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L. Merino-Martín (https://orcid.org/0000-0002-0343-0546) ✉ (luis.merino.martin@urjc.es), Depto de Biología y Geología, Física y Química inorgánica, ESCET, Univ. Rey Juan Carlos, Madrid, Spain and CEFE, Univ. Montpellier, CNRS, EPHE, IRD, Univ. Paul Valéry Montpellier 3, Montpellier, France. – D. Hernández-Cáceres (https://orcid.org/0000-0002-9459-4940), G. Zhang and A. Stokes (https://orcid.org/0000-0002-2276-0911), Univ. Montpellier, AMAP, INRAE, CIRAD, CNRS, IRD, Montpellier, France. – F. Reverchon, Red de Estudios Moleculares Avanzados, Inst. de Ecología, A.C., Pátzcuaro, Michoacán, México. – G. Angeles-Alvarez, Red de Ecología Funcional, Inst. de Ecología, A.C., Xalapa, Veracruz, México. – D. Dunoyer de Segonzac, ADNid, Montferrier sur Lez, France. – D. Dezette, Eco&Sols, Univ. Montpellier, CIRAD, INRAE, IRD, Montpellier SupAgro, Montpellier, France.

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Within a landscape, multiple habitats exist for soil microbial communities. But how these habitats shape community composition requires an understanding of the way in which microbial diversity is impacted across a broad range of spatial scales. Mountain ecosystems are excellent systems to study microbial communities, because a multitude of climate and soil variables change within a relatively small distance. We investigated microbial community structure in bulk and rhizosphere soils beneath three plant species, *Vaccinium myrtillus*, *Juniperus communis* and *Picea abies*, that structure local plant communities along an elevation gradient in the French Alps. We examined the impact that climate, soil properties, plant diversity and plant root chemical and morphological traits had on microbial α- and β-diversities. The most abundant bacterial phyla detected in both bulk and rhizosphere soils were Proteobacteria, Actinobacteria, Acidobacteria and Verrucomicrobia. Along the elevation gradient, bacterial phyla did not display a clear distribution pattern between bulk and rhizosphere soils. For fungi, dominant phyla were Ascomycota and Basidiomycota, and contrasting distribution patterns were found between bulk and rhizosphere soils. Overall, bacterial and fungal α-diversity responded differently to elevation as well to soil compartments (bulk versus rhizosphere soil), revealing no significant patterns in bulk soil beneath any of the structuring plant species, but increasing in the rhizosphere compartment of *P. abies* just below the treeline. Changes in bacterial β-diversity with elevation were related mostly to soil physical and chemical properties. Bacterial and fungal α-diversity in rhizosphere communities were more related to plant species identity, vegetation diversity and belowground plant traits compared to soil properties, whilst the opposite was found for bulk soil. Our results highlight that environmental changes at the landscape scale (e.g. associated to elevation, soil properties or climate), impact significantly soil microbial communities, but vegetation refines communities at a local scale via the rhizosphere niche.

Keywords: alpine ecosystem, *Juniperus communis*, microbial diversity, mountain ecosystem, *Picea abies*, root traits, soil properties, *Vaccinium myrtillus*

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Introduction

Soil is a hyper-heterogeneous environment and disentangling the factors that shape soil microbial community composition requires an understanding of the broad range of spatial scales involved, before any predictions about functioning in a future climate can be made (Averill et al. 2021). Mountains are excellent systems to study microbial communities because a multitude of climate and soil variables that impact belowground microbial communities change within a relatively small distance due to the elevational gradient (Sundqvist et al. 2013). As elevation increases, plant growth and vegetation distribution are mainly limited by decreasing temperature and a shorter growing season, causing a reduction of organic matter content in soils, making them nutrient-limited for plant growth (Zhou et al. 2002, Donhauser and Frey 2018, Möhl et al. 2019, Stokes et al. 2021). Thus, soil microbes present at these high elevations have to overcome oligotrophic conditions, as well as little protection against environmental stressors, due to coarse, poorly developed soil horizons and low water retention capacity (Donhauser and Frey 2018, Praeg et al. 2019). Together, these shifts in environmental conditions and extreme spatial heterogeneity should cause environmental filtering that make high-elevation soil microbial communities phylogenetically clustered and highly specialized (Donhauser and Frey 2018, Collins et al. 2020). At a smaller scale, changes in soil properties will also impact plant root trait expression along the elevation gradient (Weemstra et al. 2021), but it is not known how, or if, these changes could alter the substrate available for root-associated microbiomes, and subsequent niche dimensionality.

As soil microorganisms respond to environmental variations at disparate scales in the landscape, according to differences in climate, soil heterogeneity and vegetation type, measurements of taxonomic diversity should reflect the scale at which microbial communities are modified by abiotic and biotic factors. When aiming to understand how communities are shaped at different scales, diversity can be quantified as either: 1) the number and distribution of different species (richness) and their relative abundance (species evenness) in a particular habitat, referred to as alpha diversity (α-diversity) and 2) the similarity/dissimilarity of microbial communities among those particular habitats, referred to as beta diversity (β-diversity). Alpha diversity should reflect better environmental variations at a very local level, for example due to differences in chemical (e.g. nitrogen (N) and carbon (C) content) or morphological (e.g. root diameter) root traits, which increase the diversity of resources for microbial communities. A broad variety of root traits should therefore enhance niche dimensionality, increasing the probability that soil microorganisms can partition resources and coexist (high α-diversity). However, the link between root traits and microbial diversity also depends on the extent to which plant diversity contributes to heterogeneity in soil (Thakur et al. 2020). In a cross-continental study, Prober et al. (2015) found that plant diversity was not related to microbial α-diversity but was positively related to microbial β-diversity, that is more appropriate for describing the effects of broad variations in the landscape (e.g. climate and soil properties) and the disparity between microbial communities. In a complex landscape, both α- and β-diversity should be used to describe microbial diversity at differing scales, providing complementary information to help understand the effects of abiotic and biotic factors on community composition.

To study the effects of plant root traits on belowground microbial communities, it is necessary to examine the root-associated microbiome that is present in the rhizosphere. The rhizosphere is the narrow region of soil around plant roots, and is characterized by high concentrations of root-derived, easily degradable C sources that lead to an increase of microbial activity, and a subsequent shift in community composition compared to the bulk soil matrix (Kuzyakov and Razavi 2019). A recent study emphasised the importance of root traits as determinants of bacterial community composition in the rhizosphere, although the same traits are a less relevant driver of rhizospheric fungal community composition (Merino-Martín et al. 2020). In contrast, the plant species effect has been reported to be stronger on fungal community composition than on bacterial community composition, especially in the rhizosphere (Liu et al. 2018) and particularly in forest ecosystems (Tedersoo et al. 2014). However, the combined effect of vegetation and associated plant root traits on microbial α-diversity in the rhizosphere has not yet been elucidated. The rhizosphere favours copiotrophic bacteria, with high C decomposition rates, such as members of Proteobacteria (Fierer et al. 2007, Solís-García et al. 2021). Many soil fungi, especially members of the phyla Ascomycota and Basidiomycota, are saprotrophs and use organic matter, C sources and plant root residues (e.g. mucilage and exudates) as their primary energy source (Clemmensen et al. 2013). Mycorrhizal fungi are also abundant in the rhizosphere because of their strong symbiotic association with plant roots. Therefore, if plant root traits (such as diameter, root tissue density, C and N content) are modified in response to the elevation gradient and local soil heterogeneity, as shown by Merino-Martín et al. (2020) and Weemstra et al. (2021), we expect a strong change in β-diversity in the rhizosphere along the elevation gradient, depending on plant species identity.

At a broader scale, soil pH is a key driver of microbial diversity and dynamics (Rousk et al. 2010, Tedersoo et al. 2014, Zhalnina et al. 2015). For example, Dumbrell et al. (2010) showed that in a temperate woodland, pH was the primary factor structuring the arbuscular mycorrhizal (AM) fungal community, providing strong support that niche differentiation is based on abiotic soil factors, because host plant species identity had only a minimal effect on fungal communities. However, these authors examined β-diversity of fungal communities along a very strong pH gradient (pH 3.72–8.04), and it was not clear if the host plant effect had a direct influence on AM fungal communities, or an indirect response arising from the plants' response to soil pH. By examining several plant species and the response of their root traits to an environmental gradient, we should be able to

determine if microbial communities are affected at different spatial scales within a landscape.

To date, most of the studies in mountain ecosystems have found no clear pattern in microbial α- and β-diversity along elevational gradients, as bacteria and fungi display different behaviours. Bacterial α-diversity was shown to decrease (Bryant et al. 2008), increase (Wang et al. 2011), peak at mid-elevations (Singh et al. 2012) or present no changes (Fierer et al. 2011, Wang et al. 2012) with increasing elevation, while fungal α-diversity has been observed to decrease (Bahram et al. 2012) or show a hump-shaped trend (Miyamoto et al. 2014). This disparity in results may be due to the different elevation-related environmental factors in each study. Bacterial composition and β-diversity are mainly affected by pH (Lauber et al. 2009, Wang et al. 2015, Hu et al. 2016), but also by soil nutrient content, temperature and precipitation (Yuan et al. 2014, Rui et al. 2015, Shen et al. 2015). Soil fungi, however, respond to multiple variables (Jarvis et al. 2015, Ren et al. 2018), with plant diversity being a principal factor shaping fungal community composition and β-diversity (Peay et al. 2013, Wu et al. 2013, Chen et al. 2017). However, a single study whereby α- and β-diversity have been examined from rhizospheric to landscape spatial scales, and beneath different types of plant species and communities, has not yet been performed, to our knowledge, even though such a study is fundamental to understanding habitat partitioning and the drivers of microbial community composition.

Here, we evaluated the responses of soil bacterial and fungal diversity and abundance to an elevation gradient across distinct types of vegetation communities with differing soil physical and chemical properties. At a more refined scale, we examined whether plant species (that possessed different types of mycorrhizal associations) and associated functional root traits modified soil microbial communities by altering the range of habitats available. We hypothesized that: (H1) at the landscape level, overall microbial α-diversity will decline at higher elevations, because of a shorter growing season, reducing plant production and associated organic matter input to soil, thus leading to more specialised microorganisms when abiotic conditions are limiting. (H2) At a more local level, bulk soil microbial community structure will be strongly influenced by soil properties and will differ from that in the rhizosphere compartment, where plant species identity and functional root traits will define microbial diversity and community composition, particularly for bacterial communities. (H3) In bulk soil, bacterial community structure will be mainly driven by pH, while fungal community structure will be more influenced by the quantity and quality of the C pool.

Material and methods

Study site description and sampling design

Fieldwork was conducted at the Belledonne massif in the French Alps in June 2018 (France, 45°7′1″N, 5°53′35″E; Table 1), along an 8 km elevation gradient that comprised six altimetric bands (ranging from 1400 to 2400 m a.s.l.), separated from each other by a distance of 200 m (Stokes et al. 2021). The thermal treeline is dominated by *Pinus cembra*, *Pinus uncinata* and *Picea abies* and situated between 2000 and 2100 m (Wang et al. 2018). Below the treeline, the dominant trees shifted along the gradient from mixed forests of *Fagus sylvatica*, *Pinus sylvestris* and *Abies alba* in the montane belt to mixed forests of *Picea abies*, *P. uncinata* and *P. cembra* in the subalpine belt. Above the treeline, the vegetation was dominated by (sub)alpine heaths of Ericaceae (*Vaccinium* spp., *Rhododendron ferrugineum*, *Loiseleuria procumbens*, *Juniperus communis* subsp. *nana* and grasslands dominated by graminoid species (*Nardus stricta*, *Carex sempervirens*, *Festuca* spp.). Climatic parameters along the gradient such as mean annual temperature (MAT), mean annual precipitation (MAP) and mean annual solar radiation (MAR) were calculated during a ten-year period (2004–2014) using the meteorological AURELHY model (Piedallu et al. 2013, 2016) at each elevation band (Stokes et al. 2021, Table 1, Supporting information). MAT decreased from 8.5 ± 0.2 to 5.7 ± 0.2°C up the elevation gradient, while MAP increased from 1024 ± 41 to 1187 ± 40 mm. MAR varied from 4204 to 4339 MJ m^{-2} up

Table 1. Main characteristics of the elevation gradient. Mean values and standard deviation in brackets for climatic data, elevation and GPS coordinates measured in each of the five plots (further detail on the data description available in the data paper (Stokes et al. 2021) and raw data available at <https://entrepot.recherche.data.gouv.fr/dataverse/ecopics>).

Elevational band (m)	Elevation (m a.s.l.)	GPS coordinates	MAT (°C)	MAP (mm)	MAR (MJ m^{-2})	Life zone
1400	1366 (15)	45°08′15″N 5°85′79″E	8.5 (0.2)	1024 (41)	4204 (0)	montane
1600	1601 (12)	45°09′25″N 5°86′91″E	7.3 (0.7)	1066 (20)	4181 (0)	subalpine
1800	1799 (20)	45°10′83″N 5°89′27″E	8.1 (0.5)	1110 (7)	4575 (102)	subalpine
2000	1971 (11)	45°11′73″N 5°90′28″E	5.7 (0.7)	1155 (12)	4463 (0)	subalpine
2200	2208 (19)	45°12′73″N 5°92′29″E	3.8 (0.1)	1205 (20)	4339 (0)	alpine
2400	2405 (17)	45°12′95″N 5°93′03″E	5.7 (0.2)	1187 (40)	4339 (0)	alpine

MAT: mean annual temperature, MAP: mean annual precipitation, MAR: mean annual solar radiation.

the elevation gradient (Table 1, Supporting information; data from Stokes et al. 2021).

Within each elevational band, five plots (20 × 20 m) were selected, separated by at least 100 m from each other (Supporting information). Plots were selected to include, when present at that elevational band, three species: *P. abies*, a tall evergreen tree; *Juniperus communis*, a prostrate evergreen shrub and *Vaccinium myrtillus*, a small deciduous shrub (Supporting information). These three plant species were selected because of their different growth forms, diverse mycorrhizal symbioses and occupation of different ecological niches along the elevation gradient. *Picea abies* was the dominant tree species below the tree line; *J. communis* was abundant locally and *V. myrtillus* was present in all six elevational bands. *Picea abies* and *V. myrtillus* are both keystone species (Bjune et al. 2009, Nybakken et al. 2013) and although *J. communis* is not classified as a keystone species, its abundance above the treeline makes it an important species that structures plant communities. Therefore, these three species contribute to shaping the structure of the plant communities in which they are present, and so we term them 'structuring species.' *Juniperus communis* forms symbioses with arbuscular mycorrhizal fungi (AMF) that provide nutrients, water and trace minerals as well as resistance against pathogens (Smith and Read 2008); *P. abies* possesses ectomycorrhizal (ECM) associations that are formed mainly in woody species and deliver nutrients and water to the host plant in exchange for carbon (Bennett and Classen 2020), and *V. myrtillus* has ericoid mycorrhizas (ERM), allowing ericaceous species to grow in nutrient impoverished habitats with acidic soils, low temperatures, excessive soil moisture and slow turnover of organic matter (Leopold et al. 2021).

One well-developed individual of each structuring plant species was selected within each plot and, at the limit of its canopy, a 1.0 × 1.0 m quadrat was located for further plant and soil sampling. A botanical survey was performed in each quadrat, plants were identified (Flore Forestière Française, Montagnes, Rameau et al. 1999; Flora Helvetica, Lauber et al. 2001) and their abundance was estimated, making a visual assessment of the relative area covered by different plant species in each quadrat, using Simpson diversity index (Simpson 1949). The full sampling procedure is described in Stokes et al. (2021).

Soils beneath all three plant structuring species along the gradient were acidic with a pH ranging from 4.40 to 5.60 and the percentage of soil water content was significantly lower at 1400–1600 m than at 1800–2400 m. Soil water content, physical and chemical properties were measured in detail at each of the plots in separate studies (Stokes et al. 2021, Weemstra et al. 2021, Hernández-Cáceres et al. 2022). In general, underneath *V. myrtillus* and *P. abies*, soil nutrient content (soil organic carbon (SOC), total nitrogen (TN) and phosphorus content (P)), cation exchange capacity (CEC) and carbon/nitrogen (C:N) ratio varied little along the elevation gradient, except at 1600 m where they were significantly greater than at all other elevations. However, no significant differences in these characteristics beneath *J. communis* were found with elevation. NH_4^+ content was significantly higher beneath *P. abies* at 1600 m than at all other elevations but beneath *V. myrtillus* and *J. communis*, no significant differences were found. NO_3^- content did not show significant changes beneath any structuring species. Litter depth beneath *P. abies* decreased significantly from 1400 to 2000 m while beneath *V. myrtillus*, the main change was between 1400 and 1600 m and no trends were found beneath *J. communis* along the elevation gradient (Supporting information). Aggregate stability, expressed by mean weight diameter (MWD) in topsoil [depth: 0–0.25 m, MWD_{top}] and subsoil [depth: 0.25–0.50 m; MWD_{sub}] and hydraulic conductivity (Kfs) did not display significant shifts along the gradient under any of the three structuring plant species (Supporting information).

Bulk and rhizospheric soil sampling

At the center of each 1 × 1 m quadrat, a soil monolith (0.25 × 0.25 × 0.15 m) was excavated using a metal frame. Bulk soil was collected from the extracted monoliths and sieved at 2 mm, placed in plastic labelled bags and stored at −20°C for further microbial diversity analyses, or stored at room temperature for the assessment of soil physical and chemical properties (Stokes et al. 2021). A total number of 70 bulk soil samples were taken (six elevations × five plots × two to three plant species).

To obtain rhizospheric soil, further soil samples were obtained beneath plants in the same plots and close to the monoliths. Individuals of *V. myrtillus* were identified and root systems were carefully extracted manually with sterilised material. These root systems, including the soil attached into them, were placed into Falcon tubes containing 30 ml sterilised and fresh PBS-S buffer (130 mM NaCl, 7 mM Na_2HPO_4, 3 mM NaH_2PO_4, 0.02% Silwet L-77) in the field. In the case of *J. communis* and *P. abies*, whole individuals were not harvested. Instead, a large root was followed from the base of the trunk to a distance of about 0.5 m from the trunk, and fine roots attached to this root were collected and placed into centrifuge tubes with 30 ml sterilised and fresh PBS-S buffer. These centrifuge tubes were taken to the laboratory and stored at −20°C until further processing for microbial diversity analyses.

Soil DNA extraction and quantification

Roots collected in the Falcon tubes were treated to obtain rhizospheric soil following Bulgarelli et al. (2012, 2015). These roots were washed for 20 min in an orbital shaker at 180 rpm at room temperature. After that, roots were transferred in a new tube with fresh PBS-S buffer (10 ml) and a second wash was performed under the same conditions. The soil suspensions collected in the Falcon tubes after the first and second washing treatments were combined and centrifuged at 4000 g for 20 min at 4°C. The supernatant was discarded, and the pellet was referred to as *rhizosphere* soil and stored at −20°C until further processing.

DNA extractions were performed on both bulk and rhizosphere soil using the FastDNASpin Kit for Soil (MP Biomedicals, Santa Ana, CA, USA) following the manufacturer's recommendations. DNA extracts were quantified using Quant-iTPicoGreendsDNA Assay Kit (Molecular Probes/Invitrogen, ThermoFisher Scientific, USA).

Library preparation and amplicon analysis

Next Generation Sequencing Illumina of 16S rRNA genes and the fungal ITS region were performed at ADNid (Montferrier-sur-Lez, France). For 16S, the V4 hypervariable regions of the 16S rRNA gene was targeted using primers based upon the universal primer sequences A519F (5′-CAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACNNGGGTATCTAAT-3′). For ITS, region 2 (ITS2) was amplified utilizing the ITS3 (forward) and ITS4 (reverse) primer sequences described in White et al. (1990) (5′-GCATCGATGAAGAACGCAGC-3′ and 5′-TCCTCCGCTTATTGATATGC-3′) (<https://unite.ut.ee/primers.php>). For each sample, amplicons were generated using a hot start DNA polymerase (Type-it QIAGEN). PCR was conducted on 10 ng of template DNA in 15 µl employing an initial denaturation of 5 min at 95°C, followed by 25 for IT3-4 and A519-806 of 30 s at 95°C, 1 min at 55°C and 30 s at 72°C. A final extension of 30 min at 60°C completed the reaction. Library quality was controlled using a fragment analyzer. Sequencing runs, generating 2 × 300 bp reads, were performed on an Illumina MiSeq platform.

Sequencing data from each sample were automatically separated by the sequencer. Samples paired-end fastq.gz files were processed jointly using an CLI (Command Line Interface) pipeline based on FROGS 3.1 (<http://frogs.toulouse.inra.fr/>, Escudié et al. 2018). Bacterial and fungal sequence processing followed the same pathway: 1 – first, forward and reverse reads were merged together using the common region, 2 – obtained sequences were clustered using default swarm (Mahé et al. 2014, 2015) distance parameter, 3 – chimera were then removed, 4 – poorly represented clustered were removed (abundance < 5×10^{-05}), 4.5 – for ITS regions, ITSx program was used to extract fungal ITS sequences, 5 – OTU were generated, using rdp classifier and blastn databases in .biom format (SILVA 132 provided with FROGS for 16S, Unite 7.1 provided with FROGS for ITS). Downstream sequence processing (taxa filtering, variance stabilization, abundance estimates) was performed using phyloseq (McMurdie and Holmes 2013) and deseq2 (Love et al. 2014) R packages.

Community- and *species*-level root traits

Community level root traits

Once the monoliths were extracted, the depth of the litter layer was noted and removed. To extract all roots that represented a mixture of roots from one of the structuring species and from different plant species growing in the monoliths (hereafter termed *community* level and denoted $_{com}$), half of the monolith was dissected. These data are published and a detailed description on the procedure to obtain and measure *community* level root traits can be found in Stokes et al. (2021) and Hernández-Cáceres et al. (2022), and mean data are given in the Supporting information. Briefly, fine roots with a diameter < 2 mm were removed, washed and scanned in a tray of water and analysed (Winrhizo Pro ver. 2019). At the *community* level, mean root diameter (RD_{com}) was estimated in different diameter classes after scanning, root fresh mass (RFM_{com}) and root dry mass (RDM_{com}) (60°C for 72 h) were determined. Specific root length (SRL_{com}), root tissue density (RTD_{com}), root dry mass content ($RDMC_{com}$), root length density (RLD_{com}) and root mass density (RMD_{com}) were calculated. Root nitrogen (RNC_{com}) and carbon (RCC_{com}) content were measured on absorptive roots using an elemental analyser. In absorptive community level roots, the content of water-soluble compounds, cellulose ($cellulose_{com}$), hemicellulose ($hemicellulose_{com}$) and lignin ($lignin_{com}$) was determined using the Van Soest method (Van Soest 1990) in a fiber analyser (Fibersac 24).

Species level root traits

For root traits of individual species (as opposed to the *community* level), data were obtained from a separate study by Weemstra et al. (2021), who examined 11 plant species in the same plots and at the same dates, including the three structuring species. Roots were stored in moist plastic bags and kept refrigerated until analysed in the laboratory. Within one day but sometimes up to two days after sampling, roots were washed and undamaged absorptive roots were scanned and SRL_{sp}, $RDMC_{sp}$, RTD_{sp} at the *species* ($_{sp}$) level were calculated as described above for roots harvested at the *community* level. Mean data for species level root traits are given in the Supporting information.

Data analysis

All statistical analyses were performed using R software (<www.r-project.org>). The Shannon diversity index for bacteria and fungi was calculated for each elevational band and structuring species for bulk and rhizospheric soils using the phyloseq R package. Alpha-diversity and composition for the two microbial compartments were analysed using R analysis of variance (ANOVA) function *aov* with elevation and structuring plant species as explanatory variables and Tukey's HSD to test significant differences among elevational bands. Additionally, Hill numbers were calculated to quantify diversity in units of equivalent numbers of equally abundant species (Jost 2006) using *estimateD* function from the iNEXT package (ver. 2.0.20). Arguments in this function were chosen to compute the diversity estimates for the minimum sample size among all sites.

Soil physical and chemical properties, community- and species-level root traits and vegetation diversity along the elevational gradient, were analysed with elevational bands as explanatory variables using one-way analysis of variance (ANOVA) and Tukey's HSD to test significant differences

among elevational bands. All variables tested fulfilled ANOVA assumptions. Unbalanced two-way ANOVA (type-III sums of squares) was performed using car R package to assess the effect of elevation, plant structuring species and their interaction on the different soil physical and chemical properties, community- and species-level root traits and plant diversity.

The relationships between microbial community structure and the environmental variables (climatic data, soil properties, plant diversity and community- and species-level root traits) were tested using Spearman correlations and the alpha (Shannon diversity index and Hill-observed richness) and beta (NMDS first and second axis) diversities, along the elevation gradient for each one of the three structuring species.

Dissimilarity between microbial community structure among elevation bands, soil compartments (bulk and rhizospheric soil) and structuring plant species identity was studied by non-metric multidimensional scaling (NMDS) analysis using Bray–Curtis distance. NMDS ordinations were performed for transformed data using the function *getVarianceStabilizedData* from DESeq2 package. Permutational multivariate analysis of variance (PERMANOVA), implemented with the *adonis* function from the vegan R package, was used to assess the significance of the observed NMDS differences. A second set of PERMANOVA analyses were performed in order to test the effect of elevation and plant species identity on bacterial and fungal communities in each of the soil compartments separately.

Distance-based redundancy analysis (db-RDA) for constrained ordination based on the Bray–Curtis distance (*capscale* function of vegan R package) was carried out to determine the extent to which microbial community structure variation was explained by environmental variables along the elevational gradient. Then, a stepwise model selection using generalized Akaike information criterion (AIC, *ordistep* function of vegan R package with forward and backward direction) was performed. Finally, db-RDA plot is shown for the variables obtained from the model selection.

Variation partitioning analysis, using the function *varpart* of vegan R package (Oksanen et al. 2020), was performed to determine the relative importance of the environmental variables (soil physical and chemical properties, community- and species-level root traits and climatic data), and their contribution to the microbial community structure, which was later identified by partial redundancy analysis in Hellinger transformed data.

Structural equation modeling (SEM) was applied to determine the direct and indirect contributions of selected soil properties, plant traits and climate factors to the microbial community richness in bulk and rhizosphere soil, using the lavaan R package (Rosseel 2012). The model fit was evaluated based on a non-significant X^2-test ($p > 0.05$), the goodness-of-fit index (GFI > 0.90) and the root mean square error of approximation (RMSEA < 0.05).

Results

Microbial α-diversity and composition in bulk and rhizosphere soils along the elevation gradient

Bulk soil bacterial α-diversity (Shannon diversity index and observed richness, estimated with Hill number, $q = 0$) did not change significantly along the elevation gradient in *V. myrtillus* plots (Fig. 1a, c). However, an effect of elevation was observed for bulk soil beneath *P. abies* (Shannon diversity index; ANOVA; $p = 0.027$) and *J. communis* (Hill's observed richness; ANOVA; $p = 0.026$), even though no significant differences in α-diversity beneath the structuring species were found among altitudinal levels. Nevertheless, in the rhizosphere soil, the Hill-observed bacterial richness increased significantly at higher elevations beneath *P. abies*, with significant differences between altitudes (ANOVA; $p = 0.008$). Fungal α-diversity in the bulk soil did not vary significantly beneath the three structuring plant species. However, a significant effect of altitude was observed in rhizosphere soil beneath *P. abies* and *J. communis*, with fungal α-diversity increasing at higher elevations (1800–2000 m) for *P. abies* and decreasing for *J. communis* (Fig. 1b, d).

In terms of composition, bacterial communities in both bulk and rhizosphere soil under all three structuring species were dominated by the phyla Proteobacteria, Acidobacteria and Actinobacteria, regardless of the altitude (Fig. 2). Furthermore, some differences in the relative abundance of certain bacterial phyla were noted according to the altitude. In the bulk and rhizosphere soils, the abundance of Acidobacteria was greater in the lower altitudinal levels under *P. abies* and *V. myrtillus*, but not under *J. communis*. However, the relative abundance of Chloroflexi under *V. myrtillus* increased at higher elevations, regardless of the soil compartment (Fig. 2).

Soil fungal communities in the bulk soil beneath the three structuring species were dominated by Basidiomycota, especially in the bulk soil under *P. abies*, and by Ascomycota, regardless of the altitudinal level (Fig. 3). The same fungal phyla dominated fungal communities in the rhizosphere soil beneath *P. abies*, *V. myrtillus* and *J. communis* (Fig. 3). In the bulk soil under *J. communis*, a steep increase in the relative abundance of Ascomycota was observed with altitude, while a concomitant decrease in Basidiomycota proportion was observed. This pattern was not sustained in the rhizosphere soil of *J. communis*, but was instead present in that of *V. myrtillus*.

Influence of elevation and plant species identity on microbial community structure in bulk and rhizosphere soils

The NMDS analysis showed a marked difference in bacterial community structure (β-diversity) between the two soil compartments (bulk soil versus rhizospheric soil) (Fig. 4). Bacterial community structure changed slightly along the elevation gradient, but the soil compartment had much greater

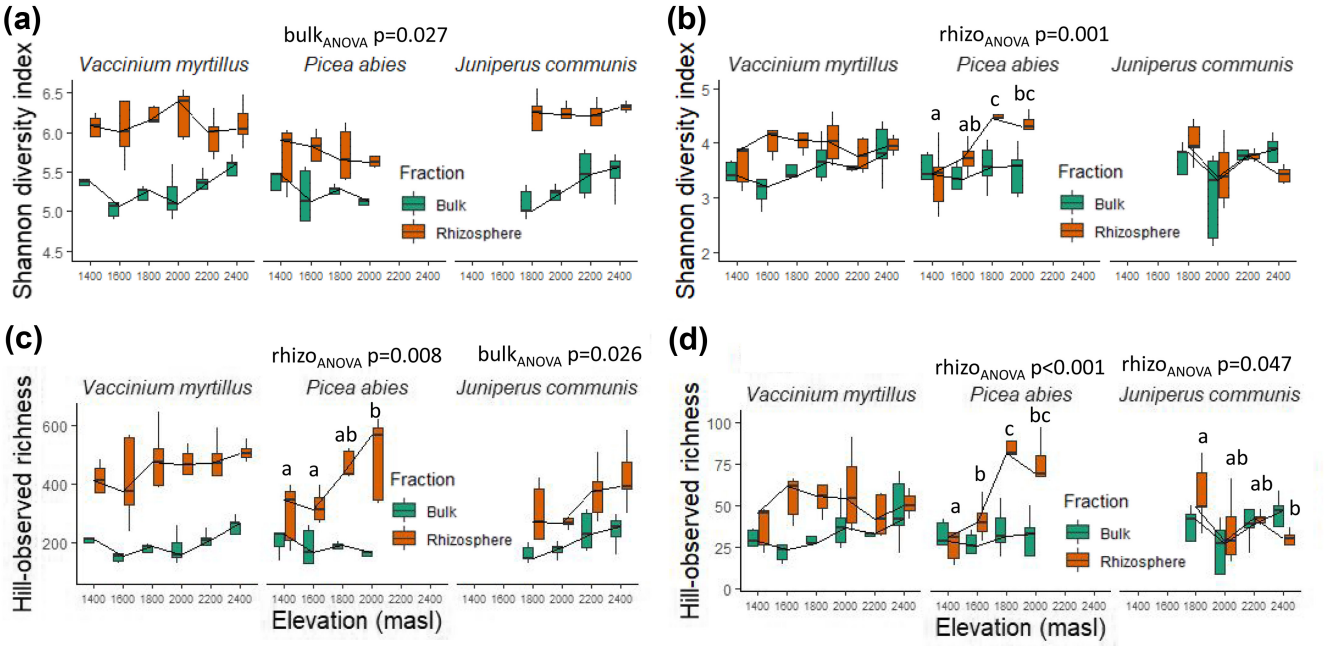

Figure 1. Box plots (median, first and third quartiles and 1.5 × inter-quartile range) of Shannon diversity and Hill-observed richness (q = 0) of bacteria (a) and (c) and fungi (b) and (d) in bulk and rhizospheric soil along the elevational gradient under the influence of *V. myrtillus*, *P. abies* and *J. communis*. Different lowercase letters indicate significant differences between elevational bands tested with Tukey's HSD test ($p < 0.05$). Significant p-values for ANOVA are shown ('bulk' for bulk soil and 'rhizo' for rhizosphere). Note that scales on the y-axis are different between plots.

effect. These findings were supported by the PERMANOVA analysis which showed a stronger influence of soil compartment ($p < 0.001$, $R^2 = 0.41$) than the effect of elevation and plant species identity ($p < 0.001$; $R^2 = 0.04$; and $p < 0.001$; $R^2 = 0.03$, respectively; Table 2). In other words, soil compartment (i.e. bulk or rhizospheric soil), explained 41% of the dissimilarity in bacterial communities (Table 2).

Regarding fungal community structure, the NMDS analysis revealed a marked influence of soil compartments, elevation and plant species identity, accounting for differences in fungal β-diversity (Fig. 4). The PERMANOVA analysis confirmed the significant and predominant effect of soil compartment ($p < 0.001$, $R^2 = 0.07$; Table 2) on fungal community structure, the significant influence of elevation and structuring plant species identity ($p < 0.001$, $R^2 = 0.04$ for both elevation and species; Table 2), as well as the significant effect of their interactions. A second PERMANOVA test, performed on each soil compartment separately, revealed similar trends in both bulk and rhizosphere soils, with a significant influence of both elevation and species identity on bacterial and fungal community structure (Table 3).

Relationships of abiotic elevation-related environmental factors on microbial diversity and composition

The partition of variance analysis showed that soil physical and chemical properties significantly explained bacterial community structure in both bulk ($p < 0.001$, adjusted $R^2 = 0.34$; Table 4, Supporting information) and rhizospheric soil ($p = 0.003$, adjusted $R^2 = 0.21$; Table 4, Supporting information). Conversely, climatic data did not explain bacterial community structure in either the bulk ($p = 0.26$, adjusted $R^2 = 0.10$; Table 4, Supporting information) or rhizospheric soils ($p = 0.59$, adjusted $R^2 = 0.07$; Table 4, Supporting information).

The relationships between bacterial communities and soil physical and chemical characteristics were complex (Fig. 5). Bacterial α-diversity (Shannon diversity index and/or Hill's observed richness) in the bulk soil beneath *V. myrtillus* was negatively and significantly correlated with SOC, TN, P, CEC, NH_4^+ and clay, and positively with sand content (Fig. 5), while that of bacteria in the rhizosphere was significantly and positively correlated with pH, but negatively with Kfs (Fig. 5). Beneath *P. abies*, bacterial α-diversity in the bulk soil was significantly and positively correlated with sand content, but negatively with P, CEC and clay content, whilst it was significantly and positively correlated with pH, water content and NO_3^- in rhizospheric soil. Under *J. communis*, bulk soil bacterial α-diversity was negatively correlated with NH_4^+ and silt (Fig. 5), while that in the rhizosphere soil was negatively correlated with P and silt but positively with sand content (Fig. 5).

As shown by the partition of variance analysis, climatic data were poorly correlated with bacterial α-diversity beneath the three structuring plant species. Bacterial α-diversity in the bulk and rhizosphere soil of *V. myrtillus* was not correlated with any climatic variable (Fig. 5). Under *P. abies*, no significant correlations were found between bacterial α-diversity in the bulk soil, but it was positively correlated with MAP and

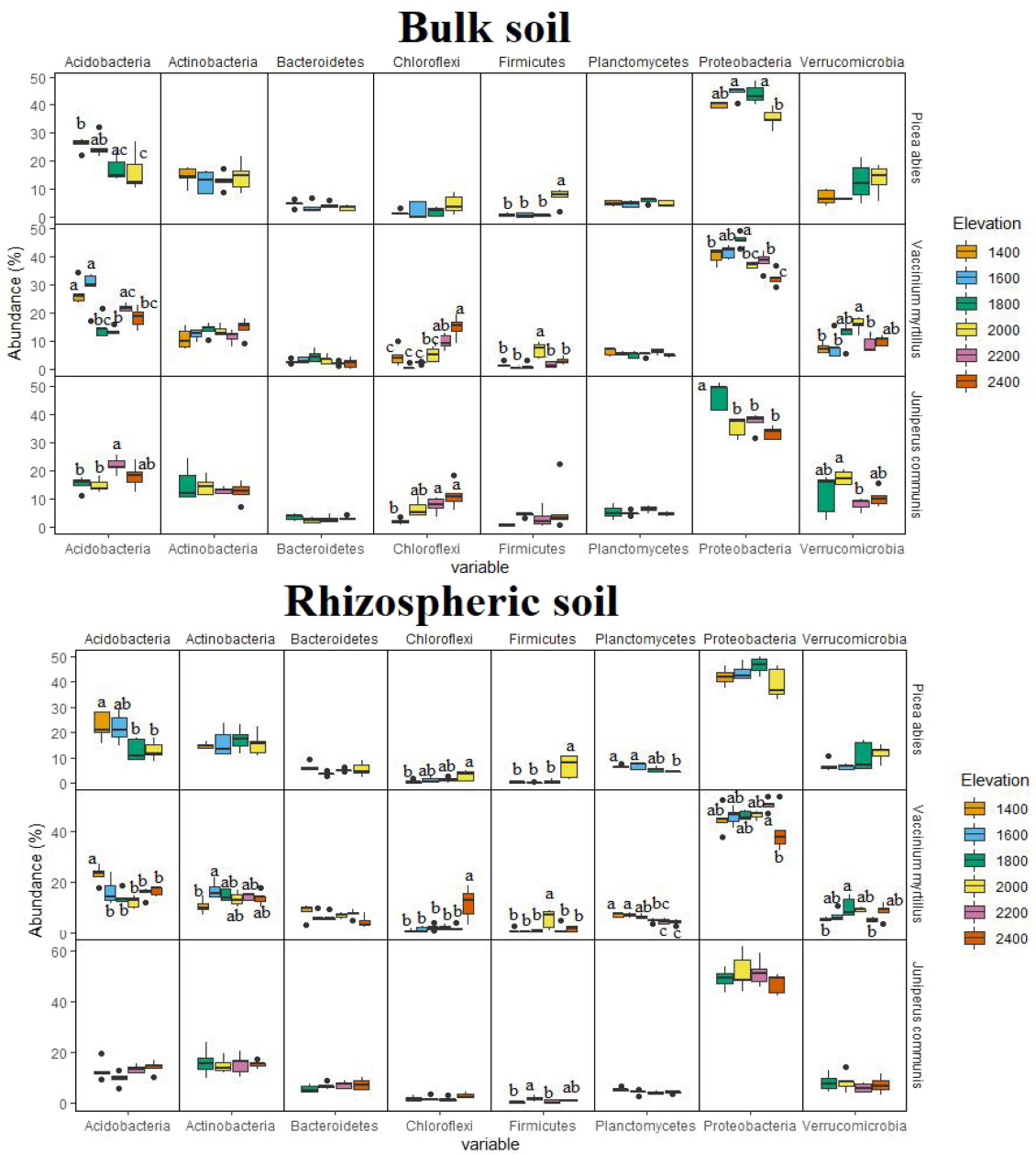

Figure 2. Relative abundance of the dominant bacterial phyla from bulk (upper panel) and rhizospheric (lower panel) soils along the elevational gradient under the influence of *P. abies*, *V. myrtillus* and *J. communis*. Boxplots represent the median, first and third quartiles and 1.5 × inter-quartile range in the data set. Different letters show post hoc Tukey honestly significant difference (HSD) results within each phylum along the elevational gradient ($p < 0.05$).

MAR and negatively with MAT in the rhizosphere. In contrast, under *J. communis*, bulk soil bacterial α-diversity was positively correlated with MAT, but negatively with MAR and MAP (Fig. 5).

The partition of variance analysis indicated that neither soil physico-chemical properties, nor climatic data, significantly explained fungal community structure in the bulk and rhizosphere soils (Table 4). However, several significant correlations were detected between soil fungal α-diversity and soil properties. Fungal α-diversity (Shannon diversity index and/or observed richness (Hill's number, $q=0$)) in the bulk soil beneath *V. myrtillus* was significantly and positively correlated with pH, water and sand contents, but negatively correlated with SOC, TN, C:N ratio, P, CEC, NH_4^+, clay content and litter depth (Fig. 6). However, rhizospheric fungal α-diversity of *V. myrtillus* was significantly and positively correlated with MWD_{top} but negatively with Kfs (Fig. 6). Beneath *J. communis*, soil fungal α-diversity in the bulk soil was positively correlated with MWD_{sub} while in the rhizospheric soil, significant positive correlations were found with litter depth and

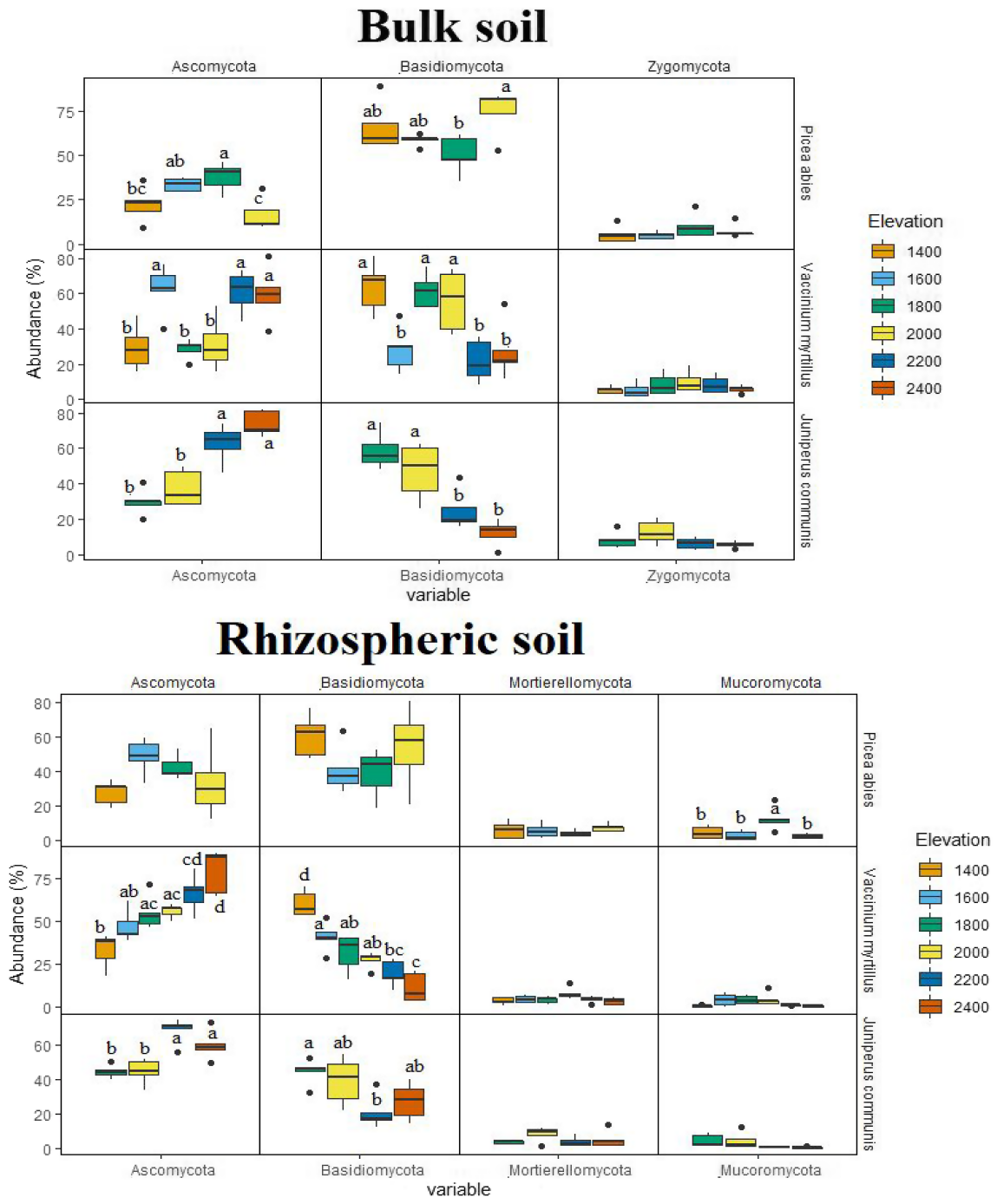

Figure 3. Relative abundance of the dominant fungal phyla from bulk (upper panel) and rhizospheric (lower panel) soil along the elevational gradient under the influence of *P. abies*, *V. myrtillus* and *J. communis*. Boxplots represent the median, first and third quartiles and 1.5 × inter-quartile range in the data set. Different letters show post hoc Tukey honestly significant difference (HSD) results within each phylum along the elevational gradient ($p < 0.05$).

NO_3^-. Under *P. abies*, soil fungal α-diversity in the bulk soil was positively correlated with water content and NO_3^-, while in the rhizospheric soil, significant positive correlations were detected with pH, water content and MWD_{top} and a negative correlation was found with C:N ratio (Fig. 6). Finally, as observed for bacterial diversity, only a few significant correlations were found between fungal α-diversity and climatic data. Beneath *V. myrtillus*, fungal α-diversity in the bulk soil was negatively correlated with MAT and positively with MAP, while that of the rhizosphere was positively correlated with MAR. Under *P. abies*, fungal α-diversity in the bulk soil was not significantly correlated with climate, but it was positively correlated with MAP and MAR and negatively with MAR in the rhizosphere. No significant correlations were found

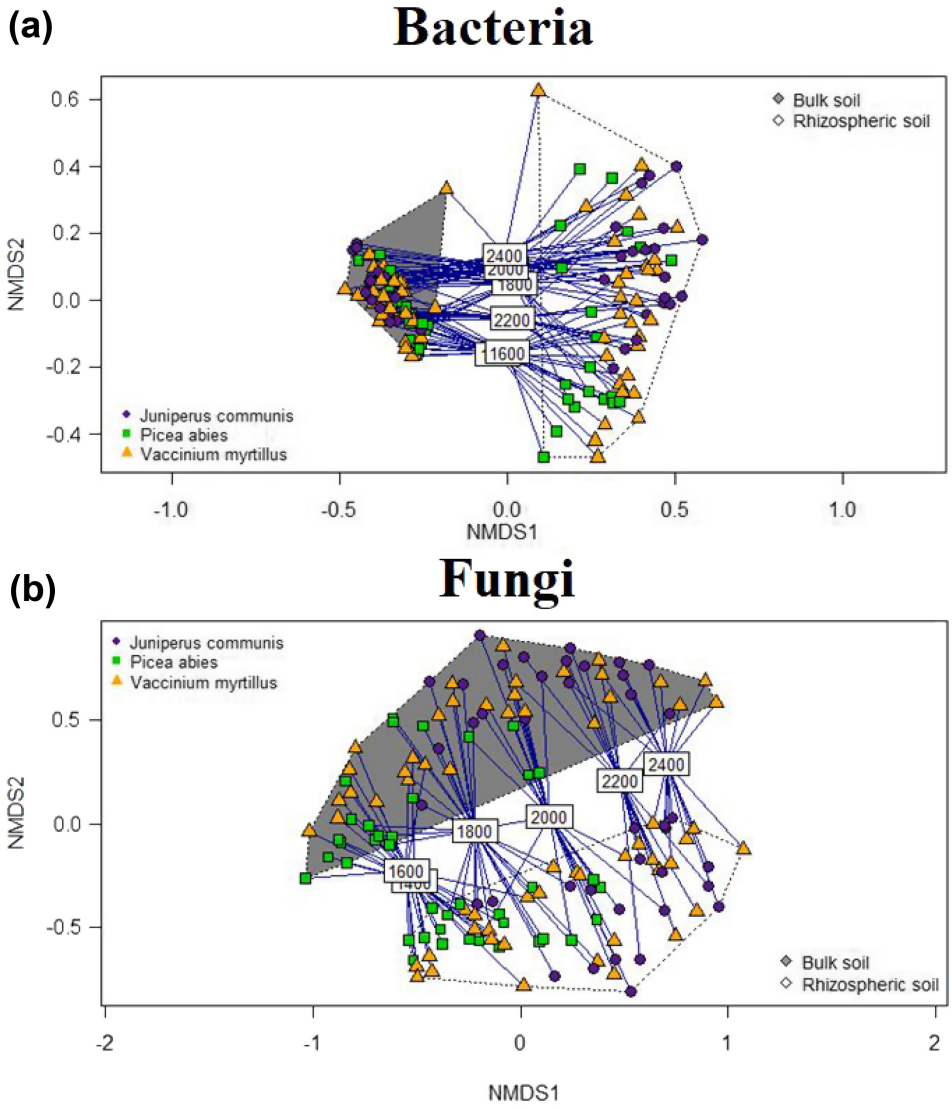

Figure 4. Non-metric multidimensional scaling (NMDS) of (a) bacterial and (b) fungal composition and diversity for six altitudinal bands (spider diagrams) for bulk (convex hull polygon grey) and rhizospheric (convex hull polygon white) soils for *Vaccinium myrtillus* (yellow triangles), *Picea abies* (green squares) and *Juniperus communis* (purple diamonds).

between fungal α-diversity in either bulk and rhizopheric soil under *J. communis* with MAT, MAR or MAP (Fig. 6).

Relationships of biotic elevation-related factors with microbial diversity and composition

The partition of variance analysis showed that root traits, at the *community* ($p=0.23$, adjusted $R^2=0.14$; $p=0.12$, adjusted $R^2=0.15$; for bulk and rhizosphere soils respectively) and *species* ($p=0.15$, adjusted $R^2=0.01$; $p=0.34$, adjusted $R^2=0.02$; for bulk and rhizosphere soils respectively) levels, did not explain significantly bacterial community structure, in either the rhizosphere or bulk soil (Table 4). Consistently, few significant correlations were detected between microbial diversity and root traits at *community*- and *species*-levels or with plant diversity. Beneath *V. myrtillus*, bacterial α-diversity (Shannon diversity index and/or Hill observed richness) in the bulk soil, positively correlated with $RDMC_{sp}$, whilst that of the rhizosphere was not correlated with any biotic variable (Fig. 5). Under *P. abies*, bacterial Hill-observed richness in the rhizosphere was negatively correlated with RD_{com}, RNC_{com}, RCC_{com} and $lignin_{com}$ and positively correlated with SRL_{com}, R $C:N_{com}$, $hemicellulose_{com}$, $cellulose_{com}$ and RLD_{com} (Fig. 5). Beneath *J. communis*, bacterial α-diversity in the bulk soil was positively correlated with SRL_{sp} and negatively with RD_{com}, while rhizospheric bacterial α-diversity was negatively correlated with RD_{com} and RTD_{com} (Fig. 5).

Regarding fungi, neither *community*, nor *species* level root traits, explained significantly the community diversity and composition in rhizosphere soil ($p=0.32$, adjusted $R^2=0.12$ and $p=0.52$, adjusted $R^2=0.01$, respectively) and bulk soil ($p=0.64$, adjusted $R^2=0.11$ and $p=0.49$,

Table 2. Effect of structuring plant species, elevation and compartment (bulk and rhizospheric soil) on (a) bacterial and (b) fungal community structure assessed with PERMANOVA. Bold values indicate $p < 0.005$ and italic values indicate $p < 0.05$.

Factors	df	Sums of sqs	Means sqs	F	R^2	p(> F)
(a) Bacteria						
Plant species	2	0.40	0.20	4.41	0.03	**0.001**
Elevation	1	0.51	0.51	11.29	0.04	**0.001**
Soil compartment	1	5.10	5.10	112.98	0.41	**0.001**
Plant species × Elevation	2	0.24	0.12	2.62	0.02	*0.01*
Plant species × Soil compartment	2	0.21	0.10	2.29	0.02	*0.02*
Elevation × Soil compartment	1	0.13	0.13	2.95	0.01	*0.03*
Plant species × Elevation × Soil compartment	2	0.09	0.04	0.97	0.01	0.42
Residuals	128	5.78	0.05		0.46	
Total	139	12.44			1	
(b) Fungi						
Plant species	2	2.43	1.21	3.55	0.04	**0.001**
Elevation	1	2.21	2.21	6.48	0.04	**0.001**
Soil compartment	1	3.77	3.77	11.02	0.07	**0.001**
Plant species × Elevation	2	1.55	0.78	2.27	0.03	**0.001**
Plant species × Soil compartment	2	1.11	0.56	1.63	0.02	**0.001**
Elevation × Soil compartment	1	0.66	0.65	1.92	0.01	**0.002**
Plant species × Elevation × Soil compartment	2	0.67	0.33	0.97	0.01	0.553
Residuals	128	43.76	0.34		0.78	
Total	139	56.16			1	

adjusted $R^2 = 0.02$, respectively) (Table 4). In the bulk soil of *V. myrtillus*, fungal α-diversity (Shannon diversity index and/or Hill's observed richness) was negatively correlated with RD_{com}, RCC_{com} and $lignin_{com}$ and positively correlated with SRL_{com}, $hemicellulose_{com}$ and $cellulose_{com}$, whilst in the rhizosphere it was only negatively correlated with RTD_{com} (Fig. 6). Under *P. abies*, fungal α-diversity in rhizosphere soil was negatively correlated with RCC_{com} and $lignin_{com}$ and positively correlated with $hemicellulose_{com}$; no correlations were found in the bulk soil (Fig. 6). Fungal α-diversity beneath *J. communis* in the bulk soil was positively correlated with RTD_{com} and negatively correlated with $RDMC_{com}$ in the rhizosphere (Fig. 6).

Finally, the distance-based redundancy analysis (db-RDA) showed the relationships between the measured environmental variables along the elevation gradient (climatic data, soil physical and chemical properties and *community*- and *species*-level root traits) that best explained the variations in microbial diversity and community structure in both bulk and rhizosphere soils (Fig. 7). Bacterial community structure was best explained by pH, sand content, $hemicellulose_{com}$ and C/N ratio, whilst the main driving factors of fungal community structure were RD_{com}, $hemicellulose_{com}$, MWD_{top}, soil water content, C:N ratio and MAR. Furthermore, SEM analysis, carried out by separating bulk and rhizosphere soil compartments, showed that bacterial α-diversity (i.e. Hill-observed

Table 3. Effect of structuring plant species identity and elevation on microbial community structure in bulk and rhizospheric soils assessed with PERMANOVA. Bold values indicate $p < 0.005$ and italic values indicate $p < 0.05$.

	Bacteria											
	Bulk soil						Rhizospheric soil					
Factors	df	Sums of sqs	Means sqs	F	R^2	p(> F)	df	Sums of sqs	Means sqs	F	R^2	p(> F)
Plant species	2	0.07	0.03	2.22	0.06	**0.015**	2	0.41	0.21	3.40	0.08	**0.001**
Elevation	1	0.11	0.11	7.09	0.09	**0.001**	1	0.52	0.52	8.60	0.10	**0.001**
Plant species × Elevation	2	0.06	0.03	1.88	0.05	*0.04*	2	0.22	0.11	1.79	0.04	*0.02*
Residuals	64	1.00	0.02		0.81		64	3.87	0.06		0.77	
Total	69	1.29			1		69	5.02			1	
	Fungi											
	Bulk soil						Rhizospheric soil					
Factors	df	Sums of Sqs	Means sqs	F	R^2	p(> F)	df	Sums of Sqs	Means sqs	F	R^2	p(> F)
Plant species	2	1.41	0.70	1.88	0.05	**0.001**	2	2.06	1.03	2.97	0.08	**0.001**
Elevation	1	1.59	1.59	4.25	0.06	**0.001**	1	1.26	1.26	3.65	0.05	**0.001**
Plant species × Elevation	2	1.10	0.55	1.46	0.04	**0.006**	2	1.10	0.55	1.59	0.04	**0.002**
Residuals	64	24.02	0.38		0.85		64	22.17	0.35		0.83	
Total	69	28.12			1		69	26.59			1.00	

Table 4. Partition of variance in constrained ordination distance-based redundancy analysis (db-RDA) for the four environmental variables (soil physical and chemical properties, community- and species-level root traits and climatic data) for bacterial and fungal community structure in bulk and rhizospheric soil samples. Bold values indicate $p < 0.005$.

	Bacteria							
	Bulk soil				Rhizospheric soil			
Environmental variables	df	R^2	R^2 adjusted	p	df	R^2	R^2 adjusted	p
Soil properties	15	0.49	0.34	**0.001**	15	0.38	0.21	**0.003**
Community level root traits	11	0.28	0.14	0.23	11	0.28	0.15	0.12
Species level root traits	3	0.05	0.01	0.15	3	0.06	0.02	0.34
Climate	3	0.14	0.10	0.26	3	0.11	0.07	0.59
	Fungi							
	Bulk soil				Rhizospheric soil			
Environmental variables	df	R^2	R^2 adjusted	p	df	R^2	R^2 adjusted	p
Soil properties	15	0.37	0.20	0.30	15	0.33	0.14	0.24
Community level root traits	11	0.26	0.12	0.32	11	0.25	0.11	0.64
Species level root traits	3	0.06	0.01	0.52	3	0.06	0.02	0.49
Climate	3	0.10	0.06	0.65	3	0.10	0.05	0.81

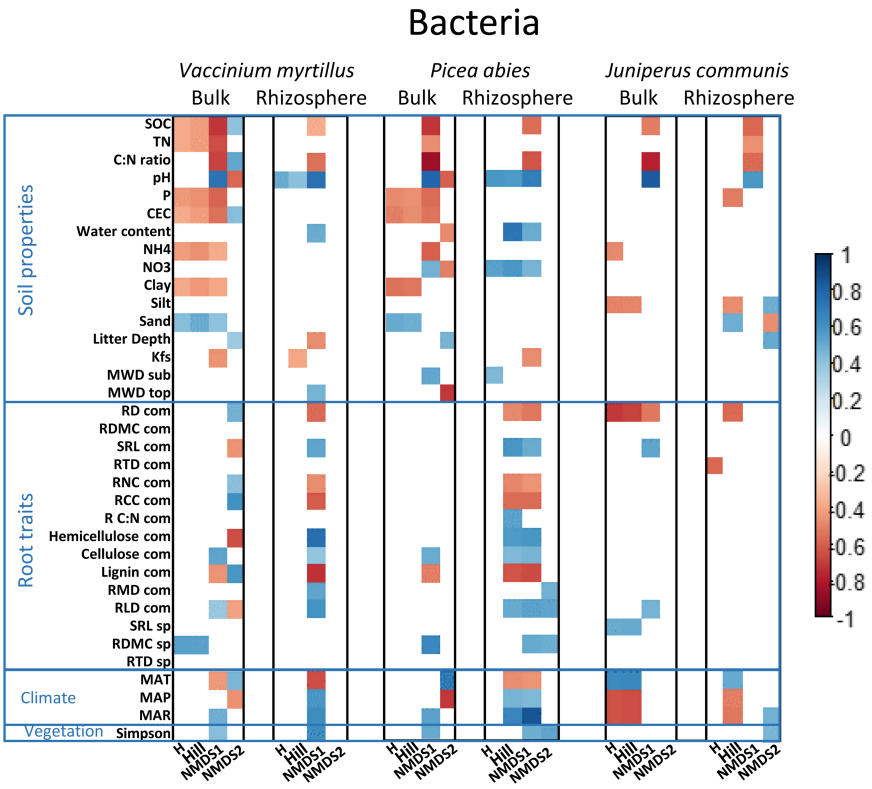

Figure 5. Spearman correlations between bacterial α-diversity (H: Shannon index and Hill: observed richness, Hill number, q = 0) and β-diversity (NMDS1 and NMDS2) in bulk and rhizospheric soils with abiotic and biotic elevation-related environmental factors beneath *V. myrtillus*, *P. abies* and *J. communis* along the elevational gradient (intensity of color denotes significance levels). Only significant correlations are shown ($p < 0.05$). SOC is soil organic carbon; TN is total nitrogen; C:N ratio is soil carbon:nitrogen ratio; CEC is cation exchange capacity; Kfs is soil hydraulic conductivity; MWD_{sub} is mean weight diameter of subsoil aggregates; MWD_{top} is mean weight diameter of topsoil aggregates. MAT is mean annual temperature; MAP is mean annual precipitation; MAR is mean annual solar radiation. RD is root diameter; RDMC is root dry mass content; SRL is specific root length; RTD is root tissue density; RMD is root mass density; RLD is root length density; RNC is root nitrogen content; RCC is root carbon content, RC:N is root carbon to nitrogen ratio; com denotes community level root traits and sp denote species level root traits.

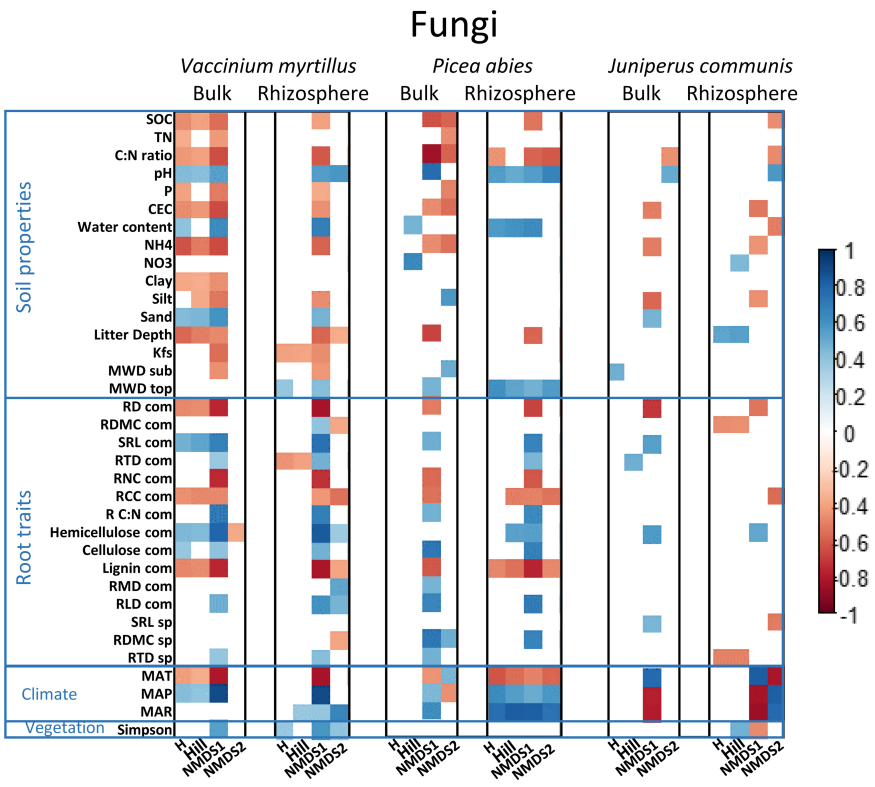

Figure 6. Spearman correlations between fungal α-diversity (H: Shannon index and Hill: observed richness, Hill number, q = 0) and β-diversity (NMDS1 and NMDS2) in bulk and rhizospheric soils with abiotic and biotic elevation-related environmental factors for *V. myrtillus*, *P. abies* and *J. communis* along the elevational gradient (intensity of colour denotes significance levels). Only significant correlations are shown ($p < 0.05$). SOC is soil organic carbon; TN is total nitrogen; C:N ratio is soil carbon:nitrogen ratio; CEC is cation exchange capacity; Kfs is soil hydraulic conductivity; MWD_{sub} is mean weight diameter of subsoil aggregates; MWD_{top} is mean weight diameter of topsoil aggregates. MAT is mean annual temperature; MAP is mean annual precipitation; MAR is mean annual solar radiation. RD is root diameter; RDMC is root dry mass content; SRL is specific root length; RTD is root tissue density; RMD is root mass density; RLD is root length density; RNC is root nitrogen content; RCC is root carbon content, RC:N is root carbon to nitrogen ratio; com denotes community level root traits and sp denote species level root traits.

richness) in the bulk soil, was mainly influenced by SOC content and the structuring species, whilst elevation, structuring species and Simpson diversity were the main driving factors of bacterial α-diversity in the rhizosphere. In contrast, fungal α-diversity in bulk soil was influenced by elevation, whilst the structuring species identity was the main driving factor of fungal α-diversity in rhizosphere soils (Supporting information).

Discussion

We found that microbial α-diversity did not differ along the elevational gradient, except in the rhizosphere of *P. abies* and *J. communis*, where it actually increased for *P. abies* and decreased for *J. communis*, therefore refuting our H1, that expected a general decrease in microbial diversity with limiting abiotic changes at the landscape level. However, our H2 was corroborated because the main factor affecting bacterial and fungal community structure was the soil compartment (i.e. bulk or rhizosphere soil compartments), with bulk soil bacterial structure better explained by soil properties than by structuring plant species identity. Also, in the rhizosphere compartment, fungal β-diversity was more strongly related to plant species than to soil properties and root traits, whereas bacterial β-diversity was better related to plant root traits. In agreement with our H3, bacterial community structure in both bulk and rhizosphere soils were best explained by soil pH, sand content, C:N ratio and $hemicellulose_{com}$, whilst the main factors influencing fungal community structure were RD_{com}, $hemicellulose_{com}$, C:N ratio, soil moisture and MAR.

Microbial community diversity and composition in bulk and rhizospheric soils along the elevation gradient

Overall, microbial (both bacterial and fungal) α-diversity in bulk soil beneath any of the three structuring species did not show any significant patterns with altitude. Although these results are in line with several studies conducted along temperate elevational gradients (Shen et al. 2013, Yuan et al. 2014), these findings refute our first hypothesis, as we expected that microbial α-diversity would decline at higher elevations. We posited that at the landscape scale, the shorter growing season

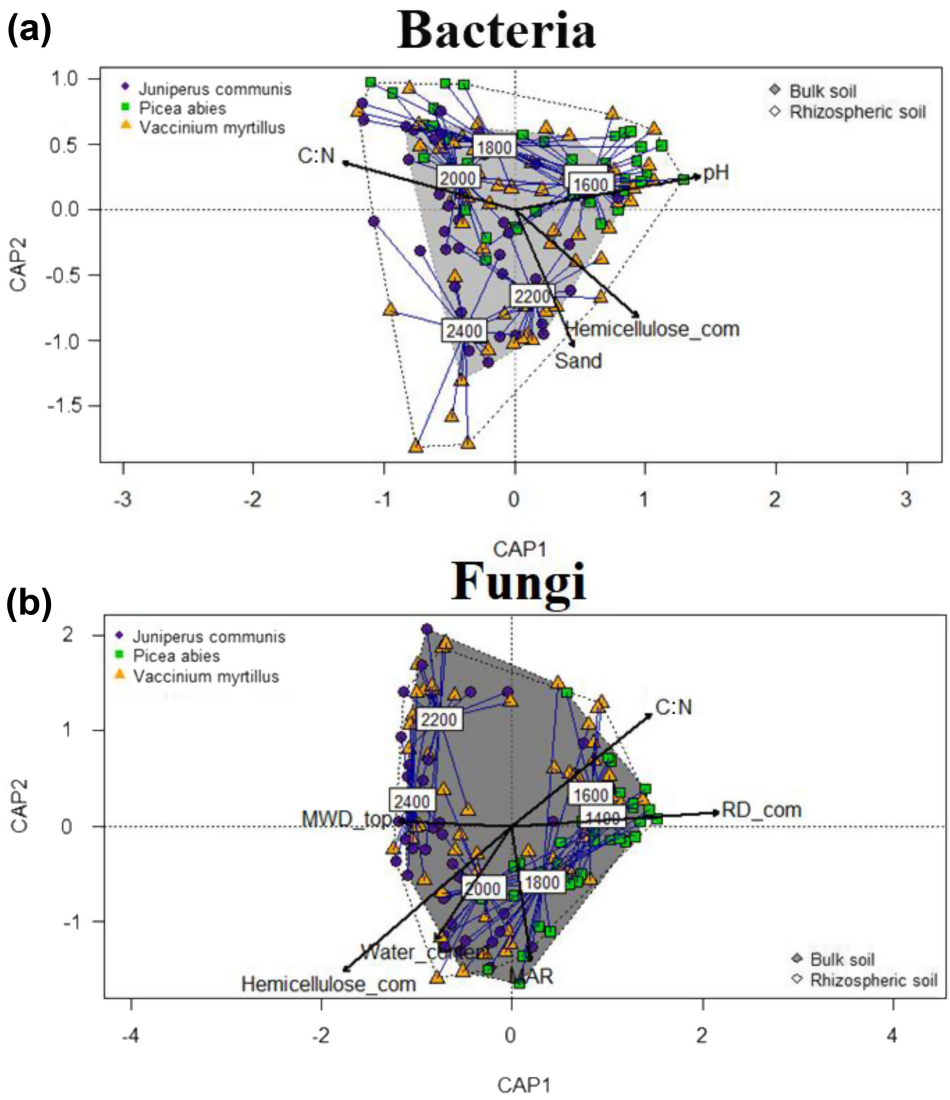

Figure 7. Distance-based redundancy analysis ordination (db-RDA) of (a) bacterial and (b) fungal composition and diversity for six altitudinal bands (spider diagrams) for bulk (convex hull polygon grey) and rhizospheric (convex hull polygon white) soils for *Vaccinium myrtillus* (yellow triangles), *Picea abies* (green squares) and *Juniperus communis* (purple diamonds). Vectors of environmental factors (soil physical and chemical properties, species- and community-level root traits and climatic data) selected by stepwise regression are shown in the constrained ordination: (a) bacteria: pH, sand content, hemicellulose$_{com}$ and C:N ratio; (b) fungi: RD$_{com}$, hemicellulose$_{com}$, MWD$_{top}$, water content, C:N ratio and MAR).

and its impact on reduced plant production and associated organic matter input to soil, as well as the lower soil water holding capacity and higher exposure to UV radiation, would cause distinct patterns in soil microbial α-diversity. However, although overall α-diversity was not impacted by elevation, we did observe changes at a more refined scale, depending on soil compartment, vegetation and fungal/bacterial diversity.

Surprisingly, we found that the Hill-observed bacterial richness in the rhizosphere compartment of *P. abies* increased at higher elevations, although no significant trend was observed beneath the other two structuring species. Our results contrast with those obtained by Cui et al. (2019), who found a hump-shaped pattern of bacterial α-diversity in the bulk soil and rhizosphere of *Abies fabri* along an elevational gradient at the Tibet-Qinghai Plateau, which the authors attributed to differences in soil factors. The increase in relative abundance of spore-forming Firmicutes that we found in the rhizosphere compartment of *P. abies* at the treeline, may enable this family to thrive at this elevation, thus increasing the bacterial population. We also found that fungal α-diversity in the rhizosphere compartment of *P. abies* peaked 200 m below the treeline, in contrast with results reported by Schön et al. (2018) for mature *P. abies* in the Bavarian Alps, where the greatest fungal diversity in the rhizosphere was found at the lowest and highest elevations. Additionally, Cui et al. (2019) found no differences in fungal α-diversity with altitude in the rhizosphere of *A. fabri*, which was attributed to an efficient spore dispersal by wind and animals. In *J. communis*,

we found a decrease in fungal α-diversity in the rhizosphere compartment at higher elevations, but no differences were detected across altitudes in that of *V. myrtillus*. As described by Praeg et al. (2019) for *Ranunculus glacialis*, the lack of any specific pattern in fungal α-diversity with elevation, may be due to the buffering effect of the rhizosphere, that is able to compensate and stabilise the impact of spatial variability of soils and temperature.

The most abundant bacterial phyla detected in our study for both bulk and rhizosphere soil under the influence of the three structuring plant species were Proteobacteria, Actinobacteria, Acidobacteria and Verrucomicrobia. These bacterial phyla have been reported as dominant in alpine soils at different altitudes (Singh et al. 2012, Yuan et al. 2014, Wang et al. 2015, Yao et al. 2017, Praeg et al. 2019, Adamczyk et al. 2020, Ren et al. 2021) and include taxa from the copiotrophic groups (Proteobacteria), as well as oligotrophic groups (Acidobacteria, Verrucomicrobia) (Fierer et al. 2007). However, despite the distinct nutrient acquisition strategies that have associated these phyla with either labile C sources or recalcitrant C compounds (Praeg et al. 2019), our results revealed that these phyla did not have a clear distribution pattern along the elevation gradient. Nor were there differences between bulk and rhizosphere soil bacterial composition at the phylum level, under any of the three structuring plant species.

With regard to fungi, dominant phyla along the elevation gradient were Ascomycota and Basidiomycota, in agreement with previous reported findings in other elevation studies (Tedersoo et al. 2014, Frey et al. 2016, Yao et al. 2017, Cui et al. 2019, Ren et al. 2021). Contrasting distribution patterns between bulk and rhizosphere soils were observed in terms of fungal composition beneath the three structuring plant species. For example, in the rhizosphere compartment of *V. myrtillus*, the relative abundance of Ascomycota increased, while that of Basidiomycota decreased linearly along the elevation gradient; a similar non-linear pattern was present in the bulk soil. *V. myrtillus* forms associations with ERM fungi, most of them belonging to the Ascomycota phylum, which prefer cold climates compared to Basidiomycota that prefer warmer sites (Zhang et al. 2016), suggesting the elevational pattern displayed in our study. Interestingly, our results contradict the oligotrophic-copiotrophic theory from Yao et al. (2017), as we found that in the rhizosphere of *V. myrtillus*, oligotrophic Basidiomycota were more abundant at lower, nutrient-rich elevations, also characterised by a greater catabolic activity than at higher elevations (Hernández-Cáceres et al. 2022). As suggested by Guo et al. (2020), the response of Basidiomycota and Ascomycota to altitude may be due to their different abilities to decompose organic compounds. Ascomycota are responsible for the breakdown of labile C compounds and are found in greater abundance during early stages of organic matter and plant residue decomposition (Osono and Takeda 2001, Voríšková and Baldrian 2013), while Basidiomycota degrade more complex and recalcitrant C compounds, being more abundant in later stages of decomposition (Hannula et al. 2010, Voríšková and Baldrian 2013). Additionally, our results show relative proportions and thus, the relative proportion of Basidiomycota is directly influenced by the abundance of Ascomycota, which is related to the ERM associations of *V. myrtillus*.

Role of soil properties and climatic data on shaping microbial composition and diversity

Our results showed a strong influence of soil properties on microbial structure and diversity in both bulk and rhizosphere soils. Overall, in bulk soil beneath the three structuring plant species, soil nutrient content (i.e. SOC, TN, P, NH_4^+ and CEC) was negatively correlated with bacterial α-diversity, indicating a general decline of bacterial α-diversity with increasing soil fertility. High levels of nutrients in soil promote the dominance of copiotrophic phyla such as Proteobacteria (Ren et al. 2021), and as we found a decrease in the dominance of Proteobacteria in bulk soil at high altitudes, we can assume this decrease was because of reduced soil C levels.

Bacterial α-diversity in the rhizosphere of *V. myrtillus* and *P. abies* was positively correlated with pH and there was also a strong influence of pH on bacterial β-diversity in both soil compartments (bulk and rhizosphere soil). This result is in line with those from several studies in temperate mountains reporting pH as the main driver of soil bacterial community structure (Fierer and Jackson 2006, Liu et al. 2016, Fierer 2017, Wu et al. 2017). The strong influence of pH on bacterial communities can be due to the narrow pH ranges for optimal growth of bacteria (Rousk et al. 2010) and its role for nutrient supply and acquisition (Praeg et al. 2019). However, fungal β-diversity in both soil compartments was not significantly influenced by soil properties, nevertheless, we detected some significant correlations between fungal α-diversity and some soil properties, especially those related with nutrient content and pH in bulk soil under *V. myrtillus*. Additionally, we showed that C:N ratio and aggregate stability in the surface soil were the soil properties that best explained differences in fungal community structure in both soil compartments. Soil C:N ratio is often used as a proxy for nutrient availability (Cleveland and Liptzin 2007), so changes in C:N ratio can influence fungal metabolism and alter soil elemental stoichiometric balance (Sinsabaugh et al. 2009, Grosso et al. 2016, Ni et al. 2018). These results agree with our (H3) hypothesis, which stated that fungal community structure in bulk soil is more related to soil nutrient content than to plant diversity or related traits. Although previous studies reported that pH often correlates with fungal diversity to a lesser extent than with bacteria (Rousk et al. 2010, Shen et al. 2014), evidence from mountain ecosystems suggests that fungal diversity, especially in bulk soil, is closely associated with soil nutrient status and C levels (Lauber et al. 2008, Tedersoo et al. 2014, Praeg et al. 2019).

Climatic data along the gradient did not contribute to explain changes in microbial community structure and diversity in either soil compartments. Also, only a few correlations existed between climatic data and microbial community

structure. Bacterial α-diversity in bulk soil under *J. communis* was positively correlated with MAT and negatively with MAR and MAP, while bacterial α-diversity in the rhizosphere under *P. abies* was negatively related to MAT and positively to MAR and MAP. Finally, fungal α-diversity was correlated to MAT and MAP in bulk soils beneath *V. myrtillus* and the rhizosphere of *P. abies*. Contrary to our results, several other studies also showed climatic factors as a main driver of microbial community structure in bulk and rhizosphere soils (Chen et al. 2017, Ren et al. 2018, Praeg et al. 2019). However, we did not sample at several periods throughout the year, nor measure real-time climate data, therefore, our data may not be exhaustive.

Impact of plant diversity and root traits on microbial diversity and community composition

We found that both *community*- and *species*-level root traits did not have a significant overall effect on bacterial community structure, in either soil compartment, when species were considered together. A significant effect of elevation on bacterial α-diversity was only detected in the rhizosphere compartment of *P. abies*. With regard to bacterial community structure, non-significant effects of root traits were found for fungal community structure in bulk and rhizosphere soil. However, in both *V. myrtillus* and *P. abies*, RTD (at the *species* level) and root C:N, SRL, RD, RNC, RCC and lignin, hemicellulose, cellulose content (at the *community* level) were correlated with fungal β-diversity in the bulk soil and rhizosphere compartments. Consistently with Merino-Martín et al. (2020), working at the same site, relationships were stronger between root traits and bacterial communities in the rhizosphere compared to the bulk soil (particularly for *P. abies*). Therefore, changes in niche dimensionality as a result of root tissue quality, rather than morphological traits, shapes bacterial communities.

Bacterial and fungal β-diversity in bulk and rhizosphere soils beneath *V. myrtillus* and *P. abies* were positively correlated with plant diversity. Plant richness can be an important driver shaping rhizosphere fungal communities (Chen et al. 2017), nevertheless, the real contribution of plant diversity to fungal community structure is still in debate. For example, some studies on elevational gradients have found that plant diversity modified bacterial, but not fungal diversity (Shen et al. 2014, Siles and Margesin 2016, Ren et al. 2018), but others have found that plant species identity and diversity were strongly related with shifts in fungal community structure (Barberán et al. 2015, Chen et al. 2017, Tian et al. 2017). Furthermore, the three species selected in this study have different mycorrhizal associations, which will influence their contribution to bacterial and fungal community structures. As recently reported by Carrara et al. (2021), AMF plants primarily depend on saprotrophic bacteria for mineral N acquisition, whereas in ECM/ERM hosts, most of the N uptake comes from organic pools and is carried out by fungal symbionts. These differences in resource acquisition strategies are likely to affect microbial communities in the rhizosphere niche, directly or indirectly through changes in litter chemistry (Cheeke et al. 2017). As an example, ECM/ERM plants have been associated with higher soil bacterial richness, but lower fungal richness than AMF plants, in a global treeline assessment (Collins et al. 2020).

Conclusion

Our study revealed that soil compartment (i.e. bulk soil versus rhizosphere) had the strongest effect on microbial community structure compared to the effects of elevation and structuring plant species. Additionally, elevation had a greater effect than structuring plant species, regardless of soil compartment. Bacterial and fungal community structure responded differently to elevation and their response depended on the soil compartment, the rhizosphere being the compartment where the most significant responses occurred. Our findings also showed that changes in bulk soil microbial communities with elevation were related mostly to soil physical and chemical properties, while shifts in rhizosphere microbial communities along the gradient were more related to plant species identity, vegetation diversity and belowground plant traits. Overall, these results highlight that changes at the landscape scale (e.g. associated to elevation, soil properties or climate), impact significantly soil microbial communities but vegetation refines communities at a local scale via the rhizosphere niche. Root chemical traits that alter the available substrate to microbial communities (especially for bacterial communities) are the main factors impacting dimensionality of the rhizosphere niche.

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Author contributions

Luis Merino-Martín: Conceptualization (equal); Data curation (equal); Formal analysis (equal); Methodology (equal); Software (equal); Supervision (equal); Writing – original draft (equal); Writing – review and editing (equal). **Daniel Hernandez-Caceres**: Data curation (equal); Formal analysis (equal); Methodology (equal); Writing – original draft (equal); Writing – review and editing (equal). **Frédérique Reverchon**: Conceptualization (equal); Formal analysis (equal); Methodology (equal); Supervision (equal); Writing

– review and editing (equal). **Guillermo Angeles-Alvarez**: Project administration (equal); Resources (equal); Writing – review and editing (equal). **Guangqi Zhang**: Formal analysis (equal). **Denis Dunoyer de Segonzac**: Methodology (equal); Software (equal). **Damien Dezette**: Methodology (equal). **Alexia Stokes**: Conceptualization (equal); Formal analysis (equal); Funding acquisition (equal); Methodology (equal); Project administration (equal); Resources (equal); Supervision (equal); Writing – original draft (equal); Writing – review and editing (equal).

Data availability statement

All the data that support the findings of this study are freely and openly available. Raw sequences are deposited at NCBI (BioProject ID: PRJNA826983; <www.ncbi.nlm.nih.gov/bioproject/PRJNA826983>) and the rest of the data (environmental and soil properties and root traits) can be accessed on Portail Data INRAE at <https://entrepot.recherche.data.gouv.fr/dataverse/ecopics>.

Supporting information

The Supporting information associated with this article is available with the online version.

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