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MACROEVOLUTIONARY PERSPECTIVES ON BIOTIC INTERACTIONS

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Plant intraspecific variation modulates nutrient cycling through its below-ground rhizospheric microbiome

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Abstract

1. Plant genetic variation, through its phenotypic display, can determine the composition of below ground microbial communities. Variation within a species is increasingly acknowledged to have substantial ecological consequences, particularly through trophic cascades. We hypothesized that the intraspecific genotypic variation of the tree host might impact the phylogenetic composition of its rhizospheric microbial communities, by favouring particular clades, that might be further reflected in ecosystem process rates.
2. We tested whether the intraspecific genotypic variation of *Pinus pinaster* modulates nutrient cycling by determining the phylogenetic structure of its symbiotic ectomycorrhizal fungi and rhizospheric bacteria. We sequenced fungal and bacterial molecular markers and reconstructed phylogenies in the rhizosphere of *P. pinaster* trees belonging to three genotypic variants (Mediterranean, Atlantic, African) in three 45-year-old common garden experiments, and measured seven soil enzymatic activities.
3. Local effects, based on differences in elevation and soil conditions across sites, were strong predictors of the ectomycorrhizal and bacterial communities thriving in tree's rhizosphere. Across-site variation also explained differences in phosphorus cycling. We detected, however, a significant effect of the plant genotype on the phylogenetic structure of the root-associated microbiota that was consistent across sites.
4. The most productive Mediterranean plant genotype sheltered the most distinct root microbiome, with the dominant Basidiomycetes and Proteobacteria having a strong influence on the phylogenetic microbial community structure and associating with an enhanced hydrolysis of celluloses, hemicelluloses and chitin. Beneath the less productive Atlantic genotype, the less abundant Ascomycetes and up to thirteen bacterial phyla shaped the phylogenetic microbial structure, and predicted the rates of peptidase. Ectomycorrhizal fungi explained the activity of cellulases and protease, and bacteria that of hemicellulases and chitinase, suggesting functional complementarity.
5. *Synthesis*. This is the first report using three-replicated long-term common gardens in mature forests to disentangle plant genotype- and site-specific drivers of the

rhizospheric microbiome and its enzymatic potential. We concluded that intraspecific variation in primary producers leaves a phylogenetic signature in mutualists and decomposers that further modulate key steps in carbon and nitrogen cycles. These results emphasize the ecological relevance of plant intraspecific diversity in determining essential plant–soil feedbacks that control ecosystem productivity and performance.

KEYWORDS
ecosystem functioning, ectomycorrhizal fungi, nutrient cycling, phylogenetic community structure, plant genotype, rhizosphere, soil bacteria

1 | INTRODUCTION

Since the emergence of community genetics, ecologists have tried to understand how particular genotypes within a single species (intraspecific variation) can influence the structure of ecological communities and the functioning of ecosystems (Vellend & Geber, 2005). A handful of studies have reported that particular genotypes of a given species may impact ecosystem functions through changes in the taxonomic composition of associated communities (Crutsinger, Souza, & Sanders, 2008; Gamfeldt, Wallén, Jonsson, Berntsson, & Havenhand, 2005; Whitham et al., 2006). Indeed, the ecological consequences of intraspecific variation can equate, or even exceed, those of interspecific variation through the cascading effects that a trophic level exerts on the composition of another trophic level (Des Roches et al., 2018). Specifically, intraspecific variation in plants may prompt shifts in the surrounding environment (soil conditions) and the composition of below-ground microbiota, potentially inducing plant–soil feedback responses (Schweitzer, Van Nuland, & Bailey, 2018). Despite increasing evidence showing that ecosystem functions are better explained by the phylogenetic (rather than taxonomic) composition of ecological communities (Cadotte, Cardinale, & Oakley, 2008; Navarro-Cano et al., 2014; Pérez-Valera, Goberna, & Verdú, 2015), there is a lack of information about the influence of intraspecific variation on the phylogenetic structure of communities and, beyond, on ecosystem performance.

A crucial assumption to use the phylogenetic composition of ecological communities as an accurate proxy of ecosystem functions is that functional traits are evolutionarily conserved across lineages. This assumption is met for many traits both in eukaryotes (Blomberg, Garland, & Ives, 2003) and prokaryotes (Goberna & Verdú, 2016). Particularly, microbial traits related to processes shaping community structure (i.e. abiotic stress tolerance and competition-related traits) and regulating metabolic functions are highly conserved across the prokaryotic phylogeny (Goberna & Verdú, 2016), and to some extent across saprotrophic or symbiotic fungal lineages (i.e. nutrient cycling-related traits) (Hugoni, Luis, Guyonnet, & Haichar, 2018; Kohler et al., 2015). This observation paves the road to use microbial community phylogenetic composition as a convenient proxy of function.

The taxonomic composition of the extraordinarily diverse communities of soil microorganisms associated with plants is partly determined by the plant genotype (van der Heijden & Schlaeppi, 2015; Korkama, Pakkanen, & Pennanen, 2006; Peiffer et al., 2013), although many other environmental factors simultaneously operate affecting these communities (Goberna, García, & Verdú, 2014; Peay et al., 2015; Rincón et al., 2015). The influence of plant genotypes on below-ground microbiota can be attributed to differences in plant growth performance, as well as in the varying amounts of carbon (C) provided to soil through litter and root exudates (van der Heijden & Schlaeppi, 2015; Hugoni et al.., 2018; Korkama et al., 2006) and through their symbionts (Gorka et al., 2019; Smith & Read, 2008). Conversely, the soil microbiome of plant genotype could influence phenotype of genetically related plants, suggesting that microbiomes can be selected to modify plant traits and coordinate changes in soil resource pools (Panke-buisse, Poole, Goodrich, Ley, & Kao-kniffin, 2015). Soil microbiota, particularly heterotrophic soil fungi and bacteria, performs essential ecosystem functions such as litter decomposition and organic matter mineralization, mainly through the production of a wide array of extracellular enzymes (Baldrian, 2014). Microorganisms are able to allocate C to produce different sets of enzymes depending on resource demands, either increasing the supply of the most limiting nutrients or attacking the most available substrates (Nicolás et al., 2018; Sinsabaugh, Hill, & Follstad Shah, 2009). Community composition may only affect processes if organisms vary in their functions, so the ways in which microorganisms allocate C can be critical for soil structure and functioning (Schimel & Schaeffer, 2012). Thus, the plant genotype, by determining the structure of its microbial partners in the rhizosphere, can be expected to exert cascading effects on ecosystem function related to nutrient cycling and C sequestration in soil. To which extent the microbial lineages that coexist in particular plant genotypes are evolutionarily related, and whether distinct microbial phylogenetic assemblages differ in essential functions, remains unknown.

Pines are obligatory ectomycorrhizal (ECM) plants and this intimate relationship strongly influences the surrounding environment (Tarkka, Drigo, & Deveau, 2018). The effects of the plant genotype on below-ground microbial communities can be ideally studied through the symbiosis between pines and ECM fungi

(Patterson, Flores-Rentería, Whipple, Whitham, & Gehring, 2018; Pérez-Izquierdo et al., 2017; Piculell, Eckhardt, & Hoeksema, 2018). Patterson et al. (2018) have recently shown that ECM community composition is under strong plant genetic control in *Pinus edulis*. We showed in a previous study that the taxonomic composition of soil ECM communities was particularly responsive to *Pinus pinaster* genotypes (Pérez-Izquierdo et al., 2017). ECM fungi depend on the C supplied by plants and, in turn, improve the uptake from soil of limiting nutrients, predominantly N and P, for the host plant (Smith & Read, 2008). The plant creates a flux of carbohydrates towards the roots to maintain the symbiosis, creating a rich environment where numerous microorganisms, such as bacteria, proliferate (Frey-Klett, Garbaye, & Tarkka, 2007; Gorka et al., 2019; Rincón et al., 2005). The community structure of these microbes is influenced by the abiotic properties of the microhabitat they inhabit, some of which are determined by the genetics of the host plant (Peiffer et al., 2013). As plant rhizodeposits are the key energy supply for rhizospheric microbiota (Lynch & de Leij, 2001), the plant modulates these inhabitants by excreting selective exudate mixtures (Hartmann, Schmid, Tuinen, & Berg, 2009; Steinauer, Chatzinotas, & Eisenhauer, 2016). Enrichment in soil organics can lead to the overrepresentation of specific microbial clades with high competitive abilities, influencing the phylogenetic structure of the whole community (Goberna, García, et al., 2014; Goldfarb et al., 2011). These observations led us to hypothesize that the genotype of the tree host might leave a phylogenetic signature in the microbial communities by favouring particular microbial clades thriving in the rhizosphere (Figure S1). Since the selection of phylogenetically distinct groups of microbes might influence biogeochemical processes (Schimel & Schaeffer, 2012), we further hypothesized that the differential phylogenetic structure of microbial communities underneath distinct plant genotypes might be reflected in ecosystem functions related to nutrient cycling (Figure S1).

To test theses hypotheses, we studied the ECM and bacterial communities associated with the rhizosphere of different genotypes of *P. pinaster* Ait. A clear genetic differentiation exists among trees coming from the three main geographic provenances, hereafter referred as Atlantic, Mediterranean and African (Alía & Moro, 1996; González-Martínez et al., 2004; Rodríguez-Quilón et al., 2016). Differences across genotypes are further reflected in the plant phenotype, in terms of stem shape, growth and biomass, pest resistance as well as frost and drought tolerance (Alía & Moro, 1996; González-Martínez et al., 2004). We analysed trees from all three genotypes that had been experimentally planted in three replicated long-term common garden experiments. Few studies have been performed on mature forests where multiple environmental variables operate (Lamit, Holeski, Flores-Rentería, Whitham, & Gehring, 2016). Unlike many other common garden experiments that often lack replication across sites, our study was conducted in three spatially distinct common gardens, allowing us to appropriately disentangle genotype- and site-specific drivers on the rhizospheric microbial communities. Our specific objectives were to study whether (a) the genotype of *P. pinaster* determines the phylogenetic community structure of symbiotic ECM fungi and rhizospheric bacteria regardless of the environmental (i.e. climatic and edaphic) conditions, and (b) the differential phylogenetic structure of the rhizospheric microbial community is further reflected in the ecosystem functioning measured as potential enzymatic activities related to C, nitrogen (N) and phosphorus (P) cycling.

2 | MATERIALS AND METHODS

2.1 | Study sites and sample collection

The study was conducted in approximately three 45-year-old common gardens of *P. pinaster* established with trees from several geographic origins (Alía & Moro, 1996). The three planting sites were located in central Spain in Cabañeros (39°22N, 4°24W), Riofrío (39°8N, 4°32W) and Espinoso del Rey (39°36N, 4°48W). General soil and climatic features of all sites are summarized in Table S1.

All sites have similar climatic conditions, mean annual temperature ranging from 10.2 to 13.4°C and precipitation from 716 to 800 mm (Table S1). Other abiotic conditions differ along the planting sites. Cabañeros is located at 1,045 m altitude, while Riofrío and Espinoso are located at 775 and 830 m, respectively (Table S1). Cabañeros shows significantly higher values of P and N content and soil moisture, as well as lower C:N ratios. Likewise, organic matter content is higher in Cabañeros, although statistical differences are only significant compared to Espinoso. Both sites show lower soil pH values compared to Riofrío (Table S1).

Plantations in all sites had a randomized complete block design with four blocks, each one including 16 *P. pinaster* individuals from several geographic origins planted 2.5 m apart (Alía & Moro, 1996). Here, we studied each *P. pinaster* provenance, which have been shown to be genotypically and phenotypically distinct (Alía & Moro, 1996; González-Martínez et al., 2004; Rodríguez-Quilón et al., 2016), by analysing trees coming from the following geographic origins: Atlantic (Galicia, NW-Spain), Mediterranean (Valencia, E-Spain) and African (Jbel Tassali, Morocco). Tree genotypes differed in their productivity in terms of biomass, the Mediterranean genotype showing the highest diameter at 1.30 m (DBH) (Mediterranean = 28.9 cm ± 1.0 a; Atlantic = 22.6 cm ± 1.7 b; African = 20.3 cm ± 0.9 b; $F_{2,26}$ = 13.9, p < 0.001). Differences in DBH associated with the plant genotypes were consistent across planting sites (site × genotype interaction: $F_{4,26}$ = 1.65, p > 0.1). We selected three trees per genotype and experimental block, making a total of 108 trees (3 sites × 3 plant genotypes × 4 blocks × 3 trees). However, due to the former opening of a firebreak in one of the sites (Espinoso del Rey), 6 trees were lacking and finally 102 trees were sampled.

Soil sampling was performed during the growing season in spring 2012, when canopy C was amply drained to the roots, leading to high bacterial and fungal activity (more details in Material and Methods S1). Four samples located one meter away from the trunk in the four cardinal points were collected below each tree by digging 10 × 10 × 20 cm (length × width × depth) after removing the litter layer. Secondary roots were traced to their link with the main root to assess their belonging to the chosen tree. The four samples taken per tree were pooled into a single composite sample. Samples

were kept at 4°C until processing, within 2 weeks. We tried to minimize the effect of opportunistic fast-growing taxa by randomly processing samples, although possible effects cannot be totally ruled out. Roots (diameter < 2 mm) were separated from soil and rhizospheric soil detached from fine roots. Roots were then gently washed with tap water over 2 and 0.5 mm sieves for collecting fine root tips. Rhizospheric soil samples were pooled by tree genotype per site and experimental block into single composite replicates for chemical and enzymatic analyses (N = 35). Rhizospheric soil aliquots were collected and stored at −20°C. The remaining bulk soil was air-dried and sieved (2 mm) for additional physical–chemical analyses (Material and Methods S1). Fine roots were observed under the stereomicroscope, and a subset of ~1.5 g (fresh weight) of randomly selected ectomycorrhizal root tips per sample was frozen with liquid N, freeze-dried and ground with mortar and pestle for further molecular analyses.

2.2 | DNA extraction, DNA metabarcoding and sequences processing

Genomic DNA was extracted from ECM root tips (50 mg of freeze-dried powder), and rhizospheric soil (500 mg) with appropriate kits (Material and Methods S1). DNA extracts belonging to the three replicated trees per plant genotype and experimental block were pooled, thus making a total of 35 ECM root tip extracts and 35 rhizospheric soil extracts. On ECM root tip DNA, the internal transcribed spacer region ITS-1 was amplified with the primer pair ITS1F-ITS2 adapted for pyrosequencing (Buée et al., 2009). On soil DNA, the 16S rRNA gene was amplified using the eubacterial primers 27F and 519R (Lane, 1991) with barcodes. Fungal and bacterial amplicon products were pooled in equimolar-independent libraries, and samples were sequenced using Roche 454-GS-FLX titanium instruments and reagents (Roche Applied Biosystems, USA) (Material and Methods S1).

A total of 106,789 and 361,880 sequences were obtained for fungal and bacterial communities, respectively. For both communities, sequences were demultiplexed according to their tags, filtered and trimmed. For fungi, ITS1 was extracted with the Fungal ITSx v1.0.3 (Bengtsson-Palme et al., 2013). Short sequences, chimeric sequences and singletons were removed (Material and Methods S1). Dereplication and clustering were performed with USEARCH v8.0.1616 software (Edgar, 2013). Operational taxonomic units (OTUs) were generated at 97% similarity threshold that were taxonomically assigned by using the Basic Local Alignment Search Tool (BLAST) against the UNITE database (Kõljalg et al., 2013) and complementarily by using RDP (Wang, Garrity, Tiedje, & Cole, 2007) at a confidence threshold of 80%. Fungal taxonomic assignment was used to classify OTUs into guilds by using FUNguild (Nguyen et al., 2016) and according to Tedersoo et al. (2014). Among them, we selected the ECM fungi that corresponded to 75% of the total sequences. For bacteria, sequences representative of each OTU were assigned to bacterial taxa using RDP at a confidence threshold of 80% (Material and Methods S1). Data were deposited in the Sequence Read Archive (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA324224).

2.3 | Phylogeny reconstruction

Fungal phylogeny from 301 ECM OTUs was estimated with the program Phylomatic as implemented in Phylocom 4.2 (Webb, Ackerly, & Kembel, 2008) and BEAST 1.5.4 (Drummond & Rambaut, 2007). We generated a fungal mega tree whose topology and age estimates for major nodes were based on the phylogenetic information available in the literature (Material and Methods S1, Table S2, Figure S2a).

The reconstruction of phylogenetic relationships in bacterial taxa was made for 2,650 bacterial OTUs, that were aligned with INFERNAL (Nawrocki & Eddy, 2013), by using RAxML 7.3.0 (Stamatakis, 2006) (Material and Methods S1).

2.4 | Phylogenetic community structure

We described the phylogenetic structure of fungal and bacterial communities by calculating a matrix (matrix P) that contains the composition of species fuzzy-weighed by their pairwise phylogenetic similarities (Pillar & Duarte, 2010) with the *PCPS* package for R (Debastiani et al., 2015). In matrix P, each OTU has a value per sample that increases as the phylogenetic distance between neighbouring OTUs decreases. Ordination techniques allow reducing matrix P to represent the phylogenetic structure at the sample level. We used principal coordinate analysis with Euclidean distances and extracted the sample scores along the first axis, which represents the principal component phylogenetic structure (PCPS1). This axis captures the deepest phylogenetic divergences among lineages (Duarte, Prieto, & Pillar, 2012) and can be used in further analyses as a single variable that describes the phylogenetic community structure (Pérez-Valera et al., 2015). We calculated the contribution of each fungal and bacterial phylum (mean ± *SE*) as the loadings of each taxon to the respective PCPS1 (Material and Methods S1).

2.5 | Ecosystem functioning

The community functioning was evaluated on rhizospheric soil (N = 35) by measuring the activity of seven hydrolytic enzymes secreted by fungi and bacteria and related to C, N and P cycling, adapting the method described by Mathieu et al. (2013). We measured the activity of β-glucosidase (BG), cellobiohydrolase (CBH), β-xylosidase (BXD) and β-glucuronidase (BGD) related to the C cycle; acid phosphatase (AP) that mobilizes P and chitinase (NAG) and L-leucine aminopeptidase (LAP), which are enzymes involved in N mobilization (Material and Methods S1).

2.6 | Statistical analyses

We tested the existence of spatial autocorrelation on the phylogenetic structure of microbial communities through Mantel tests using the *vegan* package for R 3.1.1 (Oksanen et al., 2015; R Core Team,

2014). We did not find spatial autocorrelation either for ECM fungi or bacteria (mantel correlations for all sites, $p > 0.05$), and therefore we did not include the geographical coordinates of the trees in further statistical analyses.

We assessed whether the plant genotype, the local environment and/or the interaction between both factors had an effect on the phylogenetic structure of root-associated microbial communities. To do so, we ran Bayesian generalized linear mixed models (GLMM), using the MCMCglmm package for R (Hadfield, 2010), with fungal PCPS1 or bacterial PCPS1 as dependent variables in two separate models. We used the plant "genotype" as a fixed factor, since we considered all three genotypes that exist in the common gardens (i.e. Atlantic, Mediterranean and African genotypes that differ genetically and phenotypically). We also introduced the planting "site" as a fixed factor, as we were interested in analysing the local effects and particularly whether there is a "genotype" × "site" interaction. We further introduced the 'block' as a random factor in all models. We used the default priors and ran 13,000 MCMC iterations with a burn-in period of 3,000 iterations. The statistical significance of the factors in the model was estimated by calculating the 95% credible interval of their posterior distribution. To test for the existence of a significant 'genotype' × 'site' interaction, we used the deviance information criterion for comparison of the models with and without the interaction (Table S3). The 'genotype' × 'site' interaction was not significant in any case and was not further considered.

In order to detect a global functional response, we performed a principal component analysis (PCA) including all enzymatic activities. We assessed whether the plant 'genotype', the local effects ('site') or their interaction explained the first two PCs of the enzymatic potential, in two separate models as above. We then tested whether the phylogenetic structure of microbial communities predicts the functions. To do so, we ran separate MCMCglmms using either the first two PCs of enzymatic potential or each enzymatic activity taken individually as a dependent variable. In all models, we used the 'fungal PCPS1', 'bacterial PCPS1' and planting 'site' as fixed factors, and the 'block' as a random factor in the same model. Interactions between each PCPS1 and the site were tested as above and it was found to be non-significant (Table S3).

To ensure that tree genotype differences on either the phylogenetic structure of microbial communities or ecosystem functions were not a consequence of the environmental similarity between the tree geographic origin and the planting sites, we compared the fungal and bacterial PCPS1 as well as the enzymatic activities of each genotype with the environmental distances between the planting site and the geographic origin (see Hernández-Serrano et al., 2014 for a similar procedure) (Material and Methods S1).

3 | RESULTS

A total of 75,872 and 133,581 final sequences were obtained after post-processing for ECM (2,168 ± 49 sequences per sample) and bacterial rhizospheric communities (3,817 ± 205 sequences per sample), respectively. Sequence grouping yielded a total of 301 ECM (60 ± 2 per sample) and 2,650 bacterial (501 ± 14 per sample) OTUs. ECM OTUs were assigned to the phyla Ascomycota (11%) or Basidiomycota (89%) (Table S4). *Thelephoraceae*, *Atheliaceae*, *Inocybaceae*, *Russulaceae*, *Sebacinaceae*, *Cortinariaceae* and *Bankeraceae* were among the most representative fungal families out of a total of 26 families identified (Table S4). We identified 14 bacterial phyla, including Proteobacteria (33%), Actinobacteria (18.8%), Planctomycetes (15.4%), Acidobacteria (13%) and Bacteroidetes (5.2%) (Table S5).

3.1 | Microbial phylogenetic community structure

We described the phylogenetic structure of microbial communities by constructing phylogeny-weighed community (taxon × sample) matrices (matrix P). Matrix P values showed a differential contribution of ECM and bacterial phyla, respectively, to the overall phylogenetic community structure (Figure S3). Ascomycota showed high matrix P scores indicating a tendency of these fungi to co-exist with close relatives compared to Basidiomycota. For Bacteria, OTUs assigned to Proteobacteria, Firmicutes, Acidobacteria and the candidate phylum Saccharibacteria showed the highest matrix P values on average, while those within Bacteroidetes and Planctomycetes had the lowest scores (Figure S3).

We used multivariate analysis on matrix P. The first principal component of the phylogenetic community structure (PCPS1) explained 50% and 41% of the total variance of ECM and bacterial communities, respectively. The contribution of fungal phyla to PCPS1 revealed a preponderance of Basidiomycota on the negative pole of the axis, while Ascomycota was positioned on the positive pole (Figure 1a, left panel). For bacteria, a clear segregation was observed associating negative PCPS1 with Proteobacteria and positive PCPS1 with the other phyla (Figure 1b, left panel).

3.2 | Plant genotype and site effects on microbial phylogenetic community structure

The planting site significantly explained the phylogenetic structure of both ECM and bacterial communities (Figure 1; Table S6), and this effect was similar across plant genotypes as revealed by the non-significant genotype × site interaction. Most interestingly, the plant genotype significantly explained the phylogenetic structure of ECM and bacterial communities (Figure 1; Table S6). The effect of the planting site in structuring ECM communities was ca. two- to three-fold that of the plant genotype, whereas the opposite was true for bacterial communities (Figure 1; right panel).

Specifically, we detected divergent fungal assemblages under Mediterranean trees, whose phylogenetic structure was mainly impacted by the dominant Basidiomycetes (as indicated by the negative scores on PCPS1) compared with the Atlantic genotype, where the less abundant Ascomycetes had a stronger influence (Figure 1a, right panel). The fungal assemblages of African trees did not significantly

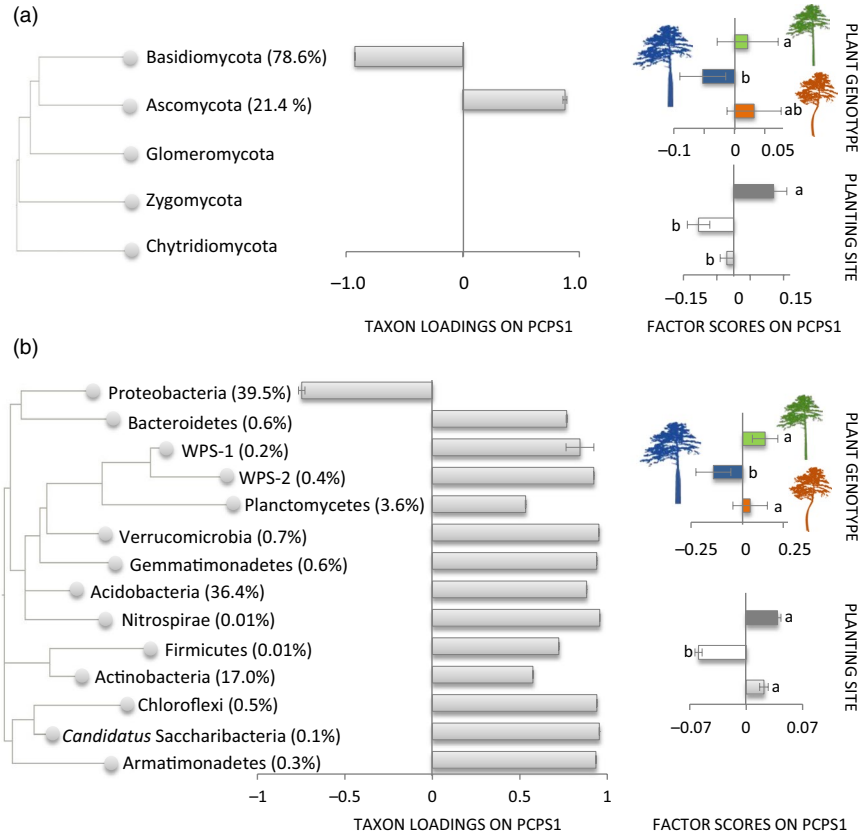

FIGURE 1 Effect of the plant genotype and planting site on the phylogenetic structure of (a) ECM and (b) bacterial communities. Panel left i) Phylogenetic trees depicting the relationships between main phyla (relative abundance across all genotypes in parentheses) and Loadings (means ± SE) of each taxon on PCPS1; panel right ii) Scores of plant genotype and planting site on PCPS1. Pine silhouettes depict different Pinus pinaster genotypes (as in Fig. 1; pine's crown size indicates differences in biomass production). Colours indicate different sites (dark grey: Cabañeros, white: Riofrío; light grey: Espinoso del Rey). Different letters denote significant differences among genotypes or sites according to Bayesian GLMs (see Supporting Information Table S6). [Colour figure can be viewed at wileyonlinelibrary.com]

differ from either of the other plant genotypes (Figure 1a, right panel). The phylogenetic structure of bacterial communities under the Mediterranean genotype was significantly different from the other two genotypes, and showed a remarkable influence of a single phylum, the dominant Proteobacteria (Figure 1b, right panel). However, the bacterial phylogenetic community structure in the rhizosphere of the Atlantic and African genotypes, which did not differ significantly was largely impacted by the other 13 phyla detected, including the abundant Acidobacteria and Actinobacteria and other less frequent phyla.

3.3 | Microbial phylogenetic community structure and enzymatic activities

The enzymatic activities in the rhizospheric soils showed great variability across plant genotypes and sites (Figure 2; Figure S4). The first two axes of the PCA grouping all the enzymatic activities accounted, respectively, for 30.9 and 26.4% of the variance (Figure 2). The enzymatic profile of the Atlantic genotype was different from that of the other two genotypes (Figure 2), and showed more potential allocation towards N and P acquisition enzymes according to the ratios BG:(NAG + LAP) and BG:AP, respectively (Table S7). The three planting sites were clearly separated from each other according to their enzymatic profiles (Figure 2) and the ratios BG:(NAG + LAP) and BG:AP were mainly different between Cabañeros and Espinoso. To explain this variability, we tested the effects of the phylogenetic structure of ECM and bacterial communities, which significantly explained the activity of several enzymes that mediate the C and N cycles (Table 1). P cycling, however, was only explained by the planting site (Table 1). Fungal PCPS1 exerted a significantly negative effect on cellobiohydrolase activity, indicating that samples overrepresented by distant-related Basidiomycetes also showed higher potential cellulose degradation (Table 1; Figure 1a). On the contrary, fungal PCPS1 was positively related to leucine activity, that is, increases in leucine activity were associated with the overrepresentation of closely associated Ascomycetes (Table 1; Figure 1a). On the other side, the bacterial phylogenetic structure explained the activity of β-glucuronidase and chitinase (Table 1). The relationship between both enzymes and the bacterial PCPS1 was negative suggesting an important contribution of Proteobacteria to their activity. Additionally, the planting site had a significant effect on chitinase (Table 1).

4 | DISCUSSION

Our long-term common garden experiments indicate that, in addition to a site effect, intraspecific genotypic differences of *P. pinaster* significantly explain the phylogenetic structure of root-associated ectomycorrhizal and bacterial communities. Shifts in the phylogenetic structure of these microbial communities have further consequences on ecosystem performance in terms of potential enzyme activities associated with nutrient cycling (Figure 3). Our study provides valuable insights into plant–microbiota interactions under well-replicated field conditions, thus overriding the limitations of studies that are performed under laboratory conditions or those focused on a single

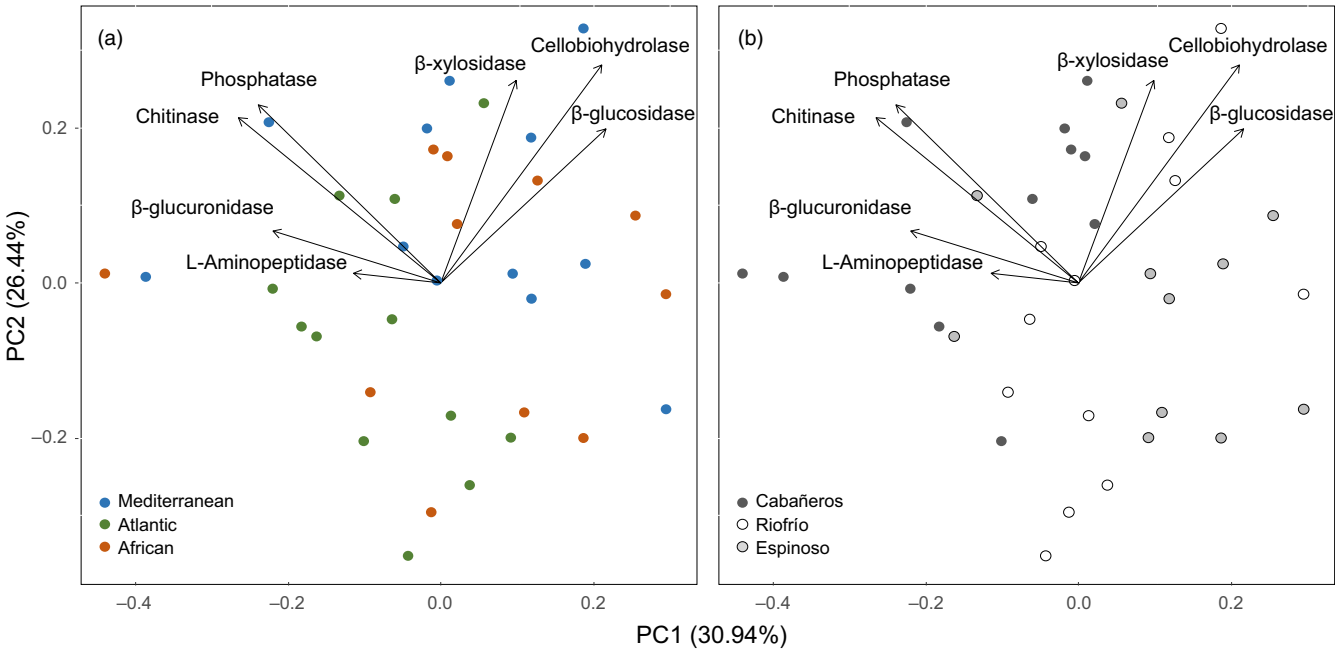

FIGURE 2 Enzymatic profile in the rhizosphere of Pinus pinaster analysed by Principal component analysis (PCA) and visualized (a) by tree genotype and (b) site. Percentages in parentheses indicate the variance explained by each axis. [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Bayesian post-mean estimates and their 95% expected credible intervals (in brackets) of the effect of ectomycorrhizal (ECM) and bacterial phylogenetic community structure (PCPS1) and planting site on enzymatic activities. Block was included as random factor in all the models. The site Cabañeros was taken as the reference in all models after checking that results were held when other sites were taken as the reference. Significant differences (i.e. credible intervals not including zero) are shown in bold type

Nutrient cycle	Enzymatic activity	Fungal PCPS1	Bacterial PCPS1	Site Espinoso	Site Riofrío
C (cellulose)	β-glucosidase	−0.25 (−0.68, 0.22)	−1.73 (−3.75, 0.40)	−0.03 (−0.18, 0.11)	−0.18 (−0.42, 0.06)
	Cellobiohydrolase	**−0.06 (−0.12, −0.001)**	−0.21 (−0.47, 0.07)	−0.003 (−0.02, 0.02)	−0.03 (−0.06, 0.003)
C (hemicellulose)	β-xylosidase	−0.002 (−0.03, 0.027)	−0.02 (−0.13, 0.10)	0.004 (−0.007, 0.014)	−0.005 (−0.02, 0.01)
	β-glucuronidase	0.40 (−0.22, 0.97)	**−2.42 (−5.50, −0.06)**	−0.12 (−0.31, 0.06)	−0.19 (−0.51, 0.15)
N (peptides)	L-Aminopeptidase	**0.029 (0.005, 0.05)**	0.019 (−0.07, 0.13)	0.006 (−0.002, 0.01)	0.002 (−0.01, 0.01)
N (chitin)	Chitinase	0.039 (−0.22, 0.32)	**−1.12 (−2.34, −0.005)**	**−0.17 (−0.26, −0.09)**	**−0.23 (−0.38, −0.10)**
P	Phosphatase	−0.29 (−2.01, 1.78)	−1.24 (−9.78, 6.94)	**−2.01 (−2.67, −1.42)**	**−1.56 (−2.58, −0.42)**

group of microbes, as discussed by van der Putten et al. (2013) and van der Heijden, Martin, Selosse, and Sanders (2015).

4.1 | Local environment shapes microbial phylogenetic community structure

The local environmental context has been recognized as the main factor determining the effect of plant species identity or genotype on the microbial community structure (Peiffer et al., 2013; Tedersoo et al., 2016). Our results also indicate that local processes exert a strong effect in phylogenetically structuring the ECM and bacterial communities thriving in the rhizosphere, as the planting site was a significant source of variation in our experiment. Despite this site effect, we detected that the plant genotype consistently determined microbial communities across planting sites. It is important to highlight that the tree genotype effects were not due to the environmental similarity between the geographic origin of the trees and the planting sites. Interestingly, we observed that the local effects were particularly relevant to determining ECM (rather than bacterial) phylogenetic structure. These results could be attributed to ECM fungi showing dispersal limitation and/or coarse-grained responses to environmental factors that significantly differed across sites, such as pH, water availability or soil nutrients (Peay, Garbelotto, & Bruns, 2010; Tedersoo et al., 2016). On the other hand, the smaller effect of the planting site (compared to the plant genotype) on the community structure of rhizospheric bacteria can be interpreted as a preferential response to differences in fine-scale factors determined, for instance, by tree exudation (Edwards et al., 2015).

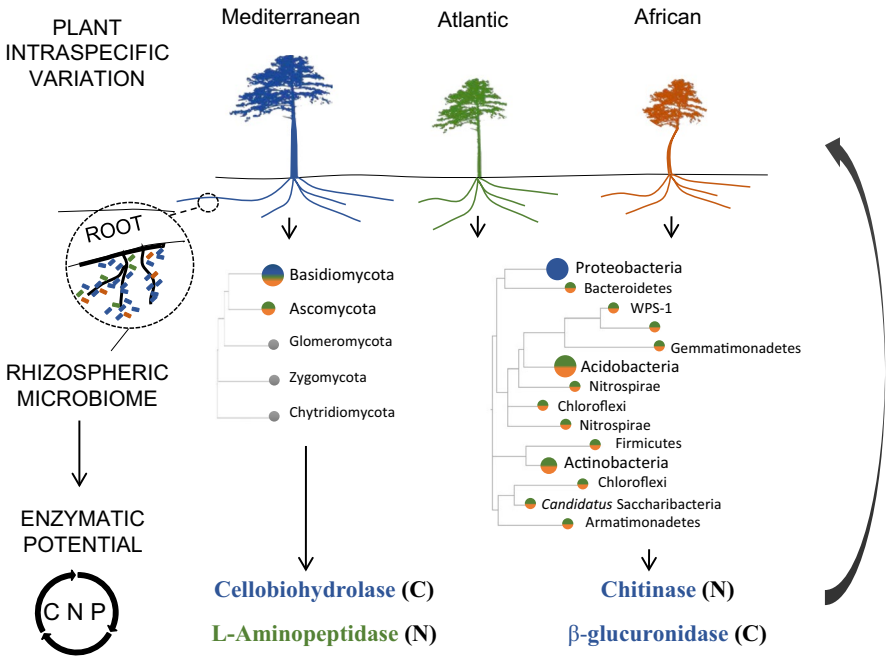

FIGURE 3 Intraspecific variations in the plant genotype determine the phylogenetic community structure of the rhizospheric microbiome that further modulates the rates of ecosystem functions related to nutrient cycling. Plant genotypes, which are depicted in different colours, have distinct phenotypes (e.g. biomass production, indicated by different crown size) that may lead to differential resource allocation to their symbiotic ECM fungi and/or exudation to the rhizosphere. This favours particular microbial clades (coloured circles in the phylogenetic tree referring to the effect of the specific genotypes) that in turn differ in their productivity in terms of enzymatic breakdown of carbon (C), nitrogen (N) and phosphorus (P) organic substrates, which might feedback tree growth. Pine silhouettes have been slightly modified from that downloaded from http://www.phylopic.org (MM Tobias) to reflect different pine genotypes. The original image is licensed under a Creative Commons 3.0 license (http://creativecommons.org/licenses/by/3.0). [Colour figure can be viewed at wileyonlinelibrary.com]

4.2 | Impact of plant genotype on rhizospheric microbial phylogenetic community structure

The genotype of *P. pinaster* significantly shaped the phylogenetic community structure of the ECM and bacterial communities in its rhizosphere, regardless of the environmental conditions. The Atlantic and Mediterranean genotypes showed the most phylogenetically distinct microbial communities. The ECM phylogenetic assemblage in the rhizosphere of Mediterranean trees was dominated by Basidiomycetes, which tended to co-exist with evolutionarily distant fungi, compared to that of Atlantic trees, which showed a marked influence of Ascomycetes that predominantly co-occurred with closer relatives. Despite the generally low specificity in mycorrhizal symbioses, the C allocated to each fungus for mycelial biomass can greatly differ depending on their exploration strategy (Agerer, 2001), nutrient mobilization ability (Talbot, Martin, Kohler, Henrissat, & Peay, 2015) and on whether they are favoured by the host (Bever, Richardson, Lawrence, Holmes, & Watson, 2009). Even environmental stressors, often associated with a reduced plant photosynthetic activity, can alter the ECM communities particularly by favouring Ascomycetes (Brown, Whitham, Morgan Ernest, & Gehring, 2001; Rincón, Santamaría-Pérez, Ocaña, & Verdú, 2014). In line with these studies, we interpret that the plant genotype, by determining resource allocation to its symbionts even at the intraspecific level, may select fungal clades with different competitive abilities influencing the phylogenetic community structure of ECM fungi.

Pinus pinaster genotype also determined the phylogenetic assembly of the bacterial community in the rhizosphere. This observation can be explained by the fact that the concentration, composition and quality of rhizodeposits determine the abundance and diversity of bacteria in the rhizosphere (Bulgarelli et al., 2013; Steinauer et al., 2016). In particular, the effect of root exudates on the structure and activity of rhizospheric bacteria has been attributed to the variation in genes responsible for the plant C allocation strategy (Aira, Gómez-Brandón, Lazcano, Bååth, & Domínguez, 2010). In addition, plant photosynthates can be transferred to soil bacteria through ectomycorrhizae (Gorka et al., 2019). In this study, we found that bacterial communities associated with the Mediterranean genotype of *P. pinaster*, which was the most productive in terms of biomass, had an overrepresentation of Proteobacteria that showed low phylogenetic distances to their neighbours. Proteobacteria includes copiotrophic microbes that feed on C sources of varying recalcitrance and outcompete distantly related bacterial lineages (Goldfarb et al., 2011). This ability leads them to dominate C-rich soil environments where they tend to co-exist with close relatives (Goberna, García, et al., 2014; Goberna, Navarro-Cano, Valiente-Banuet, García, & Verdú, 2014). Thus, our results suggest that the productive Mediterranean

genotype would be able to produce more photoassimilates and/or to redirect more in form of root exudates (Farrar, Hawes, Jones, & Lindow, 2003) promoting the proliferation of these competitive bacterial clades.

4.3 | Plant genotype–microbial feedbacks on biogeochemical functioning

The ECM and bacterial phylogenetic structure shaped by the tree genotype allowed predicting enzyme activities targeting different organic compounds. The relative production of enzymatic activities at the community level is supposed to reflect optimum resource allocation in relation to substrate availability and growth requirements (Sinsabaugh et al., 2009). We observed higher peptidase activity ascribed to the dominance of ECM Ascomycetes in the roots of the Atlantic genotype. According to the enzymatic stoichiometry, the enzymatic profile of the Atlantic genotype showed high potential allocation towards N acquisition enzymes probably indicating N limitation. Similarly, an increase in protease activity has been observed with low mineral N availability (Sinsabaugh & Moorhead, 1994). This evidence is supported by our previous results that showed higher differences in soil properties and biogeochemical functioning in bulk soil under the Atlantic genotype compared to the other two genotypes (Pérez-Izquierdo, Saint-André, Santenoise, Buée, & Rincón, 2018). Moreover, the overrepresentation of ECM Ascomycetes under the Atlantic genotype, phylogenetically related to ericoid mycorrhizal fungi, might feedback N retention through the production of melanized hyphae (Clemmensen et al., 2015).

Previous studies have demonstrated that labile C inputs to the soil, such as root exudates, result in a priming effect (Högberg & Ekblad, 1996; Keiluweit et al., 2015) and that high N availability stimulates cellulose degrading enzymes (Chen et al., 2014; Sinsabaugh, Carreiro, & Repert, 2002). Thus, the higher productive Mediterranean genotype that led to an enriched rhizospheric microbiome in Proteobacteria producing hemicellulases and chitinases and in ECM Basidiomycetes with increased cellulolytic activity, give evidence of a more fertile nutrient environment with probably more microbial turnover (Rinnan & Bååth, 2009). Similar to our results, Uroz et al. (2013) reported that the ectomycorrhizosphere of forest trees appeared significantly enriched in Proteobacteria isolates capable of hydrolysing chitin. The production of chitinases among bacteria is ecologically relevant since, apart from pathogenicity (Frederiksen et al., 2013), they are involved in N mobilization from fungal necromass. On the other hand, ECM fungi can also degrade chitin, but with opposite patterns of regulation between fungal families (Maillard, Didion, Fauchery, Bach, & Buée, 2018), which could counteract the effects measured at the phylum level in our study. In turn, our results might indicate that different functional groups of microbes can complement each other with positive effects on plant growth (van der Heijden & Hartmann, 2016).

Overall, these results suggest that genetically based differences across trees might induce changes in nutrient availability and C storage (Clemmensen et al., 2015) as a result of microbial activities that could in turn feedback trees (Pregitzer, Bailey, Hart, & Schweitzer, 2010).

5 | CONCLUSIONS

Our replicated common garden experiments show that beyond the strong local effects that determine the phylogenetic structure of soil microbiota, the plant genotype (even at the intraspecific level) leaves a phylogenetic signature in its rhizospheric microbial partners. These results provide the first evidence that the effects of the plant genotypic variation on the fungal and bacterial communities interacting in their rhizosphere are phylogenetically structured and regulate essential steps of the C and N cycles, and potentially C sequestration. This suggests that plant intraspecific genetic variation has a key ecological relevance in modulating the microbial controls of nutrient cycles. Given the paramount importance of the soil microbiome in nutrient–cycle–climate feedbacks, it seems crucial to incorporate plant intraspecific diversity into models predicting shifts on ecosystem functions under changing climatic scenarios.

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AUTHORS' CONTRIBUTIONS

A.R. and S.C.G.-M. designed the experiment; L.P.-I., M.Z.-A. and A.R. collected the data; M.B. and M.Z.-A. performed the bioinformatics analysis, M.V., M.G. and L.P.-I. performed phylogenetic and statistical analyses; L.P.-I. wrote the first draft of the manuscript, and all authors contributed substantially to revisions.

DATA ACCESSIBILITY

Data were deposited in the Sequence Read Archive (https ://www.ncbi.nlm.nih.gov/biopr oject/PRJNA 324224).

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