 2012). The latter filter communities based on the species' environmental tolerances (e.g., salinity, drought, or acidity) and ecological interactions (e.g., competition, cross-feeding), which are to a large extent a consequence of species ecological requirements and functional traits (Anthony et al., 2020). Phylogenetic information helps to delve into the processes of community assembly (Webb et al., 2002), since evolutionary distance tends to reflect ecological similarity across the tree of life (Blomberg et al., 2003; Cavender-Bares et al., 2009; Maherali & Klironomos, 2007) and particularly in prokaryotes (Goberna & Verdú, 2016; Lennon et al., 2012; Martiny et al., 2013; Zimmerman et al., 2013). Therefore, assessing the phylogenetic structure of bacterial communities may contribute to tease apart the processes governing microbial community assembly and ultimately better predict their influence on ecosystem functioning (Goberna & Verdú, 2018; Stegen et al., 2013).

Ecosystems undergoing primary succession are ideal scenarios to investigate the relative importance of ecological forces driving community assembly (Walker & del Moral, 2003). Theoretically, stochasticity drives microbial community assembly in the early stages of primary succession, generating random phylogenetic patterns, and loses relevance towards more mature stages (Dini-Andreote et al., 2015). However, current models disregard that under abiotic stress, environmentally mediated community assembly may override stochasticity, regardless of successional stage. In soils, pH has a main role determining the relevance of stochastic versus niche-based processes, deviations from neutral pH resulting in phylogenetically clustered communities composed of evolutionarily related members likely based on their shared ability to withstand acidic or alkaline stress (Tripathi et al., 2018). We presume that other abiotic conditions that influence soil microbiota (e.g., moisture, salinity) might generate similar phylogenetic patterns, since microbial traits conferring resistance to such abiotic stresses tend to be evolutionarily conserved (Goberna & Verdú, 2016).

Studies focusing on plant communities indicate that abiotic filtering is more influential in the early stages of ecological succession, due to intense UV radiation, desiccation stress, and oligotrophic conditions (Holl, 1999; Prach et al., 2007), while biotic interactions intensify along with plant development. In drylands, plant ecological interactions of opposing signs have been shown to intensify towards mature plant ontogenetic stages, including facilitative interactions between nurse plants (stress-resistant species that colonize bare soils) and the beneficiary species thriving beneath their canopy (Navarro-Cano et al., 2016) as well as competitive interactions among beneficiaries (Castillo et al., 2010). Some of these observations might also apply to soil microbial communities, since plant growth simultaneously reduces soil abiotic stress (e.g., intense radiation and desiccation) and increases the amount of organic compounds that are supplied to the soil (Navarro-Cano et al., 2015, 2018a). The rise in the quantity of organic matter along primary succession correlates with the phylogenetic turnover of soil bacteria (Dini-Andreote et al., 2015). We assume that this might be due to resource availability promoting a few bacterial clades with high relative fitness, which might competitively exclude distantly related lineages and eventually magnify phylogenetic clustering (Goberna, García, & Verdú, 2014; Goberna, Navarro-Cano, et al., 2014; Goldfarb et al., 2011).

Here, we examined the phylogenetic structure of soil bacterial communities undergoing primary succession under extremely stressful abiotic conditions, that is, in metalliferous mine tailings which constitute a new artificially created environment characterized by acid to neutral pH, low nutrient contents in soils, high salinity, and elevated concentrations of metal(oids), which add to very intense radiation and desiccation (Párraga-Aguado et al., 2013). Metal-tolerant nurse plant species able to colonize barren areas trigger primary succession and conform scattered vegetation patches surrounded by open spaces (Navarro-Cano et al., 2018a; Párraga-Aguado et al., 2013). In this context, the plant life form (a trait that captures plant morphology and canopy structure) has been shown to determine the intensity with which plant patches modify the soil properties, based on the differential inputs of organics into the soil and reduction of abiotic stressors such as metal(oids) of functionally different plant species (Colin et al., 2019; Navarro-Cano et al., 2018a). Contrasted plant–soil feedbacks underneath plant species belonging to different life forms

eventually determine distinct levels of soil microbial activity and taxonomic diversity (Colin et al., 2019, Navarro-Cano et al., 2018a).

Based on the observations above, we hypothesized that under extreme abiotic stress, environmental filters might reduce the contribution of stochasticity and result in intense phylogenetic clustering of bacterial communities all over successional gradients. Such phylogenetic clustering might be magnified towards more carbon-rich stages due to intensified biotic interactions. Particularly, competition based on relative fitness differences has been suggested to operate among large clades of heterotrophic bacteria, carbon-efficient taxa dominating under high resource availability (Goberna et al., 2016; Goberna, García, & Verdú, 2014; Goberna, Navarro-Cano, et al., 2014). We also expected that the magnitude of such a phylogenetic pattern might be modulated by the plant life forms owing to their contrasted effects on soil conditions (Grigulis et al., 2013; Legay et al., 2014; Navarro-Cano et al., 2018a). Theoretically, plant life forms with denser canopies (i.e., trees, shrubs), by alleviating the effect of some abiotic stressors (e.g., desiccation, high radiation), might reduce phylogenetic clustering compared to sparser canopies. Such an effect may be, however, canceled out by enhanced biotic filtering as the amount of organic compounds increases along the succession (Colin et al., 2019).

To test these hypotheses, we quantified the phylogenetic community structure of soil bacterial communities across successional gradients underneath 13 plant species belonging to four life forms (i.e., trees, shrubs, dwarf shrubs and perennial grasses). Among a total of 102 plant species inventoried in the study sites, these 13 species showed potential to colonize barren areas and facilitate other species underneath their canopy triggering succession (Navarro-Cano et al., 2018a). We tested the extent to which bacterial phylogenetic diversity along successional gradients responds to (1) the plant life form and plant size (i.e., ontogenic developmental stage), as well as (2) soil fertility (organic substances, mineral nutrients and moisture) and soil abiotic stress (acidity, salinity, and heavy metal contents). Finally, we analyzed the contribution of main bacterial taxa to overall phylogenetic diversity. If our expectations hold true, patterns of phylogenetic clustering might prevail from the early stages of primary succession supporting that environmental filters override stochasticity. In addition, magnified competition based on relative fitness differences should increase phylogenetic clustering when measured across scales in the phylogeny (i.e., considering whole-branch lengths) rather than at the tips of the phylogeny (i.e., terminal clustering), due to the overrepresentation of large competitive clades.

# METHODS

## Experimental design and sampling

The study area is located in the Cartagena-La Unión Mining District (Murcia, Southeastern Spain; 30 S 689151 E, 4164433 N; semiarid Mediterranean climate). In 2015 we sampled seven sites scattered across ca. 21 ha, which are mining deposits that had been abandoned ca. 30 years ago (Appendix S1: Figure S1). In total, 106 topsoil samples (0–5 cm) were collected along the size gradients of 13 plant species distributed in the seven mining deposits, including 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*, *Macrochloa tenacissima*, *Piptatherum miliaceum*, and *Hyparrhenia sinaica*). For each plant species, soil samples were collected within the same mining deposit to minimize site effects, the number of samples per deposit averaging (mean ± SE) 15.4 ± 4.6. Size gradients per species included soils in the open areas between plant patches (13 samples to characterize the antecedent situation, one per gradient), as well as soils underneath seedlings (1–-2 year old, height <10 cm), juveniles (>10 cm tall with no cues of reproduction) and adults (reproductive) of each nurse (a total of 93 soil samples taken underneath plant canopies). Plant size was measured with a tape measure as the mean canopy diameter (i.e., the average of the maximum and minimum diameter of each plant patch) 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 varied depending on the maximum plant diameter recorded for each species ($6 \leq N \leq 10$; Appendix S1: Table S1) to balance the sampling effort across the gradients of plants that can differ up to two decades in their lifespan. Unbalanced sample sizes did not cause differences across species, as we previously confirmed comparing soil data against those obtained using balanced sample sizes ($N = 6$) for all nurses (Navarro-Cano et al., 2018a). Soil samples collected beneath the canopy of plant individuals were made up of five subsamples that were taken from 10 × 10 cm quadrats located in the cardinal points and the center of each plant patch, and subsequently mixed into a ca. 1-kg sample. When five subsamples could not be collected underneath small plants, the entire soil surface in the vicinity of the plant was sampled and mixed into a ca. 1-kg sample. To collect soils from open areas, five subsamples were randomly collected covering the surface of each mine deposit to account for spatial

variability, and mixed as above. Soils were transported to the laboratory on ice, sieved through a 2-mm mesh and stored at 4°C during analyses. All materials were rinsed with 70% ethanol during sampling and sample processing to avoid cross-contaminations. Appendix S1 shows a general description of plant species and soil samples (Appendix S1: Table S1) and main soil chemical parameters, which were quantified using standard procedures (Appendix S1: Table S2, Figure S2).

## Nucleic acid extraction and sequencing of 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), starting immediately after sample arrival into the laboratory. 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 32 min, followed by 28 cycles of amplification (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). 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 using a paired-end, 2 × 300 bp cycle run on an Illumina MiSeq sequencing system and MiSeq Reagent Kit in the Fisabio sequencing service (http://fisabio.san.gva.es).

Of the 106 soil samples collected in the mine tailings, seven samples taken from open areas and six underneath plant canopies had too low DNA concentrations to allow 16S rRNA gene high-throughput sequencing despite we used 10 g soil for each extraction (40 times greater than the usual soil mass used), reflecting the low microbial load of the barren substrates and the suitability of the study area to study primary succession (Appendix S1: Table S1). 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 & Edwards, 2011). We 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., 2010). After quality filtering, our 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, only few archaeal sequences were detected in the samples. Thus, sequences belonging to the archaeal domain (1035 sequences; 0.02% of the total) or with no taxonomic affiliation at the domain level (41,277 sequences; 0.8%) were excluded from our analysis. Moreover, one sample (osqu7) with poor sequencing yield (<1000 reads) was discarded from the dataset. Then, 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). We opted for collapsing sequences into OTUs due to the computational costs of reconstructing phylogenetic trees. A total of 30,146 OTUs were identified and only OTUs represented by 15 or more reads ($N = 12{,}367$) were considered for downstream analyses. A community matrix showing the abundance of these OTUs in each of the 92 samples was constructed and their relative abundance of OTUs was corrected by the estimated number of 16S rRNA gene copies (Kembel et al., 2012). Appendix S1: Figure S2 shows main bacterial phyla whose relative abundance shifted along the plant size gradients.

## Phylogenetic reconstruction

Prior to phylogenetic inference, the 12,367 sequences representing OTUs were aligned with PyNAST by using QIIME (Caporaso et al., 2010). The Lane mask (Lane, 1991) was applied in order to remove hypervariable regions and thus to improve phylogenetic analyses (Capella-Gutiérrez et al., 2009). This step resulted in an alignment of 444 nucleotide positions, from which bacterial phylogeny was reconstructed using RAxML v 7.3.0 (Stamatakis, 2006). Specifically, three maximum-likelihood phylogenetic trees were built using the GTR-GAMMA site rate substitution model on the FinisTerrae Supercomputer at the Galicia Supercomputing Centre (CESGA). To avoid high phylogenetic uncertainty resulting from the usage of short sequences, trees were constrained according to the topology of the mega-tree built from the Silva database at the phylum level (class level for Proteobacteria) (Quast et al., 2012) and rooted

using *Archaeoglobus profundus*. We generated 100 rapid bootstrap replicates, followed by a search for the best ML tree by comparing the likelihood scores. Then, we generated nonparametric bootstrap replicates using the autoMRE option of RAxML, which runs bootstrapping until bootstrap replicates converge. The three phylogenetic trees are available in Zenodo (https://doi.org/10.5281/zenodo.19480271).

## Phylogenetic diversity and turnover

The Mean Pairwise Distance (MPD) and Mean Nearest Taxon Distance (MNTD) metrics were calculated to explore phylogenetic diversity across different phylogenetic depths (Mazel et al., 2016; Figure 1) using the picante package in R (Kembel et al., 2010). MPD measures the average phylogenetic distance among pairs of individuals drawn at random from a sample, while MNTD calculates the mean distance separating each species in each community from its closest relative and is thus less influenced by the basal structure of the phylogenetic tree (Figure 1). Since these metrics are influenced by taxonomic diversity (Kembel et al., 2010), we computed their standardized effect sizes (ses.MPD and ses.MNTD) using the following formula: $ses.X = (X_{obs} - X_{rand})/SD_{rand}$, where $X_{obs}$ represents the observed value of the metrics, $X_{rand}$ represents the mean of randomized values after shuffling the OTUs across the phylogeny using 999 randomizations, and $SD_{rand}$ represents the standard deviation of the randomized values. Negative values of ses.MPD and ses.MNTD indicate phylogenetic clustering, where coexisting OTUs are more closely related than expected by chance, while positive values indicate overdispersion with coexisting OTUs less related than expected by chance.

In addition, we estimated phylogenetic turnover between assemblages to understand main shifts along primary succession (Stegen et al., 2012). We calculated the phylogenetic turnover across short phylogenetic

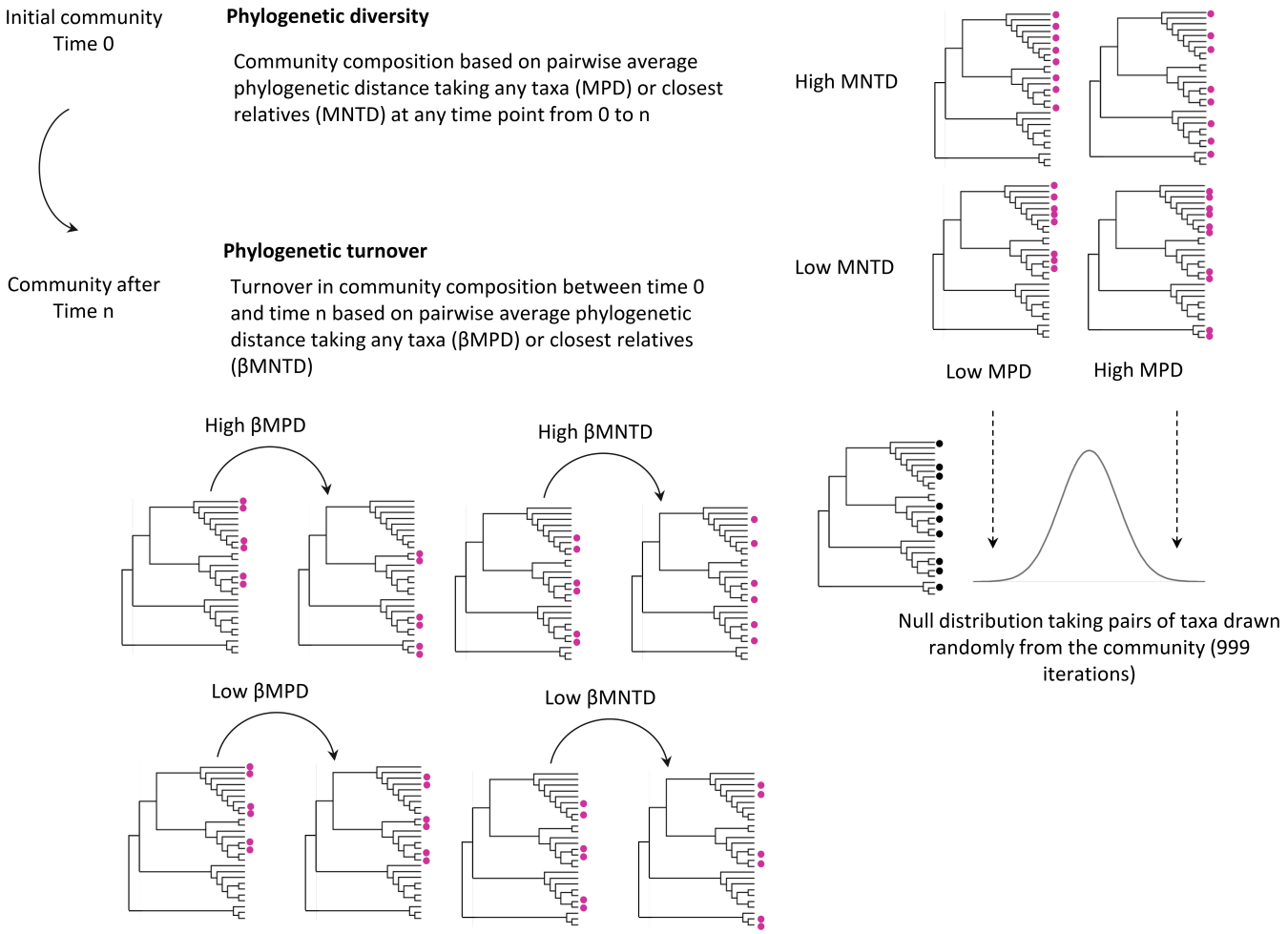


**FIGURE 1** Schematic representation of metrics of phylogenetic diversity and phylogenetic turnover across the whole phylogeny and at the tips of the phylogeny.

distances (βMNTD) using the bNTIn.p function in iCAMP (Ning et al., 2020). We compared each stage of the succession against the initial stage, that is, taking as a reference the soil collected in open areas or underneath seedlings (in the cases in which we failed to obtain any DNA from the open area, Appendix S1: Table S1), to understand the cumulative turnover throughout the succession (Figure 1). The choice of the reference community is not expected to influence the results since the levels of phylogenetic diversity in soils in gaps versus seedlings was not significantly different (data not shown). Similarly, we calculated the phylogenetic turnover across the whole phylogeny (βMPD), with the nNRIn.p function of the same package. We used a null modeling approach as above to calculate the ses.βMNTD and ses.βMPD. Positive values indicate high phylogenetic turnover, that is, replacement across communities of groups that are phylogenetically more distant than in replacement expected by chance, and negative values suggest low turnover.

## Statistical analysis

We first tested whether the phylogenetic diversity of soil bacteria departs significantly from the random expectation. We specifically performed Bayesian generalized linear mixed models (GLMM) to test the departure from zero of either ses.MPD or ses.MNTD. We included the "mining deposit" as a random factor in the models, so as to account for site effects. Since each species was sampled in a single deposit, we did not include the "species" as a random factor to avoid model over-parametrization. The use of Bayesian GLMMs allowed us to accommodate the uncertainty associated with phylogenetic reconstruction based on short sequences (Huelsenbeck et al., 2000). In total, three Bayesian GLMMs were ran with the *MCMCglmm* package for R (Hadfield, 2010), each one using the phylogenetic metrics calculated from a different tree. We used the default priors and ran 13,000 MCMC iterations with a burn-in period of 3000 iterations. Then, we integrated over the posterior samples by drawing 1000 random samples across models. The statistical significance of the factors in the model was estimated by calculating the 95% credible interval of their posterior distribution.

We then tested whether bacterial phylogenetic clustering, measured with ses.MPD or ses.MNTD, is impacted by the plant life form or plant size with Bayesian GLMMs as above. Plant size (mean canopy diameter) was natural log-transformed to better approximate a normal distribution. We additionally tested whether bacterial phylogenetic clustering, either ses.MPD or ses.MNTD, was related to an increase in soil fertility and/or a reduction in abiotic stress below the plant canopy. Principal components analyses (PCA) were carried out for soil fertility (i.e., resources for soil microorganisms: total organic carbon, total nitrogen, phosphorus, potassium, soil moisture), and abiotic stress parameters (i.e., conditions that define the stress levels in the microbial habitat: soil pH, electrical conductivity, and concentrations of arsenic, copper, cadmium, lead, zinc, aluminum, lithium) using the *FactoMineR* package for R (Lê et al., 2008; Appendix S1: Figure S2). We further used Bayesian GLMMs as above to evaluate the impact of principal components (PC1 and PC2) of both PCAs on bacterial phylogenetic diversity. We specifically tested the effect of plant life form on soil moisture using the log-transformed soil gravimetric humidity as a dependent variable, plant life form as a fixed factor, and the mine deposit as a random factor with the lme function in the *nmle* package for R (Pinheiro et al., 2025).

Finally, we identified the bacterial clades that are responsible for the changes in phylogenetic clustering through Bayesian GLMMs as above, using the phylogenetic metrics as the dependent variable and the relative abundance of each clade as independent variables in the model. Analyses were performed using R 4.2.1 (R Core Team, 2022).

# RESULTS

Overall, soil bacterial communities were phylogenetically clustered relative to the null expectation, as shown by the 95% credible intervals below zero (MCMCglmm post-mean estimate, ses.MPD = −3.37; 95% credible interval [−4.83, −1.80]; ses.MNTD: −6.70 [−7.27, −6.13]; Figure 2). The intensity of phylogenetic clustering varied widely along with primary succession. However, standardized MPD and MNTD did not correlate with each other ($r = -0.06$; $p > 0.56$) and responded to different plant and soil features, as discussed below and schematized in Figure 3.

Clustered patterns, indicating that communities were composed of bacteria more closely related than expected by chance at all stages of the succession, were concurrent with a general increase in phylogenetic turnover along primary succession, that is, a replacement of groups that were phylogenetically more distant to the initial community than expected under random replacement (Figure 4). Such an increase in turnover was evident at the tips of the phylogeny (ses.βMNTD), but not when considering the whole phylogeny (ses.βMPD; Appendix S1: Figure S4). The increase in phylogenetic turnover varied

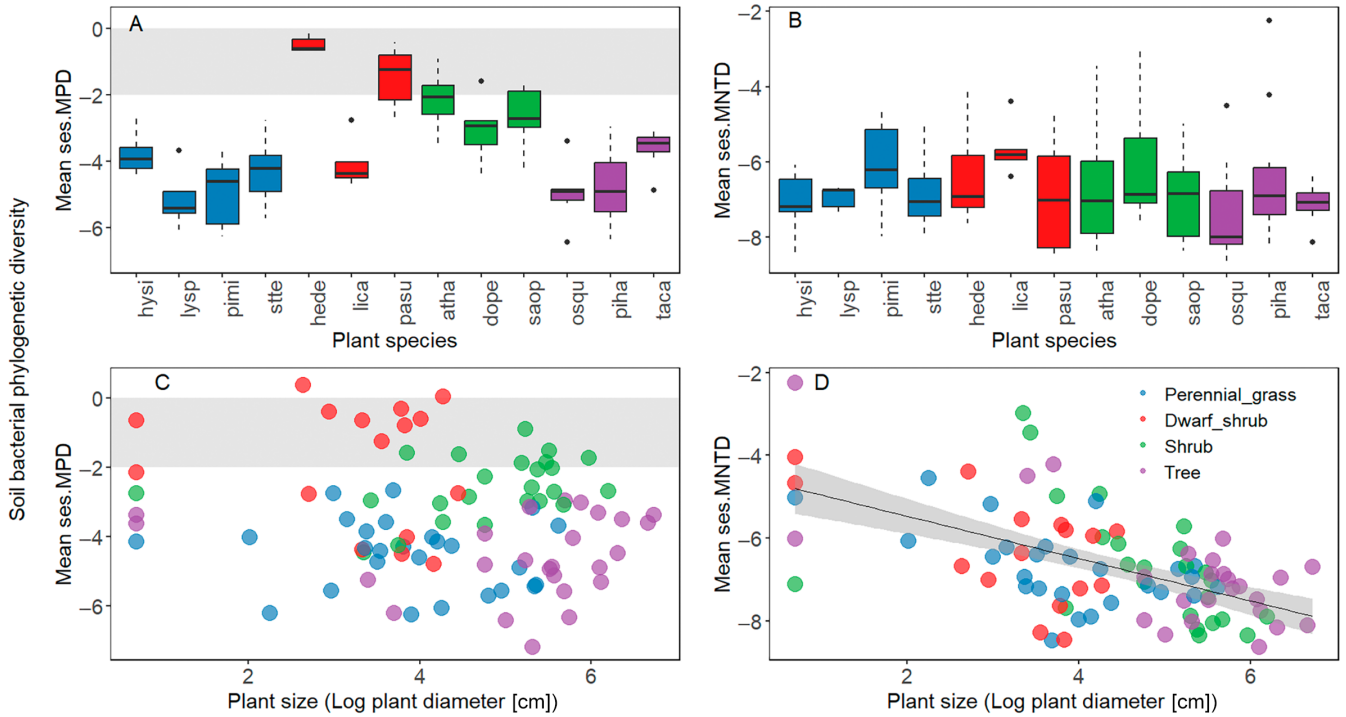


**FIGURE 2** Phylogenetic diversity of soil bacterial communities along size gradients of plant species belonging to different plant life forms. Standardized Mean Pairwise Distance (MPD) (A, C) and Mean Nearest Taxon Distance (MNTD) (B, D) are represented as averages obtained from three phylogenetic trees, and plant size as the natural logarithm of average plant diameters. Shaded areas indicate phylogenetic randomness where stochastic processes dominate, while non-shaded areas below zero represent phylogenetic clustering. Colors represent plant life form: green (shrubs), purple (trees), blue (perennial grasses), red (dwarf shrubs). Abbreviations indicate nurse plant species identity: hysi (*Hyparrhenia sinaica*), lysp (*Lygeum spartum*), pimi (*Piptatherum miliaceum*), stte (*Macrochloa tenacissima*), hede (*Helichrysum stoechas*), lica (*Limonium carthaginense*), pasu (*Paronychia suffruticosa*), atha (*Atriplex halimus*), dope (*Dorycnium pentaphyllum*), saop (*Salsola oppositifolia*), osqu (*Osyris lanceolata*), piha (*Pinus halepensis*), taca (*Tamarix canariensis*).

in magnitude depending on plant species identity but was evident in 10 out of 13 cases (with values surpassing ses.βMNTD > 2 indicating that phylogenetic turnover is more than two SDs above the null expectation; Figure 4).

## Plant effects on soil bacterial phylogenetic clustering

Plant life form significantly impacted ses.MPD, soil bacterial communities being more phylogenetically clustered below trees and perennial grasses than below shrubs (taking shrub as a reference; trees: −1.51 [−2.57, −0.53]), perennial grasses: (−1.53 [−2.53, −0.36]) (Figure 2A). Bacterial ses.MPD below dwarf shrubs did not differ significantly from any of the other plant life forms due to high interspecies variability (dwarf shrubs as a reference; trees: −0.9 [−2.3, 0.6], shrubs: 0.5 [−1.2, 2.2], perennial grasses: −0.9 [−2.5, 0.4]). On the contrary, ses.MNTD was not influenced by the plant life form (dwarf shrubs as reference; trees: 0.4 [−0.6, 1.4], shrubs: 0.3 [−1.1, 1.3], perennial grasses: −0.05 [−0.9, 0.8]) (Figure 2B). Plant size did not impact ses.MPD (0.02 [−0.21, 0.25]; Figure 2C), but it explained ses.MNTD (−0.64 [−0.90, −0.40]), soil bacterial communities below mature plant patches being more terminally clustered than those in barren soils or below seedlings (Figure 2D). The negative relationship between ses.MNTD and plant size was confirmed for each life form separately (shrubs: −0.60 [−1.14, −0.10]; trees: −0.76 [−1.06, −0.47]; perennial grasses: −0.48 [−0.89, −1.52]; and dwarf shrubs: −0.73 [−1.32, −0.14]) (Figure 2D).

## Soil effects on bacterial phylogenetic clustering

Soil fertility and abiotic stress variables were reduced through two separate PCAs. The first two PCs explained 78.3% and 58.7% of the total variance, respectively (Appendix S1: Figure S3). These PCs distinctly predicted

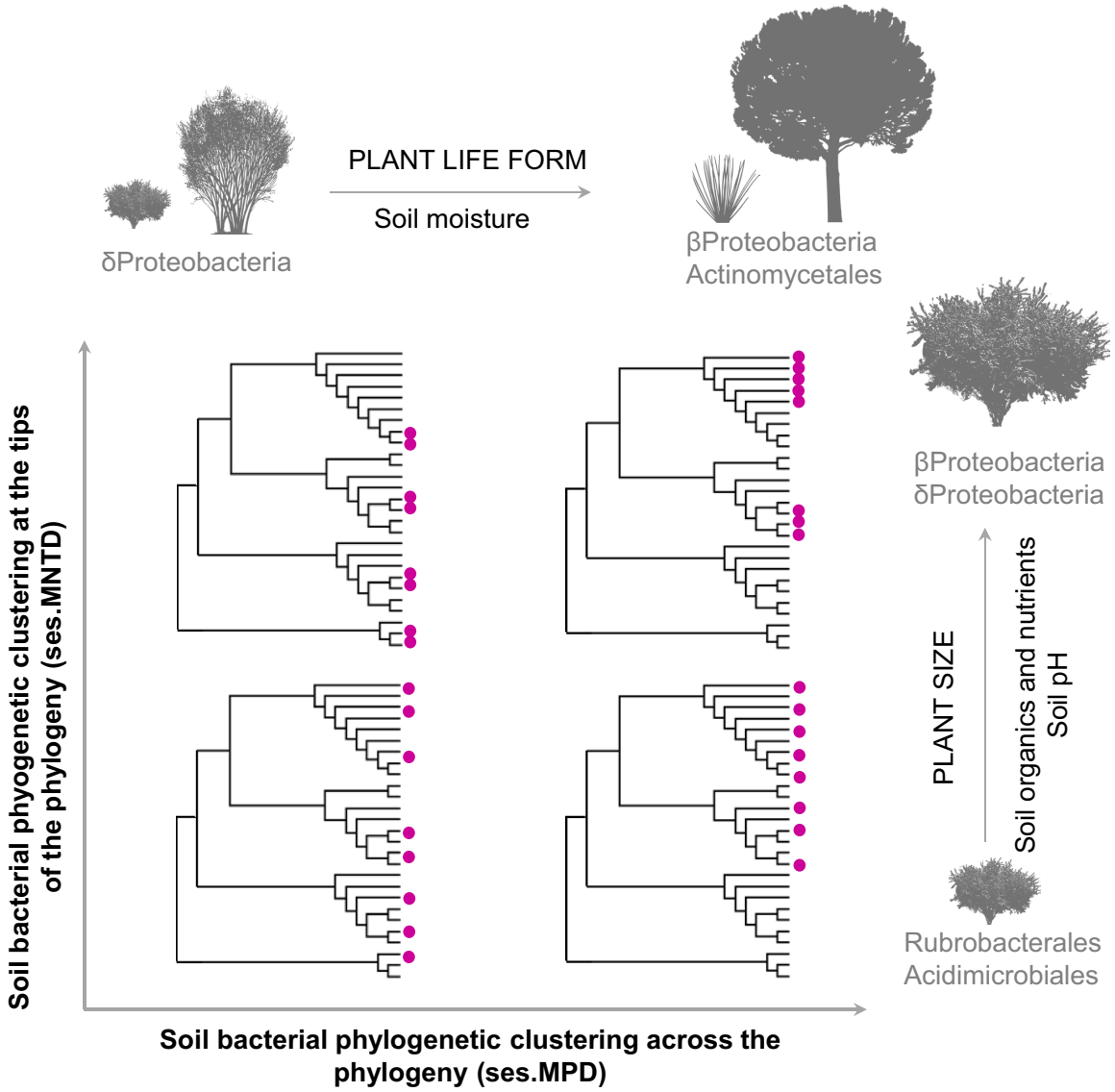


**FIGURE 3** Schematic representation of the distinct ecological processes that operate at different evolutionary scales in soil bacterial communities. In drylands under severe abiotic stress, low levels of phylogenetic diversity (clustering) measured across the phylogeny responded to different plant life forms and their impact on soil moisture regardless of primary succession. Phylogenetic clustering measured at shallow depths intensified along with plant growth and enrichment in soil organics and nutrients towards more mature stages of primary succession. Bacterial lineages that were significant predictors of the levels of phylogenetic diversity are given. Plant silhouette images were obtained from PhyloPic.org and are in the public domain.

both phylogenetic metrics (Figure 5). ses.MPD was negatively related to PC2.fert (Figure 5A), which correlated with soil moisture (Appendix S1: Figure S3A). Accordingly, dwarf shrubs, for which the highest (less negative) values of ses.MPD were observed, displayed the lowest soil moisture below their canopy ($\chi^2 = 18.9$, df = 3, $p < 0.001$) (1.5% ± 0.4%) compared to shrubs (2.2% ± 0.8%), perennial grasses (2.2% ± 0.7%), and trees (2.5% ± 1.2%) although only differences between dwarf shrubs and trees were statistically significant. No parameter associated with soil abiotic stress explained the variation in ses.MPD (Figure 5B). Terminal phylogenetic clustering (ses.MNTD) decreased with soil organic and nutrient contents (−PC1.fert.log values) while it increased with soil pH (+PC2.ab.stress values) (Figures 5C,D and S3).

## Bacterial clades contributing to phylogenetic diversity metrics

Shifts in the phylogenetic community structure were associated with changes in the relative abundance of the main bacterial phyla in soils, namely Proteobacteria and

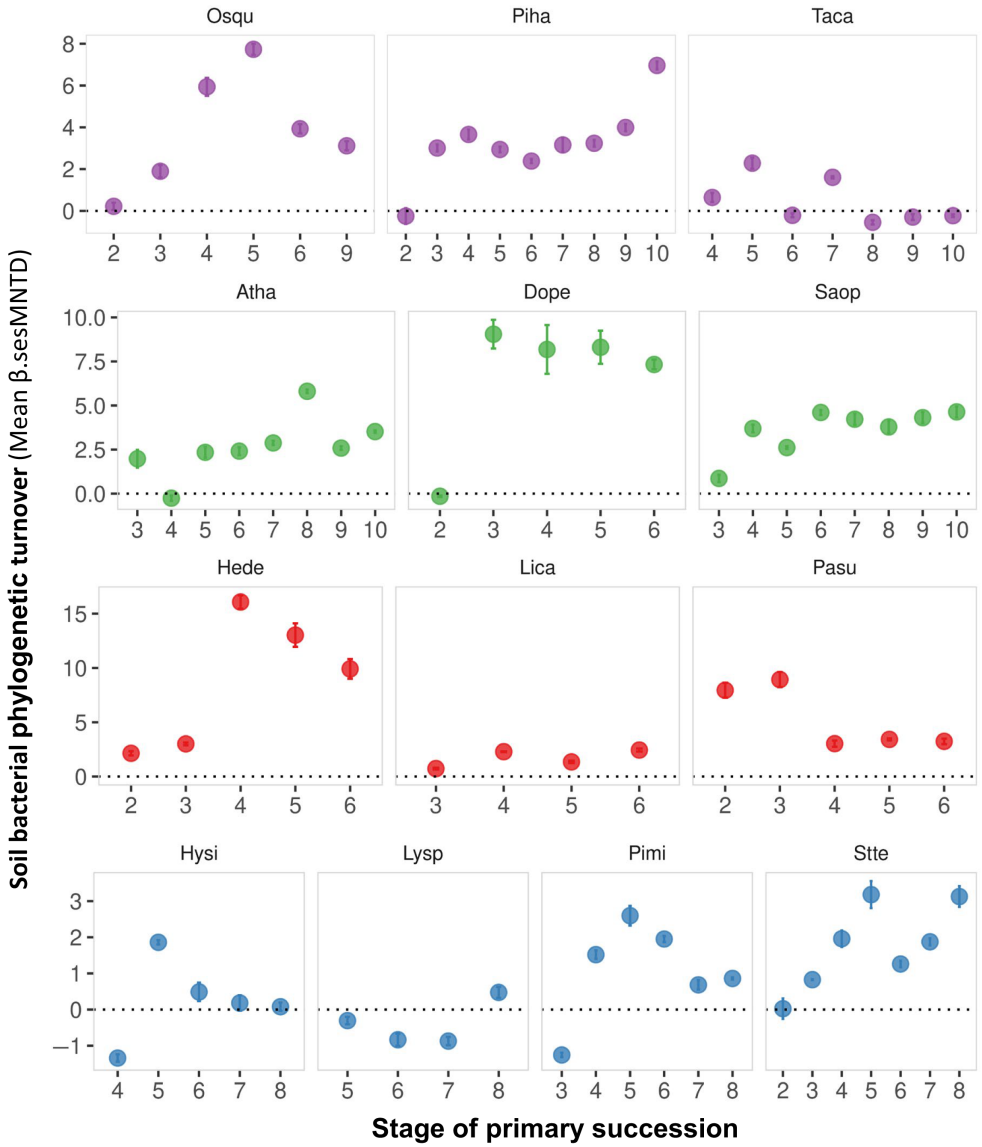


**FIGURE 4** Phylogenetic turnover of soil bacterial communities along size gradients of plant species belonging to different plant life forms. Standardized βMNTD (ses.βMNTD) is represented as averages obtained from three phylogenetic trees, and calculated for each assemblage against the initial stage, that is, taking as a reference the soil collected in gaps or underneath seedlings. Numbers represent stage of the succession from the initial stages (1, open areas; 2, seedling; 3, juvenile) up to senescent individuals (6, 8, or 10 depending on the plant life form). See main text for details on the sampling design. Colors represent plant life form: green (shrubs), purple (trees), blue (perennial grasses), red (dwarf shrubs). Abbreviations indicate nurse plant species identity as in Figure 2. Results for phylogenetic turnover when considering the whole phylogeny (ses.βMPD) are shown in Appendix S1: Figure S4.

Actinobacteria. Both metrics of bacterial phylogenetic diversity decreased in assemblages dominated by Proteobacteria (Figure 6A,B; Appendix S1: Figures S5 and S6). This pattern was driven by the overrepresentation of Betaproteobacteria (for both metrics) and Deltaproteobacteria (ses.MNTD only) (Figure 6C,D). We could not detect the orders of Betaproteobacteria behind this pattern (data not shown). In the case of Deltaproteobacteria, ses.MNTD correlated negatively with the relative abundance of Myxococcales (−2.21 [−3.08, −1.21]) and Syntrophobacterales (−5.04 [−8.82, −1.05]). Bacterial phylogenetic diversity was either negatively (ses.MPD) or positively (ses.MNTD) correlated with the relative abundance of Actinobacteria (Figure 6A,B). The contrasting patterns were related to different orders within the Actinobacteria: ses.MPD decreased with relative abundance of Actinomycetales, Solirubrobacterales, and candidate 0319-7L14, but increased with Rubrobacterales (Figure 6E); ses.MNTD increased in assemblages dominated by Rubrobacterales and Acidimicrobiales (Figure 6F).

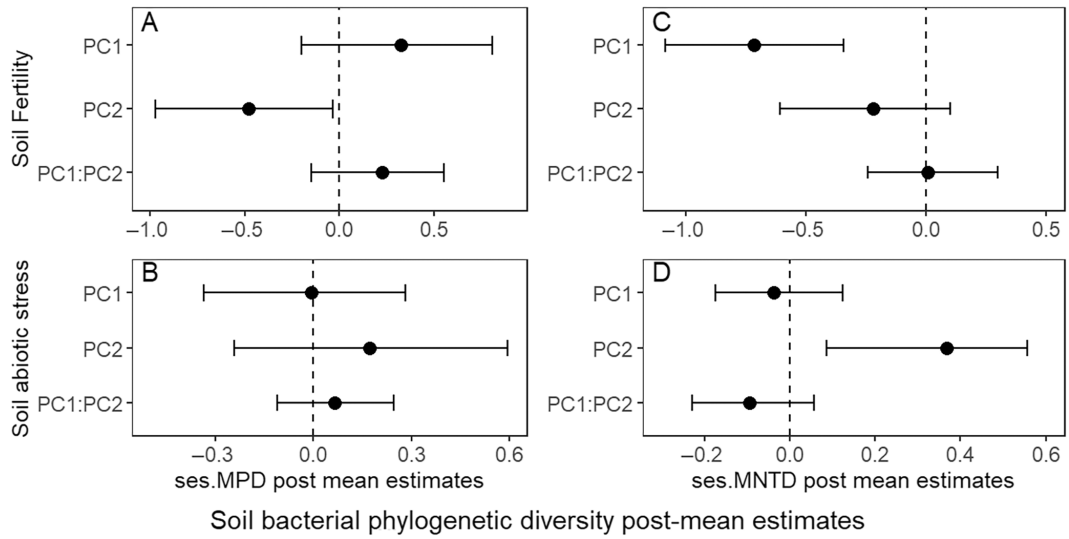


**FIGURE 5** Bayesian post-mean estimates (and their expected 95% credible intervals) explaining the effect of variables associated with soil fertility and abiotic stress on the standardized Mean Pairwise Distance (MPD) (A, B) and Mean Nearest Taxon Distance (MNTD) (C, D). Factors with intervals not including zero are significant. Principal components analyses (PCAs) are shown in Appendix S1: Figure S3.

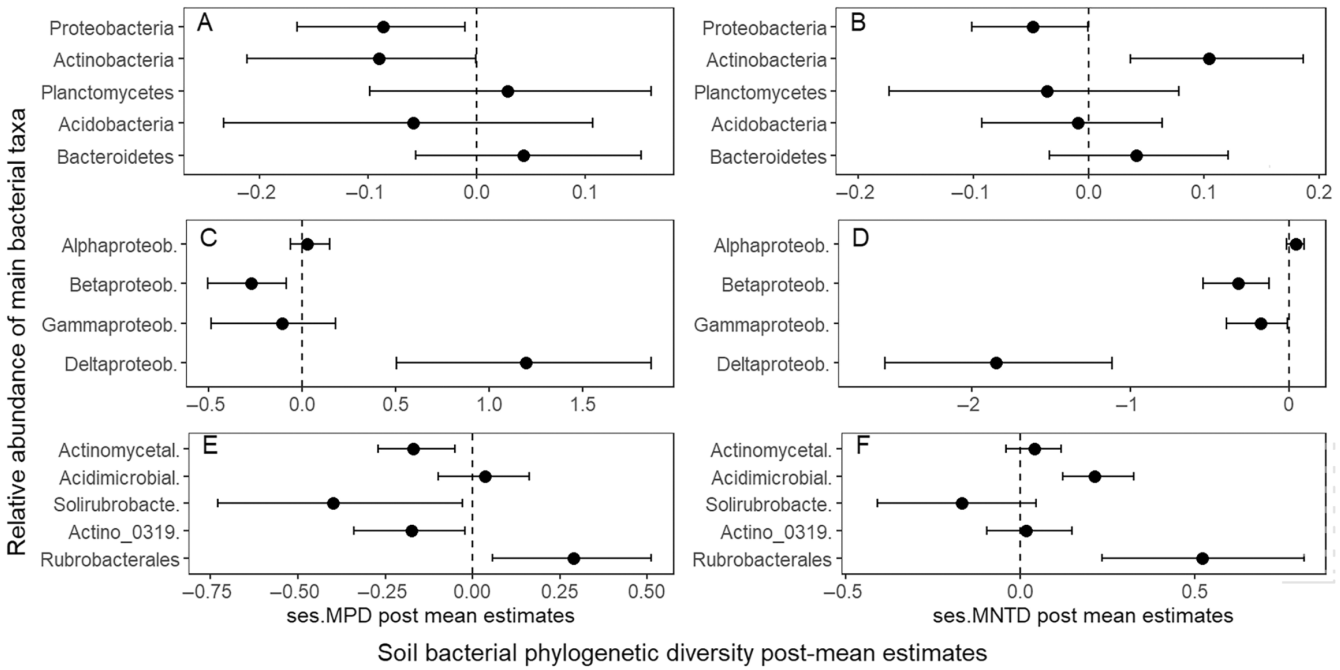


**FIGURE 6** Bayesian post-mean estimates (and their expected 95% credible intervals) explaining the effect of the relative abundance of main bacterial taxa on the standardized Mean Pairwise Distance (MPD) (left panel) and Mean Nearest Taxon Distance (MNTD) (right panel). Factors with intervals not including zero are significant. Relative abundance of all taxa is shown against both metrics in Appendix S1: Figures S4 and S5.

## DISCUSSION

Phylogenetic clustering was widespread in soil bacterial communities along primary succession gradients, both when explored across the phylogeny or only at the tips of the phylogeny, for all plant life forms and plant growth stages. These results indicate that bacterial communities were composed of taxa that are more closely related than expected by chance, as has been previously reported in soil bacterial communities (Goberna, García, & Verdú, 2014; Horner-Devine & Bohannan, 2006; Jones et al., 2009). Here, we detected significant levels of

phylogenetic clustering across primary succession, what contradicts the idea of stochasticity as a primary driver of community assembly in early assembly stages (Dini-Andreote et al., 2015). In support of our hypothesis, under abiotic stress, environmental filters override stochasticity, most likely by allowing the establishment of lineages sharing evolutionarily conserved traits to cope with abiotic conditions and by filtering out less stress-tolerant taxa (HilleRisLambers et al., 2012; Webb et al., 2002). Stressors such as desiccation, acidity, or the contents of certain metal(oid)s may be responsible for such patterns in our study sites. Along the successional gradients, we detected large levels of phylogenetic turnover, that is, a large replacement of groups that were phylogenetically more and more distant from the initial community. Such a large turnover was evident in 10 out of 13 successional gradients. We could not identify any common features in terms of plant life form or study site in the gradients that did not follow the general trend. Turnover was not evident when explored across the phylogeny (ses.βMPD), but rather at the tips of the phylogeny (ses.βMNTD), because main deeply rooting lineages were present across the succession. Large phylogenetic turnover led to changes in composition towards communities composed of closely related taxa, indicated by the intensification of phylogenetic clustering detected at the tips of the phylogeny (ses.MNTD). Based on the influence of certain taxa on the metrics of phylogenetic diversity, we speculate that both competitive and cooperative interactions shift along primary succession, as discussed below.

Both metrics of phylogenetic diversity (ses.MPD and ses.MNTD) revealed significant levels of phylogenetic clustering. However, they did not correlate with each other and responded to distinct abiotic and biotic drivers, as schematized in Figure 3. Using simulations and empirical data, Mazel et al. (2016) evidenced that both metrics do not always covary in biological communities owing to key differences in how they are computed and in the processes they reflect. While MPD reflects evolutionary relatedness across the whole phylogeny and is greatly impacted by major clades branching at deep nodes, MNTD measures relatedness in terminal branches descending from superficial nodes (Webb et al., 2002). As a consequence, patterns of clustering and overdispersion may occur at different phylogenetic scales in the same biological community, and assumptions based on a single metric are thus likely to be limited in capturing and interpreting the assembly of complex biological communities (Freilich & Connolly, 2015; Mazel et al., 2016; Qian et al., 2017).

The intensity of clustering across the phylogeny (ses.MPD) responded to the plant life form, irrespective of the stage of primary succession (Figure 3). Specifically, plant patches nursed by trees and perennial grasses sheltered more closely related soil bacteria compared to (dwarf) shrubs. We interpret that differences across life forms were due to their differential impacts on soil moisture, which most likely occur through different levels of plant canopy interception and reduction of intense evaporation. Moisture is a key parameter that profoundly alters soil microbial communities composition (Schimel et al., 2007). Microbial traits that confer tolerance to desiccation are phylogenetically conserved, and so is the response of soil bacterial communities to experimental drought (Amend et al., 2016; Goberna, Navarro-Cano, et al., 2014). Experimental evidence shows that dry-adapted bacteria tend to have wider niche breadths, that is, they tend to be generalists that tolerate a broader range of soil moisture conditions, while wet-adapted bacteria tend to be specialists with a metabolism restricted to humid conditions (Lennon et al., 2012). This suggests that larger moisture levels, by overrepresenting microorganisms with a shared tolerance to wet conditions, will induce phylogenetic clustering. This prediction fits well with our observations, since higher soil moisture led to more intense phylogenetic clustering driven by the overrepresentation of several lineages of Proteobacteria and Actinobacteria. The explanation, however, might be context-dependent and not generalizable as illustrated by the opposite results found by Lee et al. (2018) in artic soils, where increasingly wet conditions led to overdispersed patterns. These authors invoked the role of endemic Cyanobacteria, an ancient lineage whose overrepresentation might remarkably increase the levels of phylogenetic diversity under wet conditions. Beyond being a resource that impacts microbial physiological state, moisture also determines the physical connectivity of the soil matrix, which has important implications for resource availability, motility of soil biota, and biological interactions (Erktan et al., 2020; Pett-Ridge & Firestone, 2005; Treves et al., 2003). In our study, bacterial communities related with high basal phylogenetic clustering (ses.MPD) and high soil moisture harbored a greater relative abundance of Betaproteobacteria and Actinomycetales. These lineages include wet-adapted organisms (Lennon et al., 2012) which are strong competitors able to exclude distant lineages (Goldfarb et al., 2011), what may eventually lower phylogenetic diversity measured across the whole phylogeny.

Towards the tips of the bacterial phylogeny (ses.MNTD), soil bacterial communities were increasingly clustered associated with plant growth regardless of the plant life form (Figure 3). That is to say, plant development exacerbated the clustered patterns of soil bacteria by promoting the coexistence of close relatives, contrasting with

the increase in taxonomic diversity observed across the same gradients of primary succession (Colin et al., 2019). Such changes correlated with shifts in the composition of bacterial communities from bare soil to full-grown vegetation patches. Specifically, higher (but still strongly clustered) terminal phylogenetic diversity during the early stages of primary succession responded to the overrepresentation of thermotolerant, extreme radiation tolerant, acidophilic, and chemolithotrophic Actinobacteria (i.e., Rubrobacterales and Acidimicrobiales) (Norris, 2015; Stackebrandt & Schumann, 2006), coherent with the prevalence of abiotic filters. The decrease in terminal phylogenetic diversity along with plant growth was positively correlated to the larger contents in soil organic carbon and nutrients underneath larger plants. Labile carbon inputs (such as glycine or sucrose) enrich bacterial communities in fast-growing copiotrophs, such as Betaproteobacteria of the orders Burkholderiales and Rhodocyclales, leading to phylogenetically clustered patterns at the shallow scale in the phylogeny (Goldfarb et al., 2011) as we also detected. Altogether, these results suggest that pulses of easily assimilable organic carbon, such as those provided by rhizosphere exudates, are likely to support more taxonomically diverse communities composed by more closely related taxa. This supply of limiting resources might allow the coexistence of taxa with similar niches that tend to compete with each other for nutrients in oligotrophic unvegetated soils (Morin, 2000; Stevens & Carson, 2002). In our case, the levels of terminal clustering were further magnified as succession progressed responding to cooperative Deltaproteobacteria (Syntrophobacterales and Myxococcales), whose members tend to aggregate responding to different ecological processes. Syntrophic bacteria often form structured consortia with their kin and with their obligate mutualistic archaea (Morris et al., 2013), while members of Myxococcales swarm cooperatively and produce multicellular fruiting structures (Fiegna & Velicer, 2005). Altogether, these data contradict our expectation of increased competition based on relative fitness differences towards carbon-enriched mature stages, which decrease phylogenetic diversity across scales rather than in the tips of the phylogeny (Goberna, Navarro-Cano, et al., 2014; Mayfield & Levine, 2010). Instead, our results suggest a reduction in the levels of competition based on limiting similarity, that is, the classical Darwinian competition where closely related microbial taxa compete more intensely as they share similar traits, and a concomitant promotion of cooperative behaviors towards more mature stages of primary succession.

A final important picture emerging from our results is that the taxonomic scale matters when assessing phylogenetic diversity. When we tried to link changes in phylogenetic diversity to shifts in community composition at coarse taxonomic scales (phyla or classes), specific lineages exhibited contrasting trends depending on the phylogenetic metrics considered. Actinobacteria constitute a striking example, since their relative abundance was positively correlated with terminal phylogenetic diversity but inversely correlated with whole-phylogenetic diversity. Investigating such changes at a finer taxonomic scale revealed that these two patterns were actually related to different orders within the Actinobacteria, whose members are ecologically distinct in terms of tolerance to abiotic stress, metabolism, and competitive abilities. Our results show that a detailed analysis of the lineages that areoverrepresented under particular conditions, and how they impact the metrics of diversity, is therefore key to understanding the meaning of the phylogenetic structure in bacterial communities.

## CONCLUSIONS

Against current models of primary succession, our data indicate that environmental filters can override stochasticity from the early stages of community assembly under severe abiotic stress. Such filtering generates clustered patterns of phylogenetic diversity, which are exacerbated as the succession proceeds and shallow-diverging bacterial lineages become progressively more closely related. These patterns are generated based on the enrichment across the succession in a few deep-divergent lineages, which are composed of numerous closely related taxa. Examining the influence of lineage composition on metrics of evolutionary relatedness suggests that both cooperative and competitive biotic interactions underpin the magnified levels of terminal phylogenetic clustering in mature stages of community assembly. Ultimately, such shifts in phylogenetic patterns may determine key microbial-driven ecosystem functions, such as decomposition and nutrient cycling (Goberna & Verdú, 2018). Our study exemplifies how combining phylogenetically informed measures of diversity that quantify relatedness at different evolutionary scales, and testing the influence of microbial lineages on such metrics across the taxonomic hierarchy, can help to unravel the driving forces of microbial community assembly.

### AUTHOR CONTRIBUTIONS

Marta Goberna, Miguel Verdú, and Jose A. Navarro-Cano obtained funds. All authors designed the study. Jose A. Navarro-Cano and Marta Goberna collected field data and samples. Yannick Colin and Marta Goberna

processed and analyzed data and wrote the first draft. All authors contributed to the final version of the manuscript.

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## CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

## DATA AVAILABILITY STATEMENT

Phylogenetic trees generated in this study are available in Colin et al. (2026) in Zenodo at https://doi.org/10.5281/zenodo.19480271. These trees have been reconstructed from sequences from Colin et al. (2019) which are deposited in the European Nucleotide Archive at www.ebi.ac.uk/ena/data/view/PRJEB23564. Soil data from Navarro Cano et al. (2018b) are available in Navarro-Cano et al. (2018a) in Dryad at https://doi.org/10.5061/dryad.j70qf.

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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