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RESEARCH ARTICLE

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Unearthing the soil-borne microbiome of land plants

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Abstract

Plant–soil biodiversity interactions are fundamental for the functioning of terrestrial ecosystems. Yet, the existence of a set of globally distributed topsoil microbial and small invertebrate organisms consistently associated with land plants (i.e., their consistent soil-borne microbiome), together with the environmental preferences and functional capabilities of these organisms, remains unknown. We conducted a standardized field survey under 150 species of land plants, including 58 species of bryophytes and 92 of vascular plants, across 124 locations from all continents. We found that, despite the immense biodiversity of soil organisms, the land plants evaluated only shared a small fraction (less than 1%) of all microbial and invertebrate taxa that were present across contrasting climatic and soil conditions and vegetation types. These consistent taxa were dominated by generalist decomposers and phagotrophs and their presence was positively correlated with the abundance of functional genes linked to mineralization. Finally, we showed that crossing environmental thresholds in aridity (aridity index of 0.65, i.e., the transition from mesic to dry ecosystems), soil pH (5.5; i.e., the transition from acidic to strongly acidic soils), and carbon (less than 2%, the lower limit of fertile soils) can result in drastic disruptions in the associations between land plants and soil organisms, with potential implications for the delivery of soil ecosystem processes under ongoing global environmental change.

KEYWORDS
belowground networks, environmental thresholds, moss microbiome, plant microbiome, plant–soil interactions

For affiliations refer to page 14.

1 | INTRODUCTION

Land plants (i.e., embryophytes, including vascular and non-vascular plants) invariably establish tight interactions with microbial and invertebrate taxa that, together, constitute their associated microbiome (Vandenkoornhuyse et al., 2015). Plant-associated soil-borne organisms such as bacteria, fungi, and small invertebrates have been studied for decades due to their control over economically important processes such as food, feed, and fiber production (Soudzilovskaia et al., 2019; Steidinger et al., 2019; Vandenkoornhuyse et al., 2015). This is particularly true in the case of vascular plants, but less so in the case of non-vascular plants such as bryophytes. These associations can be either beneficial (symbionts and decomposers) or detrimental (pathogens) and, together, determine plant health (van der Putten et al., 2016; Vandenkoornhuyse et al., 2015).

The roots of plants and the fraction of soil influenced by them, particularly the rhizosphere, are a hotspot of plant-microbial interactions (Berendsen et al., 2012). For example, we know that the roots of vascular plants and their associated rhizosphere are frequently colonized by diverse bacterial communities, including members of the phyla Actinomycetota and Proteobacteriota (Lundberg et al., 2012), and that the roots of around 90% of vascular plants are colonized by endo- and ectomycorrhizal fungi belonging to the phyla Ascomycota, Basidiomycota, and Mucoromycota, which contribute to increase the volume of soil explored by plant roots (van der Heijden et al., 2015). Similarly, the surface of mosses is frequently colonized by highly biodiverse microbial communities, including members of the phylum Cyanobacteriota, which are known for their ability to fix atmospheric nitrogen, allowing them to thrive under stressful environmental conditions (DeLuca et al., 2002; Rousk, DeLuca, & Rousk, 2013).

More recently, coordinated studies have demonstrated that climate (Delgado-Baquerizo et al., 2018; Maestre et al., 2015; Tedersoo et al., 2014), vegetation type (Delgado-Baquerizo et al., 2016a; Delgado-Baquerizo et al., 2018), and soil properties such as texture, organic matter, and pH (Delgado-Baquerizo et al., 2016b; Delgado-Baquerizo et al., 2018; Fierer et al., 2009) are major environmental drivers of soil biodiversity patterns at global scale, as well as the role of soil biodiversity for ecosystem functioning and the supply of services (Delgado-Baquerizo et al., 2016b). We also know now that bacterial diversity is maximized in organic soils with neutral pH under more stable climatic conditions (Delgado-Baquerizo et al., 2016a; Fierer & Jackson, 2006), while the diversity of fungal communities peaks in tropical ecosystems and is highly driven by plant community composition (e.g., endomycorrhizal vs ectomycorrhizal trees; Crowther et al., 2019; Tedersoo et al., 2014). The greater diversity of protistan communities is, in turn, more determined by higher precipitations (Oliverio et al., 2023). However, while considerable effort has been recently directed to understanding the diversity, ecology, and association patterns of soil communities, including plant-associated soil-borne communities, across the globe (Delgado-Baquerizo et al., 2018; Tedersoo et al., 2014), we still do not know whether there is set of taxa that are consistently present in topsoils associated with land plants (i.e., their consistent soil-borne microbiome) across contrasting climates, soil conditions, and vegetation types globally, as well as their identity, and functional characteristics.

The identification of a consistent land plant-associated soil-borne microbiome, together with their main environmental preferences and functional capabilities, is important for three main reasons: (i) to better understand and predict the generality of land plant–soil biodiversity associations and belowground networks in a broader ecological and evolutionary context, which may allow us to unravel whether there is a common template defining plant-associated soil ecosystems; (ii) to resolve the importance of such uniquely associated soil-borne taxa to support ecosystem functions, with potential implications for ecological restoration and the intensification of production systems; and (iii) to identify the potential susceptibilities of land plant-associated soil-borne microbiomes to changing environmental conditions (Maestre et al., 2015). For instance, while studies suggest that terrestrial ecosystems can respond to changing environmental conditions in nonlinear ways (Berdugo et al., 2020), we still do not know whether the responses of soil-borne plant-associated communities to natural or human-induced environmental changes will be gradual or abrupt (e.g., threshold-like; Groffman et al., 2006). Given that land plants are highly dependent upon their associations with soil organisms for their nutrition, immunity, and ability to tolerate stressful conditions (Soudzilovskaia et al., 2019), understanding the nonlinear mechanisms underlying land plant–soil microbiome interactions is critical if we are to avoid potentially rapid catastrophic shifts that could threaten important soil processes that support life on Earth.

Here, we conducted a standardized field survey across 124 sampling sites globally distributed (Figure 1; Figures S1 and S2), and analyzed 364 composite soil samples collected underneath coexisting vascular plants, mosses, and unvegetated patches to: (i) investigate the individual soil taxa that are associated with land plants (i.e., vascular plants and/or mosses) across the globe; and (ii) evaluate whether their responses to changing environmental conditions are linear or threshold-like. Topsoil samples were collected from all continents, including Antarctica (Figure 1), covering a wide range of environmental conditions supporting land plants on Earth, from natural ecosystems (71 locations) to urban ecosystems (53 locations). We also considered a wide range of soil properties and vegetation types, including grasslands (29), shrublands (16), and forests (74; Table S1). Our global survey comprises 150 vascular plant species (92) and moss species (58) from 49 families, including a representative range of land plant lineages such as Bryophyta (i.e., mosses), gymnosperms, angiosperm monocots (Poales), and most of the main dicot lineages (Rosids, Caryophyllales, and Asterids; Leebens-Mack et al., 2019; see Table S2 for a complete list of vascular plant and moss species). However, our study did not account for important groups of bryophytes like hornworts (Anthocerotopsida) and liverworts (Marchantiopsida), as well as ferns (Lycophyta and Monilophyta), minoritarian gymnosperm groups like Cycadophyta and Gnetophyta, and early dicots.

Given the more than 400 million of years of independent evolution separating vascular and mosses (Leebens-Mack et al., 2019), and their contrasting lifestyles (e.g., early cf. later succession, rhizoids cf.

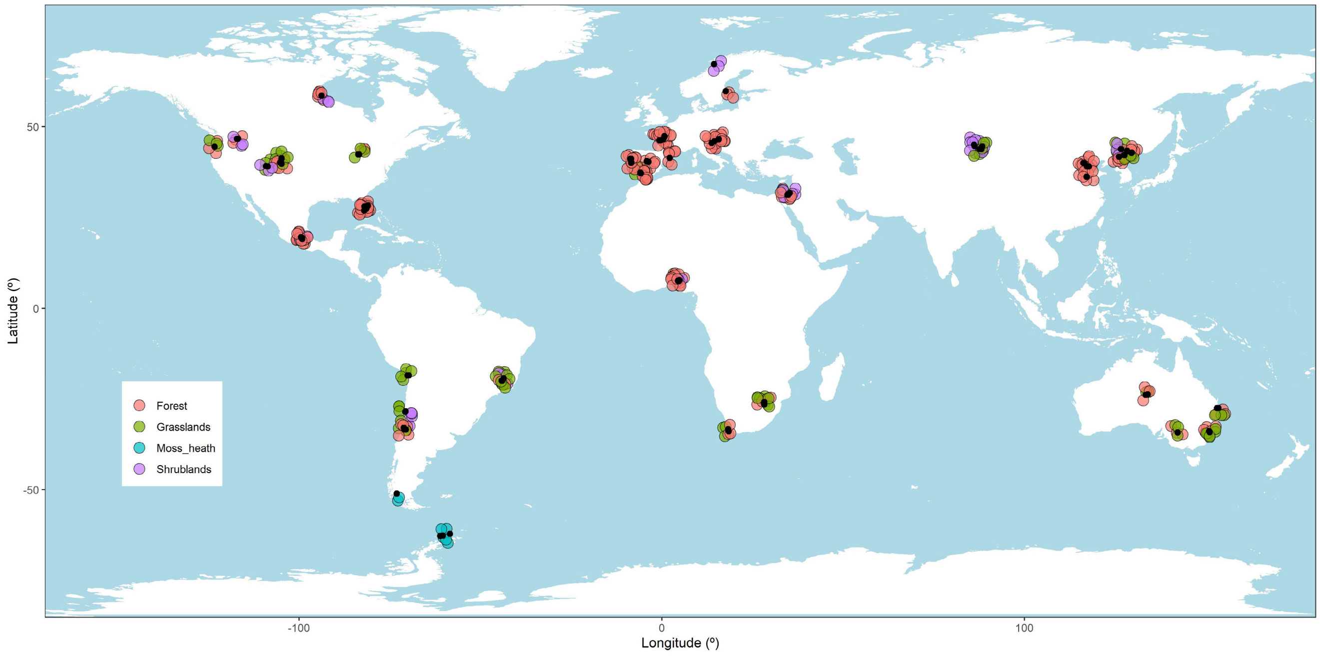

FIGURE 1 Location of the 124 sampling sites and the main vegetation type of each site.

developed root systems), we hypothesized that soils associated with either vascular plants or mosses would have developed their own uniquely associated microbiomes. Moreover, given the importance of environmental thresholds in driving plant communities and ecosystem properties worldwide (Berdugo et al., 2020), we expected that the proportion of land plant microbiomes would change abruptly under certain climatic and soil conditions. Investigating the identity of those soil-borne taxa involved in these thresholds and their sensitivity to abrupt shifts could provide insights into the role of land plant-associated soil-borne microbiomes in controlling drastic vegetation regime shifts (Berdugo et al., 2020; Groffman et al., 2006), helping us to link such shifts with alterations in soil functioning.

2 | MATERIALS AND METHODS

2.1 | Study sites

We collected topsoil samples (ca. 0–5 cm from five soil cores) underneath coexisting vascular plants, mosses, and unvegetated patches across 124 globally distributed ecosystems to test the hypothesis that land plants support unique soil microbiomes and microbial networks across the globe. We focused on surface soils because this layer is typically the most biologically active in terms of plant–soil interactions, microbial biomass and diversity, labile nutrient pools, and C exchange with the atmosphere, and also to allow direct comparison between mosses and vascular plants. A total of 368 soil samples were collected in this study, however, we reduced the analyses to 364 samples due to missing information from four locations. We collected these samples at sites from all continents, including Antarctica (Figure 1), covering a wide range of environmental conditions, from natural to managed ecosystems (e.g., forests and city parks), and from polar to tropical ecosystems. We also considered a wide range of soil properties and vegetation types (grasslands, shrublands, and forests). Soil samples were collected between February 2017 and August 2020, depending on the climatic conditions of each site and the main growing season for mosses (Table S1). Most samples were collected either in summer (41 sites) or autumn (36 sites), and to a lesser extent in winter (29 sites) and spring (18 sites). Despite the likely influence of sampling season and year of sampling on the composition of the soil microbiome, approaches like ours are still considered as highly valuable when evaluating macroecological patterns at large scales (Zhang et al., 2020).

Our global survey comprises vascular plant and moss species from 150 species from 49 families (Table S2). Mean annual rainfall across our locations ranged from 210–1577 mm and temperature 3.1–26.4°C. At each location, we established a 30 × 30 m plot comprising three, equally spaced 30 m transects. Soil samples were collected from within three microsites: (1) underneath the most common perennial vegetation type at each location (generally a tree, shrub, or grass), (2) underneath mosses, and (3) in bare soils, defined as patches devoid of vegetation and not colonized by plant roots. Five composite soil cores were collected from each microsite using a trowel, bulked into plastic Ziplock bags, and further divided into Ziplock bags containing two separate sub-samples; one that was immediately frozen (−20°C) for molecular analyses and another one that was air-dried for chemical analyses. Three sites from Antarctica and one from Chile had samples only from bare and moss surfaces. Each sample from each site was unequivocally assigned to only one species of either vascular plant or bryophyte. Tools were cleaned between microsites to avoid contamination.

2.2 | Soil microbiome

Soil microbial biodiversity (bacteria, protists, and invertebrates) was measured via amplicon sequencing using the Illumina MiSeq platform (Illumina, Inc., CA, USA) in all soils associated with vascular plants, mosses, and bare soils. Once the soils were collected and received in a central lab, soil DNA was extracted using the DNeasy PowerSoil Kit—QIAGEN (Qiagen, Hilden, Germany) according to the manufacturer's instructions. DNA was then shipped to the University of Colorado Boulder where all samples were analyzed using the same standardized protocol. To characterize the community of bacteria, protists and invertebrates, a portion of the prokaryotic 16S (bacteria) and eukaryotic (protists and invertebrates) 18S rRNA genes were sequenced using the 515F/806R and Euk1391f/EukBr primer sets (Herlemann et al., 2011; Ihrmark et al., 2012), respectively. Fungi were determined via 18S-full ITS amplicon sequencing using the primers ITS9mun/ITS4ngsUni and PacBio Sequel II platform in the University of Tartu, Estonia, as described in Tedersoo et al. (2020). The proportion of fungal functional groups was determined from the ITS data using the FUNGuild database (Nguyen et al., 2016). All ASV representative sequences selected in this study are available in Tables S3–S5.

Bioinformatic processing was performed using DADA2 v1.16 (Callahan et al., 2016). Phylotypes (i.e., amplicon sequence variants; ASVs) were identified at the 100% identity level. The ASV abundance tables were rarefied at 5000 (bacteria via 16S rRNA gene), 2000 (protists via 18S rRNA gene) and 250 (invertebrates via 18S rRNA gene) sequences per sample, respectively, to ensure even sampling depth within each belowground group of organisms. Protists are defined as all eukaryotic taxa, except fungi, invertebrates (Metazoa), and Streptophyta. Bioinformatic processing was performed as explained above. Representative sequences of 16S ASVs were annotated against the GTDB database (v214.1). The 18S taxonomy annotation used the Protist Ribosomal Reference database (PR2, https://pr2-database.org/). Taxonomic assignments for ITS ASVs were done using UNITE (https://unite.ut.ee). The fungi ASVs abundance table was rarefied at 1000 sequences per sample.

2.3 | Environmental factors

Solar radiation and climatic information, including mean annual temperature, seasonal temperature, diurnal temperature range, precipitation and precipitation seasonality, were extracted from the WorldClim v2 database (https://www.worldclim.org/data/index.html). Aridity index (AI) was extracted from the Global Aridity Index (Global-Aridity_ET0) datasets. Lower aridity index values indicate more arid sites. Soil pH was measured in all the soil samples with a pH meter in a 1: 2.5 mass:volume soil and water suspension. Sand content was also determined in the lab as done in Kettler et al. (2001). Soil organic carbon content was measured using a CN analyzer (C/N Flash EA 112 Series-Leco Truspec) after removing inorganic carbon.

2.4 | Functional genes

Real-time PCR quantifications of representative genes for nitrogen fixation (*nifH*), nitrogen mineralization (*chiA*), nitrification (*amoA* of ammonia-oxidizing archaea), denitrification (*nosZ*), acid phosphatase production (*phoC*), alkaline phosphatase production (*phoD*), carbon fixation (*cbbL*), fungal ligninase production (Mn-peroxidase), chitinase production (GH18), particulate methane monooxygenase gene (*pmoA*), and sulfur metabolism (*apsA*) were used to estimate the density of functional communities involved in soil nitrogen, phosphorus, carbon, and sulfur cycling by using primers described in Table S6. However, we acknowledge that, despite being among the most commonly abundant functional genes in soils driving the biogeochemical cycling of carbon, nitrogen, phosphorus, and sulfur, the genes analyzed only represent a subset of the existing ones and could, therefore, provide a skewed representation of the metabolic potential of our soils. This is particularly true for nitrifying genes, which are also widely present in bacteria (AOB and comammox; Martikainen, 2022).

All reactions were carried out using SensiFAST SYBR No-ROX (Bioline, USA). Each sample was quantified in duplicate in a 10 µL reaction using the Bio-Rad C1000 Touch thermal cycler CFX96 Real-Time System (Bio-Rad Laboratories, USA). Briefly, all reaction mixtures contained 5 µL of SensiFAST SYBR No-ROX (1X), 0.2 µL of each primer (0.4 µM), 0.2 µL of BSA (0.4 mg/mL) and 2 µL of diluted template DNA (0.5–3.00 ng µL − 1) for gene targets. Results were analyzed using ABI Prism software. Raw data were analyzed using the default settings (threshold = 0.2) of the software. Standard curves for real-time PCR assays were developed by PCR amplifying the respective genes by their specific primers. PCR products were purified using a PCR cleanup kit (Axygen Bioscience, Union City, CA, USA) and cloned into the pGEM-T Easy Vector (Promega Corp.). The resulting ligation mix was transformed into *E. coli* JM109 competent cells (Promega Corp.) following the manufacturer's instructions. Plasmids used as standards for quantitative analyses were extracted from the correct insert clones of each target gene and sent for Sanger sequencing. The plasmid DNA concentration was determined on a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE, USA), and copy numbers of target genes were calculated directly from the concentration of the extracted plasmid DNA. Tenfold serial dilutions (108–101 copies per µL) of the plasmid DNA were subjected to a qPCR assay in triplicate to generate an external standard curve and to check the amplification efficiency. Standard curve regression coefficients were consistently above 0.98 and melt curve analysis verified a single amplicon per reaction in all the cases. Samples and standards were assessed in at least two different runs to confirm reproducibility of the quantification. Target copy numbers for each reaction were calculated from the standard curve and were used to ascertain the number of copies per µg of DNA.

2.5 | Statistical analyses

2.5.1 | Characterizing the soil-borne microbiome of land plants

We used the "multipatt" function from the *indicspecies* package in R (De Cáceres et al., 2012) to identify the soil phylotypes that were uniquely associated with land plants, vascular plants, or mosses, which could occur through their roots/rhizoids, litter, or microhabitats in soils across the globe. The "multipatt" function is based on indicator values for each species, as defined by Dufrêne and Legendre (1997). Indicator analyses are based on the criteria of exclusiveness and abundance (Bakker, 2008). Statistical significance was based on 999 permutations. We also included unvegetated soil samples in these analyses to account for soil phylotypes that are naturally abundant in all soils, but that are not unequivocal indicators of vegetated microhabitats. We then filtered out these results to keep only those taxa that were present in at least three of the seven continents and >50% of climates (tropical, arid, temperate, polar, continental) to identify microbial assemblages which are characteristic of vascular plants and mosses worldwide (we refer to this as the moderate filter). We compared these results against using no filters and against a much stricter filter (taxa present in five continents and four climates). We also used linear mixed effect models to evaluate the effect of soil microhabitat on the relative abundance of bacterial, fungal, protistan, and invertebrate taxa and functional groups. Samples were nested within sites. For this, we use the "lme" function from the *nlme* package in R (Pinheiro et al., 2017). Given the number of variables to be tested, we compared these results after applying a false discovery rate correction based on (Pike, 2011).

We then used linear models to determine whether the mean of the standardized abundance of indicator taxa (mean = 0, standard deviation = 1) differed across plant functional groups (e.g., N-fixers vs. nonfixers) and land uses (e.g., urban vs. nonurban) within vascular plants and mosses separately. Relative abundances were standardized across indicator phylotypes. For this analysis, we used the "lm" function of the *stats* package. After this, we used Spearman correlation analyses to investigate the associations between the mean of the standardized abundances of indicator taxa for land plants and environmental variables, as well as with functional genes associated with the cycling of carbon, nitrogen, phosphorus, and sulfur. For this, we used the "rcorr" function from the *Hmisc* package (Harrell Jr, 2020).

Finally, we used co-occurrence network analyses to investigate associations within the soil-borne land plant microbiome. Co-occurrence was based on Spearman correlations, and only indicator phylotypes based on the moderate filter were considered. For this, we used the "graph_from_adjacency_matrix" and "cluster_walktrap" functions from the *igraph* package in R (Csárdi & Nepusz, 2006). We used null models based on 999 permutations of our dataset to establish the overall connectivity of the networks based on indicator phylotypes. Connectivity was defined as the proportion of highly significant ($p < .001$) links across the network.

2.5.2 | Environmental thresholds

We fitted linear and nonlinear (quadratic and general additive models [GAM]) regressions to the relationships between the relative abundance of consistent land plant microbiomes and selected environmental variables and used the Akaike information criterion (AIC) to select the model that provided the best fit in each case. This criterion penalizes model fit when more parameters (as used in nonlinear regressions) are used, so that the most likely model has the lowest AIC value. In general, differences in AIC higher than 2 indicate that the models are different in terms of likelihood. Thresholds may be present only when nonlinear regressions were a better fit to the data. Additional information regarding the use of thresholds can be found in Appendix S1.

3 | RESULTS

3.1 | Phylotypes comprising the consistent soil-borne land plant-associated microbiome

Across the 124 global locations sampled, we showed that, when no filters were applied, 135, 855, and 362 soil microbial and invertebrate phylotypes were consistently associated with land plants (i.e., mosses and vascular plants together), vascular plants, and mosses, respectively (Figures 2 and 3; Tables S7–S14). More specifically, we found that 116, 724, and 309 bacteria, and 13, 96, and 34 protists, 1, 25, and 7 fungi, and 5, 10, and 12 invertebrates were linked with land plants, vascular plants, and mosses, respectively. These numbers were reduced to 121, 395, and 221 soil microbial and invertebrate phylotypes consistently associated with land plants, vascular plants, and mosses, respectively, when the moderate geographical and climatic filters were applied (Figures 2 and 3; Tables S7–S14). Out of these, 106, 322, and 192 bacteria, and 13, 59, and 20 protists, but only 0, 7, and 2 fungi and 3, 7, and 7 invertebrates were linked with land plants, vascular plants, and mosses, respectively. These soil taxa, based on the moderate filter, accounted for less than one percent of all retrieved soil organisms. Specifically, these soil taxa represented, on average, 0.58%, 0.56%, and 0.43% of all bacterial phylotypes, 0%, 0.08%, and 0.51% of all fungal phylotypes, 0.24%, 0.35%, and 1.86% of all protistan phylotypes, and 1.18%, 1.50%, and 0.41% of all invertebrate phylotypes found in land plant-associated, vascular plant-associated, and moss-associated soils, respectively. When the strictest geographical and climatic filters were applied, these numbers were further reduced to only 50 land plant-associated phylotypes (45 bacteria and 5 protists), 66 vascular plant-associated phylotypes (53 bacteria, 12 protists, and 1 fungus), and 47 moss-associated phylotypes (40 bacteria, 6 protists, and 1 invertebrate). From here onwards, we will describe only the results obtained after applying the moderate filtering.

The consistent soil-borne bacterial microbiome of land plants was dominated by Alphaproteobacteria (20%), Planctomycetota (13%), Actinomycetota (13%), Verrucomicrobiota (12%), and Bacteroidota

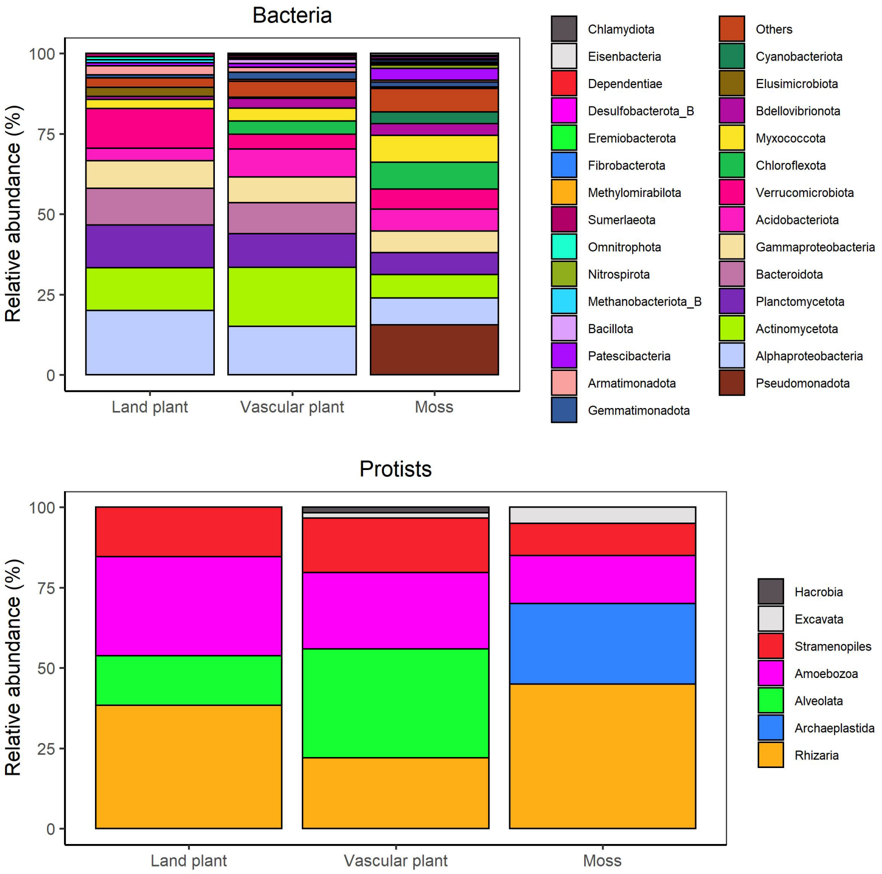

FIGURE 2 Relative abundance of major bacterial phyla and protistan supergroups belonging to the consistent microbiome of land plants (mosses + vascular plants), vascular plants, and mosses. Fungi and small invertebrates are not shown due to the low number of phylotypes selected.

(11%; Figure 2; Table S7). The consistent soil-borne protistan microbiome of land plants was dominated by Rhizaria (38%) and Amoebozoa (31%; Figure 2; Table S8). The consistent soil-borne bacterial microbiome of vascular plants was dominated by phylotypes belonging to six main taxonomic groups comprising 73% of all phylotypes: (i) Actinomycetota (18%), (ii) Alphaproteobacteria (15%), (iii) Planctomycetota (11%), (iv) Bacteroidota (10%), (v) Acidobacteriota (9%), and (vi) Gammaproteobacteria (8%; Figure 2; Table S9). Many of these taxa included nonsporulating aerobic chemoheterotrophs. The consistent vascular plant-associated microbiome was also particularly enriched in members of the family Rhizobiaceae. The consistent vascular plant-fungal microbiome was dominated by members of the Dothideomycetes family (43%) and was enriched in saprotrophic fungi, while the consistent protistan microbiome was overwhelmingly dominated by phagotrophic taxa belonging to the supergroups Alveolata (34%), Amoebozoa (24%), and Rhizaria (22%; Tables S10–S11). The consistent soil-borne bacterial microbiome of mosses was dominated by Pseudomonadota (16%), Chloroflexota (8%), and Myxococcota (8%; Figure 2; Table S12). The consistent moss-associated microbiome was also particularly enriched in Cyanobacteria (4%), as compared with vascular plants. The consistent moss protistan microbiome was dominated by phagotrophic Rhizaria (45%) and, to a lesser extent, phototrophic Archeplastida (i.e., green algae; 25%) and Amoebozoa (15%; Figure 2; Table S13).

The proportion of the consistent soil-borne vascular plant microbiome was maintained regardless of the taxonomic category (i.e., family) of vascular plants ($p = .53$) or their functional types, including mycorrhizal types ($p = .77$) and N-fixers ($p = .37$), and across climatic zones ($p = .43$) and land use types (i.e., urban vs. natural ecosystem; $p = .13$; Figure 3; Figure S3). The proportion of the consistent soil-borne microbiome of mosses was also maintained regardless of family ($p = .24$), growth type ($p = .24$), and land use types ($p = .40$; Figure S4). In contrast, ephemeral mosses, as compared with perennial mosses, were associated with a more developed consistent moss-associated microbiome ($p < .01$; Figure S4). Our analyses further revealed that the consistent soil-borne land plant-associated microbiome is organized into well-defined belowground networks, with greater connections among individual taxa than expected by chance (Figures 4–6). We only found positive relationships ($p < .001$) among soil taxa within these land plant–soil networks. Nodes from these networks could be further grouped into seventeen, eighteen, and twenty-four major clusters, in the case of land plants, vascular plants, and mosses, respectively (Tables S7–S14).

3.2 | Main groups of organisms associated with the soil-borne microbiome of plants

At higher taxonomic levels (phylum, class, order, family, and genus), we found that, across the 124 plots and seven continents sampled, soils associated with vascular plants were enriched in phylotypes

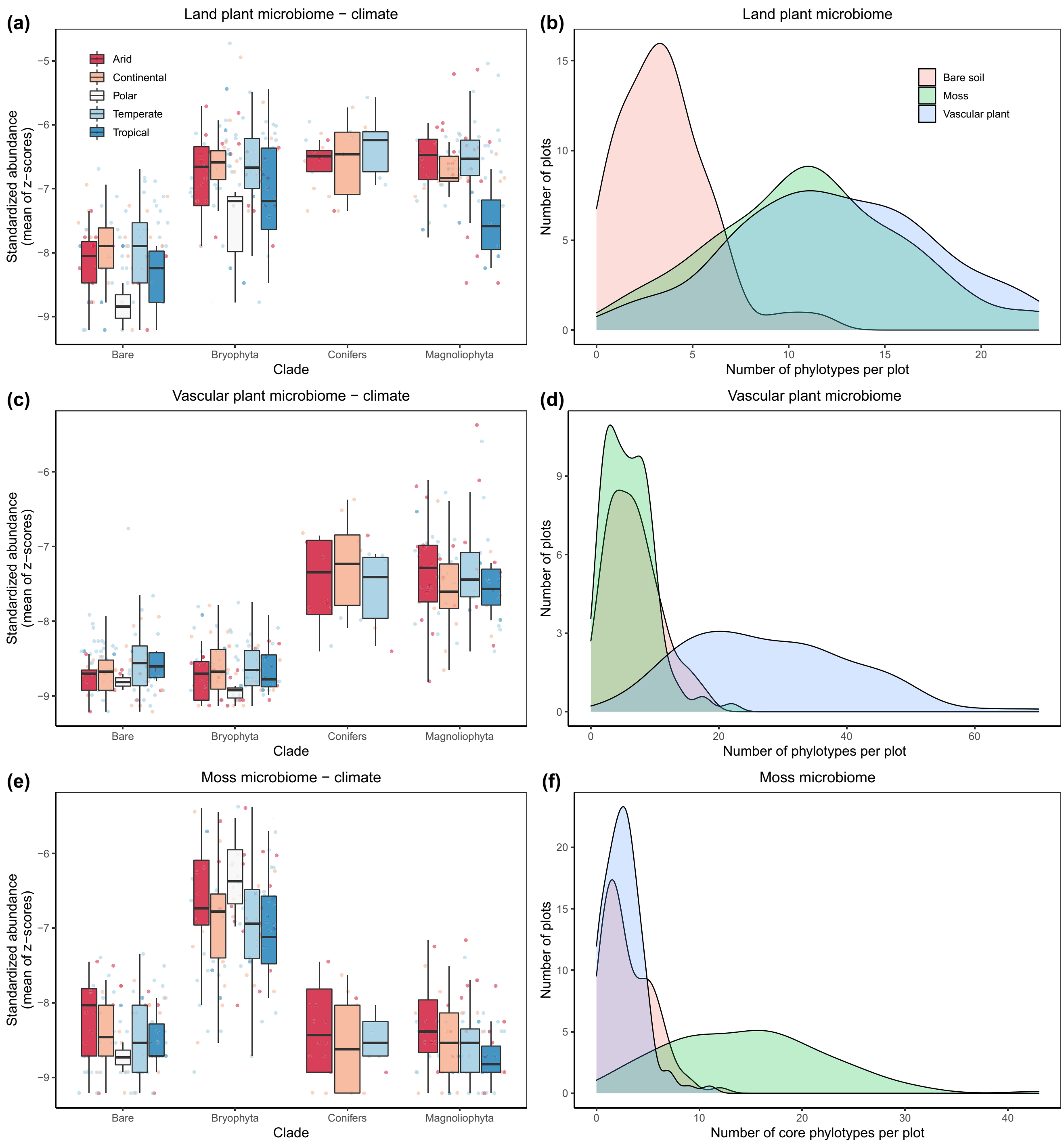

FIGURE 3 Standardized relative abundance (i.e., mean of z-scores) of bacterial and eukaryotic phylotypes that are identified as part of the consistent (a, b) land plant-associated, (c, d) vascular plant-associated and (e, f) moss-associated microbiomes as a function of climate type. *p*-values associated with microhabitat type (i.e., bare, moss, and vascular plant) are <0.001 in all cases.

belonging to one phylum, Pseudomonadota, one class, Actinomycetia, eight orders, including Planctomycetales, Streptomycetales, and Verrucomicrobiales, and twenty-three families, including Akkermansiaceae, Burkholderiaceae, Nitrosomonadaceae, the N-fixing Rhizobiaceae, and Streptomycetaceae (Figure S5 and Table S15). Soils associated with vascular plants were also characterized by a greater proportion of fungal saprotrophs and members of the free-living nematode genus *Panagrolaimus* (Figure S5 and Table S15). Soils associated with mosses had a greater proportion of one bacterial phylum (Armatimonadota), three classes (Anaerolineae [Chloroflexota], Fimbriimonadia [Armatimonadota], and Thermoanaerobaculia [Acidobacteriota]), and five orders, including Aggregatilineales [Chloroflexota], Fimbriimonadales, and Rickettsiales [Pseudomonadota], than surrounding plant-associated soils and bare soils (Figure S6 and Table S15). Moss-associated soils were also enriched in eight known bacterial genera, including *Paenibacillus* and *Spirosoma* (Figure S6 and Table S15). Finally, moss-associated soils had a greater proportion of one fungal class (Pucciniomycetes), one family (Hygrophoraceae), and two genera (*Hygrocybe* and *Lamprospora*), as well as two families (Prasiolales and Ctenocladales), and four genera of green algae (*Chloroidium*, *Diplosphaera*, *Leptosira*, and *Stichococcus*; Figure S6 and Table S15).

3.3 | Linking the consistent soil-borne microbiome of land plants and soil functioning

Next, we sought to deepen into the prospective functional capabilities of the reported plant-associated soil-borne microbiomes. We found that the proportion of soil-borne land plant microbiomes was significantly correlated with the total abundance of functional genes associated with the biogeochemical cycling of carbon, nitrogen, phosphorus, and sulfur in terrestrial ecosystems, as measured using quantitative PCR (Figure 7). Specifically, the proportion of the soil-borne land plant microbiome (i.e.,

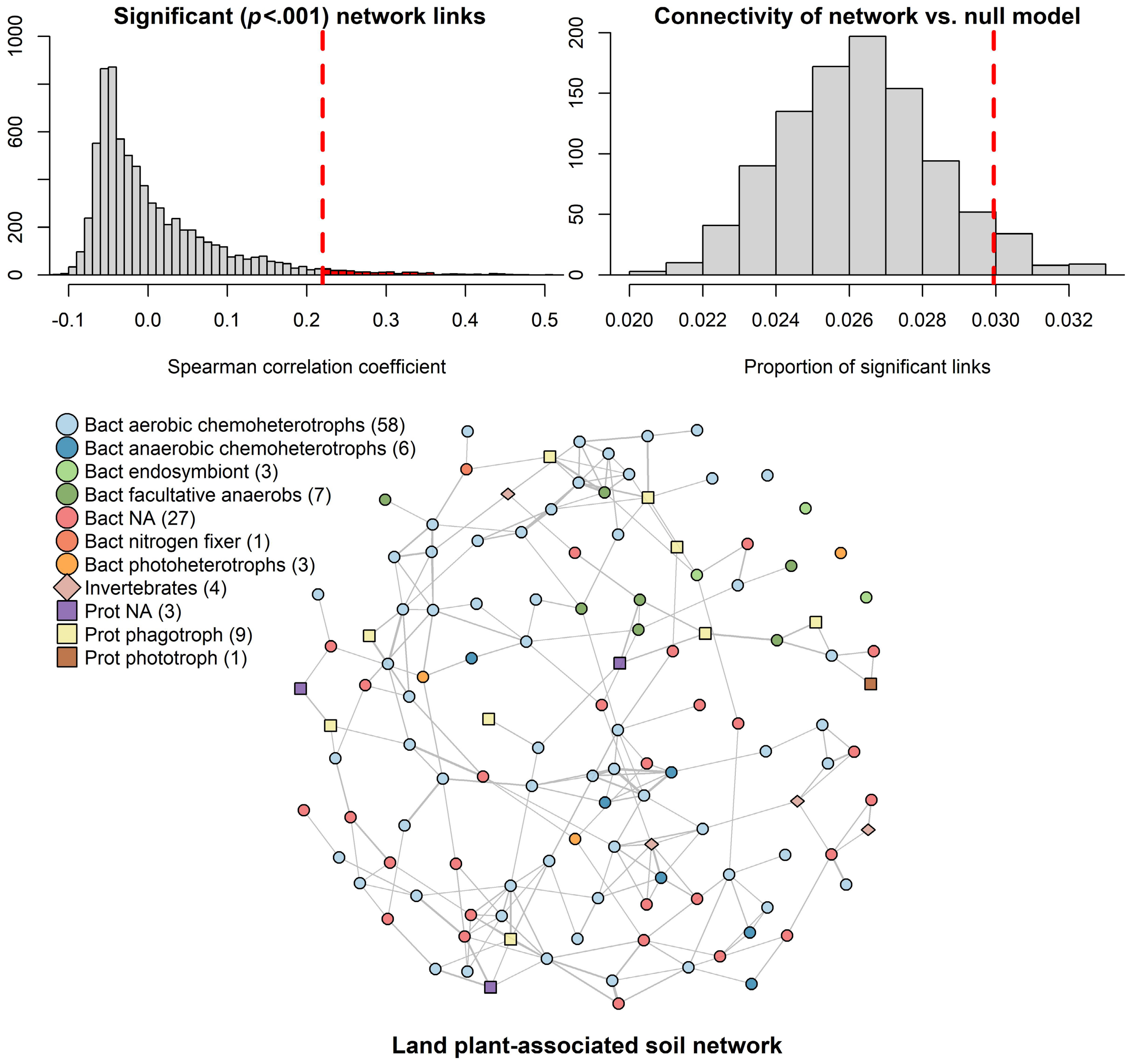

FIGURE 4 Microbial co-occurrence networks associated with land plants. Information regarding the lifestyle of phylotypes is also included. This information was retrieved from publications (see Table S7 for references). For each network, the histograms on the left shows the proportion of links that were significant at $p < .001$ (from the red vertical line to the right) based on the total possible links, while the histograms on the right shows the connectivity of the networks, based on the proportion of significant links (red vertical line), and the distribution of a null network ($n = 999$) based on the randomization of standardized abundances.

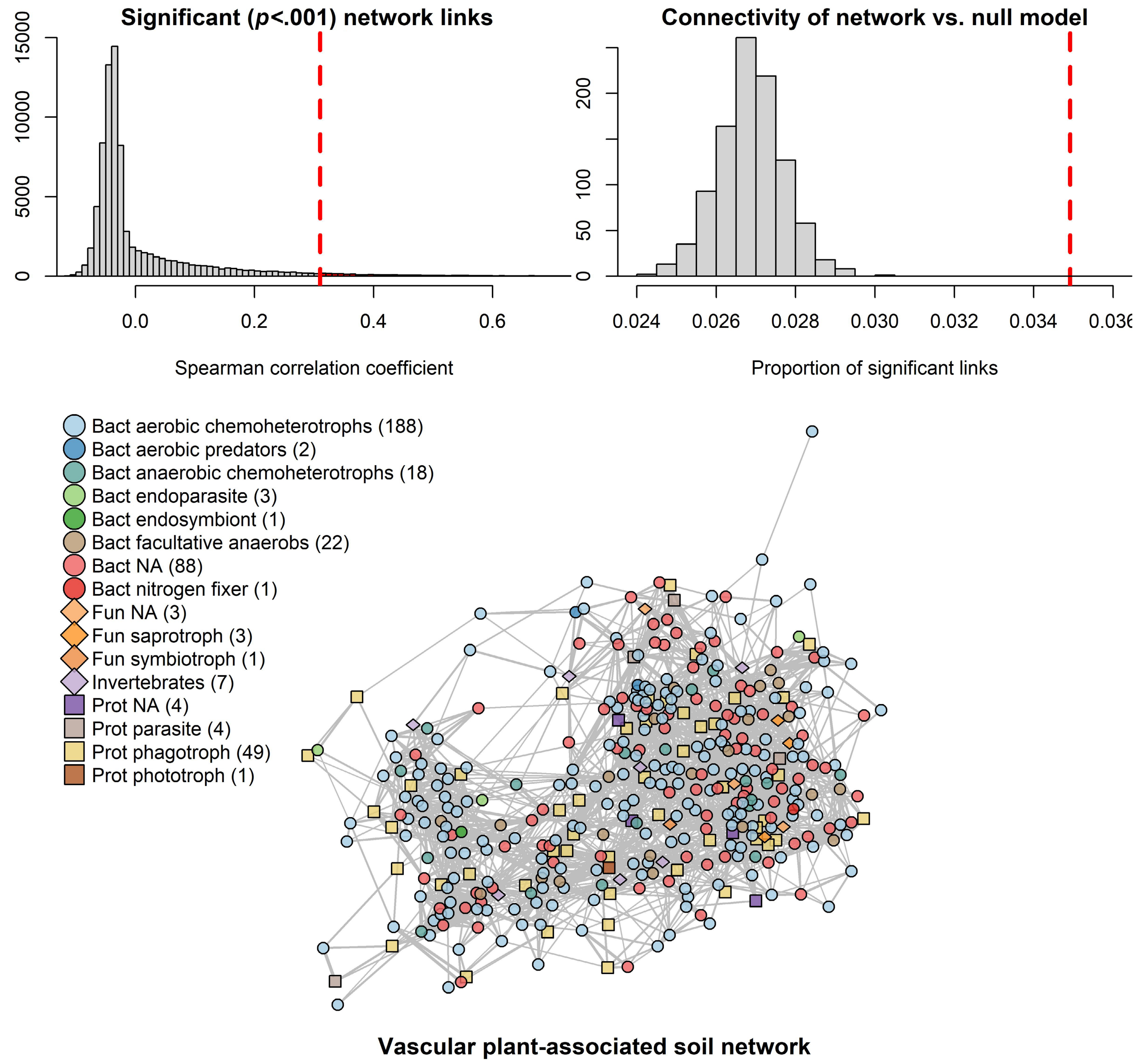

FIGURE 5 Microbial co-occurrence networks associated with vascular plants. Information regarding the lifestyle of phylotypes is also included. This information was retrieved from publications (see Table S9 for references). For each network, the histograms on the left shows the proportion of links that were significant at $p < .001$ (from the red vertical line to the right) based on the total possible links, while the histograms on the right shows the connectivity of the networks, based on the proportion of significant links (red vertical line), and the distribution of a null network ($n = 999$) based on the randomization of standardized abundances.

when both vascular plants and mosses were considered simultaneously) was positively related to the abundance of *amoA* genes (Spearman's $\rho = 0.17$, $p = .02$), involved in the oxidation of nitrite to nitrate (i.e., linked with nitrogen cycling), and negatively to genes linked to methanotrophy (*pmoA*; Spearman's $\rho = -0.19$, $p < .01$), and denitrification (*nosZ*; Spearman's $\rho = -0.15$, $p = .03$). The soil-borne microbiome of vascular plants was positively associated with genes involved in ammonium oxidation (Spearman's $\rho = 0.20$, $p < .001$). The proportion of the soil-borne microbiome of mosses was positively correlated with the absolute abundance of genes associated with phosphatase (*phoD*; Spearman's $\rho = 0.20$, $p = .04$), and sulphatase activities (*apsA*; Spearman's $\rho = 0.26$, $p = .01$), as well as with methanotrophy (Spearman's $\rho = 0.23$, $p = .03$), that is, functions linked with the metabolism of phosphorus, sulfur, and carbon.

3.4 | Role of environmental thresholds in driving the consistent soil-borne microbiome of land plants

Finally, we aimed to quantify the potential sensitivity of soil-borne land plant microbiomes to changes in environmental conditions.

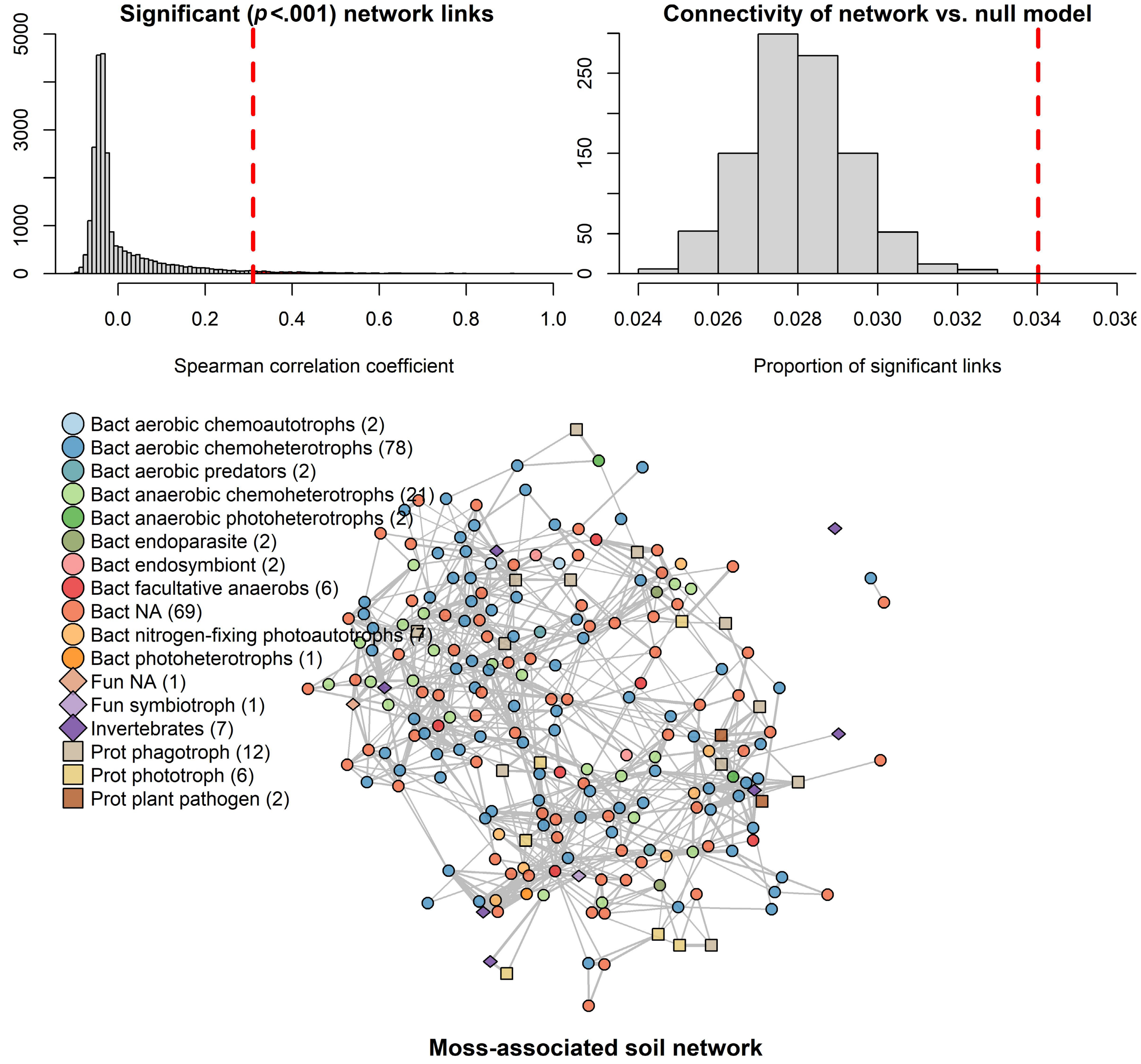

FIGURE 6 Microbial co-occurrence networks associated with mosses. Information regarding the lifestyle of phylotypes is also included. This information was retrieved from publications (see Table S12 for references). For each network, the histograms on the left shows the proportion of links that were significant at $p < .001$ (from the red vertical line to the right) based on the total possible links, while the histograms on the right shows the connectivity of the networks, based on the proportion of significant links (red vertical line), and the distribution of a null network ($n = 999$) based on the randomization of standardized abundances.

We found that the proportion of the soil-borne microbiomes of land plants, vascular plants, and mosses varied across different environments (Figure 8; Table S16). For example, we showed that the soil-borne microbiome of vascular plants declined abruptly in environments where soil carbon was less than ~2% (Figure 8c; Table S16). For mosses, their microbiome harbored a greater relative abundance of associated species in soils with pH values exceeding 5.5, the threshold between slightly acidic and highly acidic soils, and was abruptly more abundant under conditions of greater aridity (from AI ca. 0.65, the transition zone between mesic and dryland ecosystems; Figure 5e,f; Table S16).

4 | DISCUSSION

Our study provides novel evidence that, despite the enormous biodiversity of soils, land plants from a wide range of families only share a few hundred soil microbial and invertebrate species. These taxa may, thus, support the universal complex belowground networks thriving under plants across contrasting climates, management regimes, and vegetation types. Based on the greater number of indicator species, we posit that bacteria, as compared to fungi and protists, dominate the consistent soil-borne microbiome of lands plants. This most likely indicates that land plant-bacterial associations across contrasting land

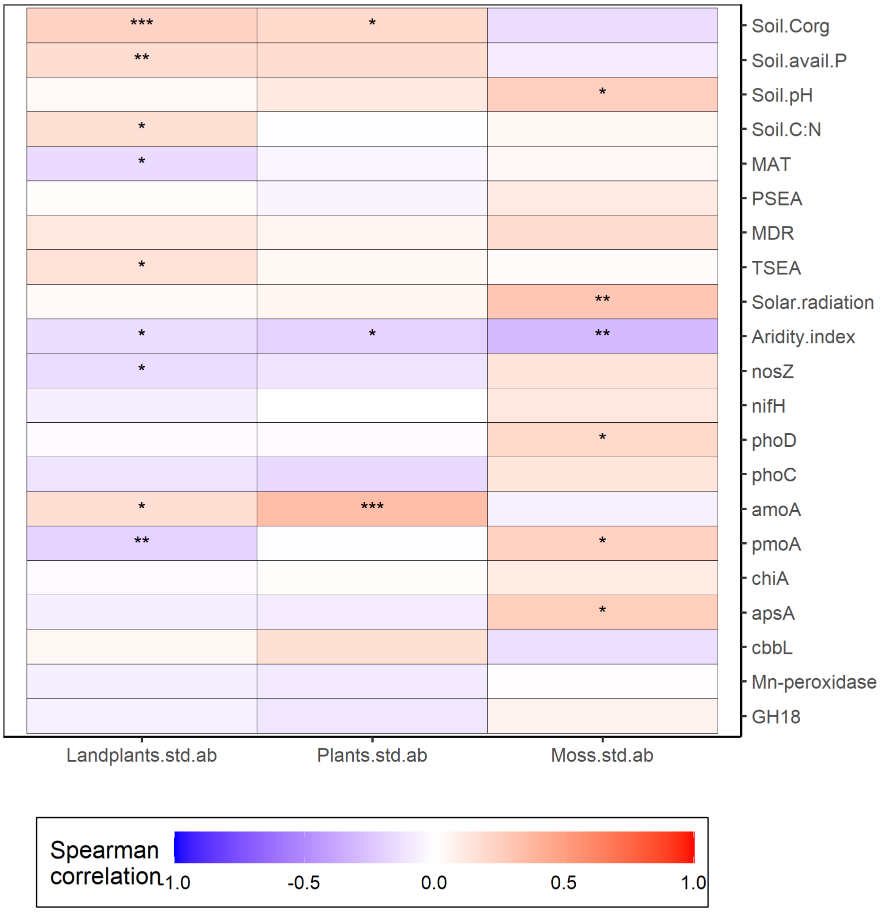

FIGURE 7 Relationships between the standardized relative abundance of phylotypes belonging to the land plant–soil microbiomes with environmental variables and microbial functions. MAT, mean annual temperature. PSEA/TSEA, seasonality of temperature and precipitation. MDR, mean diurnal T range. Microbial functions are based on real-time PCR quantifications of genes for nitrogen fixation (*nifH*), nitrogen mineralization (*chiA*), nitrification (*amoA* of ammonia-oxidizing archaea), denitrification (*nosZ*), acid phosphatase production (*phoC*), alkaline phosphatase production (*phoD*), carbon fixation (*cbbL*), fungal ligninase production (Mn-peroxidase), chitinase production (GH18), particulate methane monooxygenase gene (*pmoA*), and sulfur metabolism (*apsA*). *$p<.05$; **$p<.01$; ***$p<.001$.

plant species and environmental conditions are more generalist than land plant-fungal associations, which are highly host specific (Maciá-Vicente & Popa, 2022). The greater number of bacterial members of the consistent soil-borne microbiome of land plants could also be due to the fact that bacterial phylotypes disperse more easily across the globe than protists and fungi do (Egidi et al., 2019). Moreover, these soil taxa accounted for less than one percent of all retrieved soil organisms, which is in agreement with the percentage of soil bacteria shared across biomes within the Americas (Lauber et al., 2009). Taken together, our results provide unprecedented evidence of the existence of a reduced group of individual soil taxa, predominantly bacteria, that are consistently associated with land plants worldwide.

4.1 | Characterizing the soil-borne microbiome of land plants

The consistent plant-associated microbiome was enriched in certain microbial groups. For example, the consistent vascular plant-associated bacterial microbiome included nonsporulating aerobic chemoheterotrophs and was particularly enriched in members of the family Rhizobiaceae, which includes important nitrogen fixers (Carareto Alves et al., 2014). This result was also supported by a greater relative abundance of members of the Rhizobiaceae family in vascular plant-associated soils. In particular, we found one phylotype of *Pararhizobium* that was consistently present across locations, making it a good candidate for a generalist nitrogen fixing species. Burkholderiaceae, commonly found in disease-suppressive plant-associated soils (Carrión et al., 2018), were also particularly overrepresented in the consistent microbiome of vascular plants. In contrast, the role of Akkermansiaceae in plant-associated microbiomes is less known, despite their importance for mucin degradation and anti-inflammatory control in the human gut (González et al., 2023). Moreover, the greater relative abundance of Actinomycetota in vascular plant-associated soils is in agreement with previous studies evaluating the composition of bacterial communities from rhizosphere soils (Lundberg et al., 2012), indicating the important role of plant roots in determining the composition of the soil bacterial microbiome of vascular plants. Actinomycetota are well-known for their ability to synthesize enzymes, phytohormones, growth factors, and vitamins that are critical for the adequate development, growth, and immunity of plants (Narsing Rao et al., 2022). Moreover, bacterial groups consistently associated with vascular plants and mosses such as Proteobacteriota, Myxococcota, and Bacteroidota are also dominant in association with animals, where they are critical regulators of host health, indicating that these may be ubiquitous members of a consistent microbiome across biological domains and kingdoms (Song et al., 2020; Strandwitz et al., 2019). The consistent vascular plant-fungal microbiome was dominated by saprotrophic fungi (i.e., decomposers), while the protistan microbiome was dominated by

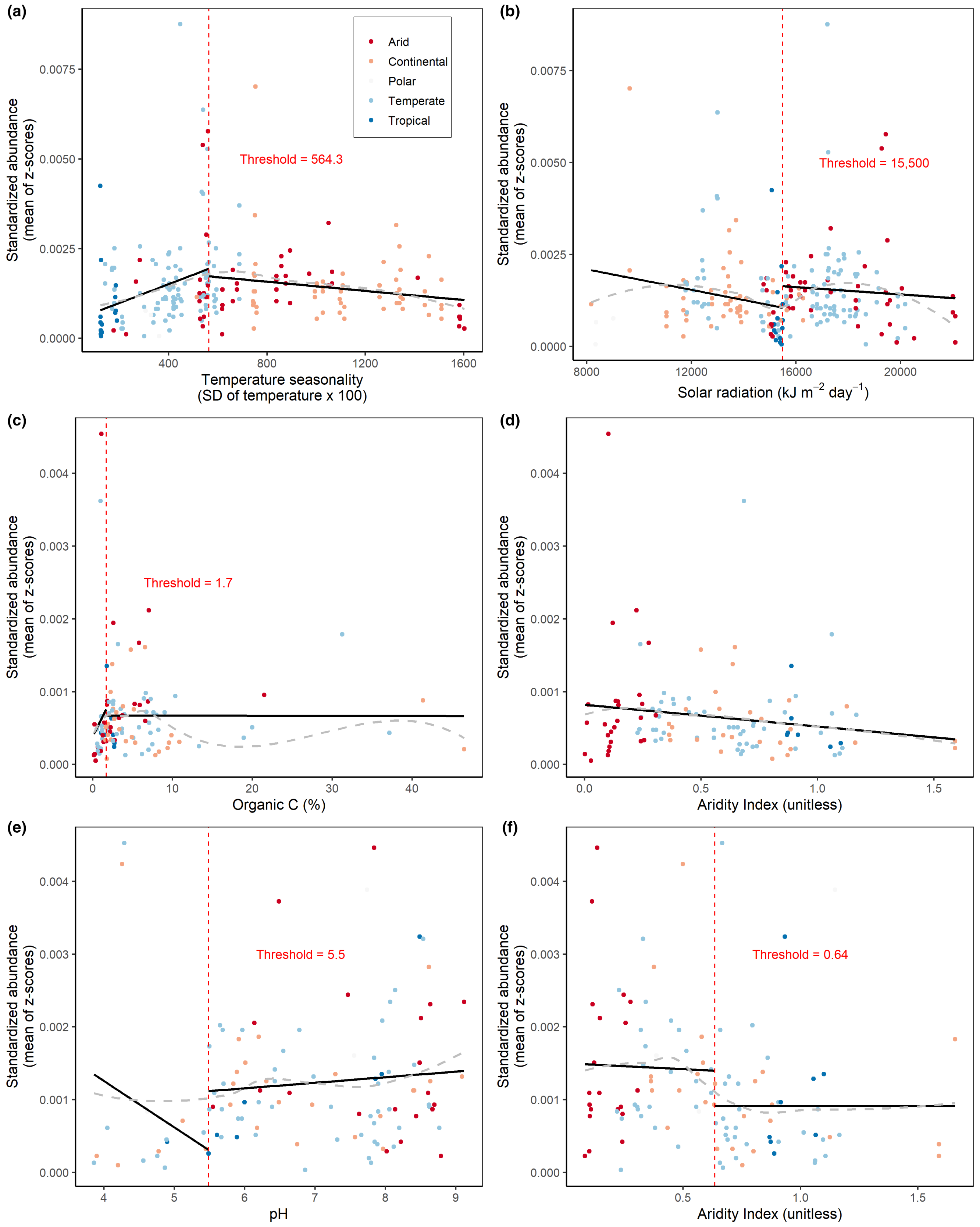

FIGURE 8 Environmental thresholds and linear associations driving the microbiomes that were uniquely associated with (a, b) land plants, (c, d) vascular plants, and (e, f) mosses. Thresholds are shown when the AIC of the segmented model is lower than that of the linear model (see Methods). Relationships in (a) and (c) are representative of continuous thresholds, while relationships in (b), (e), and (f) are representative of discontinuous thresholds. Lower aridity index values indicate more arid sites. *$p < .05$; **$p < .01$; ***$p < .001$.

phagotrophic taxa. The importance of saprotrophs in soils associated with vascular plants is also supported by the greater relative abundance of this functional group, which is likely driven by greater plant litter inputs, both above- and belowground, and rhizodeposits in this microhabitat.

Overall, these results suggest that land plant-associated soil networks are dominated by organisms that are potentially able to synthesize beneficial compounds for plants such as Actinomycetota, as well as by generalist bacterial and fungal decomposers, and phagotrophs. We hypothesize that these organisms may be part of universal complex soil food webs and ecological networks thriving under contrasting land plant species across different climatic, soil, and vegetation conditions. Importantly, we only found positive relationships among soil taxa within these land plant–soil networks, suggesting that the associations within these networks are mostly synergistic (Jared et al., 2021). However, the fact that nodes from these networks could be further grouped into major clusters suggests that some soil phylotypes are more likely to co-exist than others. Moreover, the fact that individual plants had a mean of 10–15 (land plants and mosses) or 20–30 (vascular plants) of these organisms implies that not all phylotypes may need to be present simultaneously to determine plant health.

In contrast to vascular plants, the consistent moss-associated microbiome was particularly enriched in Cyanobacteriota, including from the genera *Nostoc*, *Tolypothrix*, and *Brasilonema*, suggesting the widespread prevalence of moss-cyanobacterial associations (Rousk, Jones, & DeLuca, 2013), which are beneficial in providing reduced mineral nitrogen in otherwise low-nitrogen soils from high-latitude, dryland, and tropical ecosystems (Arróniz-Crespo et al., 2014; DeLuca et al., 2002; Ochoa-Hueso & Manrique, 2013; Rousk, Jones, & DeLuca, 2013). The moss-associated soil-borne microbiome was also enriched in green algae, particularly from the family Trebouxiophyceae, which are known for their ability to form stable symbiotic relationships with other organisms, such as with fungi in lichens (Muggia et al., 2020). The fact that ephemeral mosses, as compared with perennial mosses, were associated with a more developed consistent moss-associated microbiome may, in turn, be linked to their important role in driving the initial stages of primary and secondary succession, during which cyanobacteria and green algae are also known to play an important role (Vilmundardóttir et al., 2018).

Interestingly, soils associated with mosses were also enriched in two known bacterial genera, *Paenibacillus* and *Spirosoma*, both previously isolated from mosses and biocrusts, and widely known for their great potential in bioremediation (Hassan & Ganai, 2023; Yang et al., 2016; Zhang et al., 2019; Zhou et al., 2015). This suggests the role of bryophytes as an untapped source of microbial metabolites with biotechnological applications. In addition, the overrepresentation of plant pathogens such as rusts (Pucciniomycetes; Zhu et al., 2017) and *Phytium* (Oomycota; Martin & Loper, 1999), may mean that mosses may serve as a reservoir for plant pathogenic agents, which may be also associated with an evolutionary strategy of mosses to use fungi and pseudofungi as biological weapons to compete with vascular plants (Lehtonen et al., 2012). Actually, bryophytes are well-known for being barely infected by fungi and for harboring a biodiverse assembly of bacterial taxa with abilities to produce antifungal agents that help maintain fungal growth at bay, thus keeping bryophytes protected (Opelt et al., 2007). Moreover, this is, to our knowledge, the first time that the bacterial phylum Armatimonadota has been identified as an indicator of moss-associated soils. Considering the unknown role of Armatimonadota in soils, we suggest that understanding their function in soils may be particularly relevant to untap the biotechnological potential of mosses such as in ecosystem restoration projects.

4.2 | Linking the consistent soil-borne microbiome of land plants and soil functioning

The consistent soil-borne microbiome of land plants was linked with the absolute abundance of genes linked to soil metabolism. For example, the positive association between the proportion of the soil-borne microbiome of mosses and the abundance of genes associated with functions linked with phosphorus, sulfur, and carbon metabolism, may be highly indicative of stress resilience (Graham et al., 2017). This could account for their preeminence in primary successional processes during landscape development (Vilmundardóttir et al., 2018). In contrast, the tight association between the consistent soil-borne microbiome of vascular plants and greater abundance of genes linked with nitrogen cycling is consistent with the reported dominance of decomposers and nitrogen fixers. Thus, our findings provide not only an inventory of soil organisms that are consistently and uniquely associated with the soil underneath land plants, but also improve our understanding of how soil organisms and land plants can interact, and the implications of such interactions for soil functioning. This information also lays the foundation for future work aimed at investigating the functional links between land plants and soil organisms and their shared ecological history. This is further relevant to investigating the connection between the consistent land plant-associated soil microbiome and ecosystem services such as nutrient cycling, carbon sequestration, plant immune defense, and food production.

4.3 | Role of environmental thresholds in driving the consistent soil-borne microbiome of land plants

Quantifying the potential sensitivity of the soil-borne land plant microbiome to changes in environmental conditions may be fundamental to forecast potential disruptions in the soil-borne microbiome of land plants, with undescribed consequences for plant productivity and health worldwide. In the case of mosses, their microbiome was abruptly more abundant under conditions of greater aridity, starting at AI of ca. 0.65, a zone that differentiates drylands from mesic ecosystems (Huang et al., 2016), which likely indicates the importance of the consistent soil-borne microbiome of mosses to tolerate harsh environmental conditions. In contrast, the consistent soil-borne microbiome of vascular plants declined abruptly in environments where soil carbon was less than ~2%, the lower threshold of optimal plant growth (Oldfield et al., 2019; Patrick et al., 2013). Taken together, our results highlight the widespread existence of environmental thresholds governing land plant–soil biodiversity associations, indicating the potential vulnerability of land plant-microbial associations under ongoing and future global change (Cheng et al., 2019). This knowledge could be used to further improve our understanding on how to restore functional terrestrial ecosystems by reinstating the uniquely associated soil-borne microbiome of land plants to hasten environmental recovery. Our results also indicate that environmental changes associated with ecosystem succession, management practices, changes in soil fertility, and increases in aridity, could have important implications for the maintenance of functional land plant-microbial associations. The greater abundance of moss-associated soil taxa under harsh environmental conditions also suggests a strong coevolution between moss-like early embryophytes and soil microbes that may have been key to the eventual colonization of terrestrial environments by land plants more than 470 million years ago (Graham et al., 2017; Humphreys et al., 2010).

4.4 | Conclusions

In summary, we provide solid evidence, from a global field survey, that despite the incalculable biodiversity of soils, land plants only share a small fraction (around or less than 1%) of multi-kingdom soil-borne bacterial, fungal, protistan, and invertebrate taxa. These taxa are, however, consistently present in land plants across contrasting climates, vegetation types, and management types, correlate with important ecosystem functions, and may constitute the foundational organisms of belowground networks thriving in topsoils under land plants worldwide. We also show that despite their global prevalence, the consistent soil-borne microbiome of land plants may be highly vulnerable to environmental changes due to nonlinear responses to increases in aridity and changes in soil pH (e.g., due to acidification), and carbon content. Our findings are integral to better understanding the identity and vulnerability of the essential land plant–soil biodiversity interactions that maintain life on planet Earth.

AUTHOR CONTRIBUTIONS

Raúl Ochoa Hueso: Conceptualization; formal analysis; investigation; methodology; visualization; writing – original draft; writing – review and editing. **David J. Eldridge:** Conceptualization; data curation; investigation; methodology; writing – review and editing. **Miguel Berdugo:** Formal analysis; investigation; writing – review and editing. **Pankaj Trivedi:** Formal analysis; investigation; writing – review and editing. **Blessing Sokoya:** Formal analysis; investigation; writing – review and editing. **Concha Cano-Díaz:** Formal analysis; writing – review and editing. **Sebastian Abades:** Investigation; writing – review and editing. **Fernando Alfaro:** Investigation; writing – review and editing. **Adebola R. Bamigboye:** Investigation; writing – review and editing. **Felipe Bastida:** Investigation; writing – review and editing. **José L. Blanco-Pastor:** Investigation; writing – review and editing. **Asunción de los Rios:** Investigation; writing – review and editing. **Jorge Durán:** Investigation; writing – review and editing. **Stefan Geisen:** Investigation; writing – review and editing. **Tine Grebenc:** Investigation; writing – review and editing. **Yu-Rong Liu:** Investigation; writing – review and editing. **Steven Mamet:** Investigation; writing – review and editing. **Ana Rey:** Investigation; writing – review and editing. **Leho Tedersoo:** Investigation; writing – review and editing. **Manuel Delgado-Baquerizo:** Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; resources; writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data from this manuscript is available at 10.6084/m9.figshare.25254673.

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