Soil Biology and Biochemistry 165 (2022) 108485

Contents lists available at ScienceDirect

Soil Biology and Biochemistry

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

Vegetation creates microenvironments that influence soil microbial activity and functional diversity along an elevation gradient

Daniel Hernández-Cáceres [a], Alexia Stokes [a,*], Guillermo Angeles-Alvarez [b], Josiane Abadie [c], Fabien Anthelme [a], Manon Bounous [a], Grégoire T. Freschet [d], Catherine Roumet [e], Monique Weemstra [a,e], Luis Merino-Martín [f], Frédérique Reverchon [g]

[a] University Montpellier, AMAP, INRAE, CIRAD, CNRS, IRD, Montpellier, France
[b] Red de Ecología Funcional, Instituto de Ecología, A.C., Xalapa, Veracruz, Mexico
[c] Eco&Sols, Univ Montpellier, CIRAD, INRAE, IRD, Montpellier SupAgro, Montpellier, France
[d] Station d'Ecologie Théorique et Expérimentale, CNRS, 2 route du CNRS, Moulis, 09200, France
[e] CEFE, Univ Montpellier, CNRS, EPHE, IRD, Montpellier, France
[f] Departamento de Biología y Geología, Física y Química inorgánica, ESCET, Universidad Rey Juan Carlos, C/Tulipán s/n, Móstoles, C.P. 28933, Madrid, Spain
[g] Red de Estudios Moleculares Avanzados, Instituto de Ecología, A.C., Pátzcuaro, Michoacán, Mexico

ARTICLE INFO

Keywords:
Alpine
Catabolic activity
Catabolic diversity
Juniperus communis
Multiple substrate-induced respiration
Picea abies
Root traits
Vaccinium myrtillus

ABSTRACT

Soil microbial communities are responsive to abiotic and biotic conditions within the heterogeneous soil environment. In montane plant communities, vegetation can create distinctive microenvironments that have unique microbial responses. Here, we ask how soil microbial activity and functional diversity were influenced by the type and diversity of montane plant species, and the morphological and chemical traits of their associated root systems, that are expected to influence soil properties. Along an elevational gradient (1400-2400 m a.s.l.) in the French Alps, we investigated microbial global catabolic activity (i.e. microbial activity) and catabolic diversity (i.e. functional diversity) in bulk and rhizosphere soil beneath three plant species (*Vaccinium myrtillus*, *Juniperus communis* and *Picea abies*) using multiple substrate-induced respiration. We also measured soil physical and chemical properties, plant diversity, climatic factors and morphological and chemical traits of roots in bulk soil ('*community*' level traits, where several plant species were pooled together) and of individual plants ('*species*' level, where roots of single species were excavated). At lower elevations, global catabolic activity in the rhizosphere was higher than in bulk soil, but converged in the nutrient-poor, colder soils found at higher elevations, although changes in catabolic diversity were negligible. Variations in soil texture, cation exchange capacity, carbon and nitrogen content and pH were associated with the global catabolic activity, but these soil properties had minimal effects on catabolic diversity. Climatic variables were related to microbial activity beneath *V. myrtillus* only and warmer mean annual temperatures increased activity. Plant root traits at the *community* level in bulk soil had less effect on global catabolic activity than abiotic factors, with thicker roots, high root lignin content and low cellulose content influencing microbial activity, but not altering catabolic diversity. At the *species* level, more dense root tissue decreased global catabolic activity, reflecting changes in chemical composition. Overall, our results show that soil physical and chemical properties were the main drivers of microbial activity, but that vegetation created distinctive microenvironments that refined these relationships, mainly through modifications in root chemical traits.

1. Introduction

Mountainous regions are characterised by abrupt changes in physical, edaphic and biotic environments over short geographic distances because of the presence of elevational gradients. These gradients create a variety of ecological habitats (Körner et al., 2011; Abbott and Brennan, 2014; Hofmann et al., 2016), which makes them ideal for studying biodiversity patterns and their drivers. Several studies have emphasised the importance of investigating soil microbial community structure and function along elevation gradients, as soil microorganisms are often

* Corresponding author.
E-mail address: alexia.stokes@cirad.fr (A. Stokes).

https://doi.org/10.1016/j.soilbio.2021.108485
Received 9 April 2021; Received in revised form 1 November 2021; Accepted 6 November 2021
Available online 9 November 2021

more responsive to environmental changes than other organisms (Shen et al., 2015; Cui et al., 2019; Bardgett and Caruso 2020). Soil microorganisms play a key role in a wide range of ecosystem processes, such as decomposition of organic matter, nutrient cycling, erosion control through their action on soil aggregate stability, soil development and maintenance of structure (Prosser et al., 2007; Maron et al., 2011; Aislabie et al., 2013; Xu et al., 2019; Merino-Martín et al., 2021). As recently emphasised by several studies, there is a crucial need to consider belowground processes and integrate them with vegetation data in order to better predict soil dynamics and response to climate change (Cavicchioli et al., 2019; Hagedorn et al., 2019; D'Alò et al., 2021). We ask therefore, if it is possible to determine how climatic factors alter microbial activity and functional diversity, depending on soil and vegetation type, in a complex mountainous environment.

In temperate latitudes, (sub)alpine soils are very heterogeneous and are typically rocky, acidic and shallow with poorly developed horizons (Price and Harden, 2013). As elevation increases, plant growth and cover are mainly limited by climatic conditions and a shorter growing season, causing a reduction of organic matter content in soils at higher elevations and making soils nutrient-limited for plant growth (Zhou et al., 2002; Donhauser and Frey, 2018; Möhl et al., 2019; Stokes et al., 2021). Microclimatic conditions strongly influence soil microbial communities (Donhauser and Frey, 2018; Collins et al., 2020) and therefore microbial activity and functional diversity. Here, we define microbial activity as the global catabolic activity, an indicator of the functional capacity of microbial communities to decompose organic matter. Aditionally, we define microbial functional diversity as the catabolic diversity, i.e. the capacity of a microbial community to use different substrates for respiration, as measured through catabolic response profiles (Degens and Harris, 1997; Degens et al., 2001). Catabolic activity and diversity are strongly influenced by soil variables, especially pH (Moscatelli et al., 2018) and total and labile organic carbon (C) contents, assessed mainly through studies of increased fertilisation by organic amendments across a wide range of land uses (Degens et al., 2000; Margesin et al., 2009; Tian et al., 2015; Bongiorno et al., 2020). Moreover, catabolic activity and diversity increase with warmer temperatures in different grasslands (Grayston et al., 2001; Papatheodorou et al., 2004) and alpine climates (D'Alò et al., 2021). As climatic conditions are also determined by topography, they further influence variables such as soil nutrient availability and the quantity of litter input to the soil. Together, these environmental variables will control the catabolic activity and diversity of soil microorganisms (Kang et al., 2009). However, the linkages between catabolic activity, diversity and soil properties are not yet fully elucidated, particularly in a complex, heterogeneous landscape, where soil type, climate and vegetation all interact to modify microbial community structure and function.

Vegetation can alter global catabolic activity and diversity either at the community level or through specific species effects. Several studies have shown that catabolic diversity increased with higher levels of plant diversity e.g., along a longitudinal gradient in temperate grasslands (Liu et al., 2008) and along an elevational gradient in temperate forests (Klimek et al., 2015). These effects were mainly attributed to increases in plant biomass and mediated by soil factors. The influence of increased plant diversity on microbial catabolic activity and diversity is likely through an increase in the variety of C sources, promoting a heterogeneous distribution of soil properties (Reverchon et al., 2015). However, individual plant species also alter the structure and function of soil microbial communities, through (i) shoot and root litter production (Xu et al., 2018), (ii) root exudation patterns (Williams et al., 2021) and (iii) above- and below-ground functional traits (Spitzer et al., 2021; Sweeney et al., 2021). Microbial activity and functional diversity are enhanced in litter-rich soil because of the large amount of C available as substrate for decomposers (Nsabimana et al., 2004; Nuccio et al., 2020), although the quality of litter, especially its content in soluble C, also has a determining effect on microbial catabolic activity (Fanin et al., 2014). In particular, root nitrogen (N) and C content should increase microbial respiration and decomposition of soil organic matter (Han et al., 2020), whereas roots that are rich in more recalcitrant compounds such as lignin and cellulose could limit microbial activity (Poirier et al., 2018).

Within bulk soil, microbial processes can be altered by the proximity of plant roots. Plant roots release organic compounds that modify microbial catabolic activity and functional diversity, since many microorganisms use these root exudates and mucilage as their main energy source (Liu et al., 2008; Hobbie and Hobbie, 2013). These interactions take place with a greater intensity in the rhizosphere, a narrow zone around the root with high concentrations of easily degradable carbon sources, leading to an inflated rate of microbial activity and a greater functional diversity compared to bulk soil (Baudoin et al., 2002; Yang et al., 2013; Kuzyakov and Razavi, 2019; Nuccio et al., 2020). Therefore, plant root traits that promote rhizosphere dimensions, e.g., long, thin roots that have a large surface area, should also enhance microbial catabolic activity and diversity. Woody root traits that have the strongest effect on microbial catabolic activity and biomass include specific root length (SRL: length/mass ratio), root branching intensity and root diameter, whereas root chemical composition (C, phosphorus, calcium and magnesium content) has been found to have less effect (Khlifa et al., 2017; Sweeney et al., 2021). Thicker roots increase the volume of cortex available for mycorrhizal fungi colonisation, which would enhance nutrient uptake and soil nutrient cycling (Burke et al., 2011; Xiao et al., 2019; McCormack and Iversen, 2019), hence potentially promoting microbial activity in the rhizosphere (Chen et al., 2018). However, although root exudation is positively correlated to root diameter in grassland species (Williams et al., 2021), it is negatively related to root diameter in trees (Han et al., 2020). We therefore expect that rhizosphere microbial communities relying on root exudates as a substrate should be less active around thicker woody roots with relatively small rhizosphere dimensions compared to highly branched roots or roots with a high SRL.

We aimed to unravel the effect that biotic and abiotic factors exert on global catabolic activity and diversity in bulk and rhizosphere soils in a heterogeneous environment. To do this, we examined changes in climatic parameters, soil properties, plant species diversity and root traits along an elevation gradient (without considering microtopography), and their relationships with global catabolic activity and diversity. We investigated the global catabolic activity and diversity in soils beneath three different plant species, in both rhizosphere soil and bulk soil where a mixture of roots from other plant species would be present. We hypothesized that microbial catabolic activity and diversity would: (H1) decrease in colder and nutrient-poor soils from higher elevations, where reduced plant growth limits organic matter supply to soil, compared to soils at lower altitudes; (H2) increase at higher levels of plant diversity, especially in bulk soils, because of the broader variety of C sources available from litter and root exudates, and (H3) increase around roots possessing traits that enlarge rhizosphere dimensions i.e. longer, thinner roots, that have large exchange surface areas and potentially more available exudates. However, roots that are rich in lignin and cellulose, could limit microbial activity, because these compounds are highly recalcitrant.

2. Material and methods

2.1. Study site and plant species

Fieldwork was conducted along an 8 km elevational gradient located in the Belledonne massif in the French Alps (France, N 45° 7′ 1″ E 5° 53′ 35′, Table 1). The elevation gradient had six altimetric bands, each 200 m, and ranged from 1400 to 2400 m a.s.l. The current thermal treeline is situated between 2000 and 2100 m (Wang et al., 2018a,b). Below the treeline, the dominant trees shifted along the gradient from mixed forest of *Fagus sylvatica*, *Pinus sylvestris* and *Abies alba* in the montane belt to mixed forests of *Picea abies*, *Pinus uncinata* and *Pinus cembra* in the subalpine belt. Above the treeline, the vegetation was dominated by

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 details on data are available in the data paper Stokes et al., 2021 and raw data are available at https://data.inrae.fr/dataverse/ecopics).

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

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

(sub)alpine heaths of Ericaceae (*Vaccinium* spp, *Rhododendron ferrugineum*, *Loiseleuria procumbens* (L.) Loisel.) and *Juniperus communis* subsp. *nana* and grasslands dominated by graminoid species (*Nardus stricta*, *Carex sempervirens*, *Festuca* spp). Mean annual temperature (MAT), mean annual precipitation (MAP) and mean annual solar radiation (MAR) were calculated using the meteorological AURELHY model (Piedallu et al., 2013, 2016) during a ten year period (2004–2014) at each elevation (Stokes et al., 2021, Table 1). MAT decreased from 8.5 ± 0.2 °C to 5.7 ± 0.2 °C up the elevation gradient, while MAP increased from 1024 ± 41 mm to 1187 ± 40 mm. MAR varied from 4204 MJ/m^2 to 4339 MJ/m^2 up along the elevation gradient (Table 1, Fig. S1).

Five plots (20 × 20 m) were selected within each altitudinal band, with a lateral distance of at least 100 m between each plot (Fig. 1a and b) in June 2018. Plots were selected if they included two or three species: *P. abies*, a tall evergreen tree; *Juniperus communis* L., a prostrate evergreen shrub and *Vaccinium myrtillus* L., a small deciduous shrub (if these species were present at that elevational band, Table S1). These three plant species were selected because of their different growth forms and occupation of different ecological niches along the elevational gradient. *P. abies* was the dominant tree species below the tree line; *J. communis* was abundant above 1800m and *V. myrtillus* was one of the most frequent species above the treeline, as well as being present in all six elevational bands. *P. abies* and *V. myrtillus* are both keystone species (Bjune et al., 2009; Nybakken et al., 2013) and although *J. communis* is not classed as a keystone species, its abundance above the treeline makes it an important member of the plant community. Therefore, these three species contribute to shaping the structure of the plant communities in which they are present, and we term them 'structuring species'.

Within each plot, one well-developed individual of each structuring species was selected and at the limit of its canopy, on the downslope side, a 1.0 × 1.0 m quadrat was located for further plant and soil sampling. A botanical survey was first performed in each quadrat (data are provided in Stokes et al., 2021). Plants were identified using two floras (*Flore Forestière Française*, Montagnes, Rameau et al., 1999 and *Flora Helvetica*, Laufen et al., 2001) and their abundance was estimated from a visual assessment of the relative area covered by different plant species in each quadrat. Simpson diversity index (*S*) was calculated as following:

$$S = 1 - \frac{\sum n(n-1)}{N(N-1)} \quad (1)$$

where *n* is the number of individuals of each species and *N* the total number of individuals of all species.

2.2. Bulk and rhizosphere 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 (Fig. 1, c). Bulk soil was collected from the monolith's four lateral faces, sieved at 2 mm, air-dried and stored at room temperature before further analyses. These analyses comprised the assessment of soil physical and chemical properties and catabolic activity and diversity (Stokes et al., 2021). In total, 70 bulk soil samples were taken (six elevations × five plots × two to three plant species).

Rhizosphere soil was taken from plant roots of the three structuring

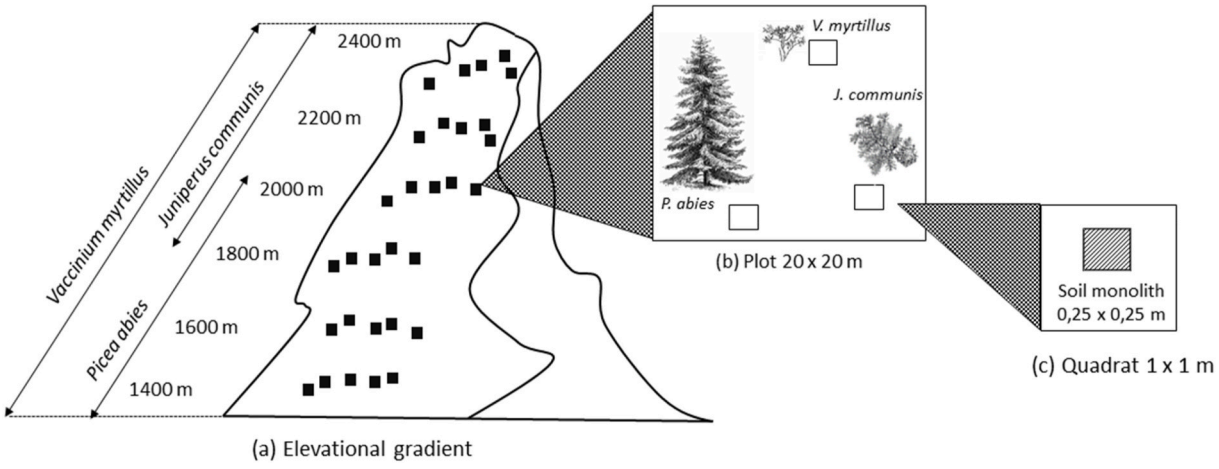

Fig. 1. Sampling design along the elevational gradient. (a) Six elevational bands, situated at 200 m from each other, were located along the gradient, ranging from 1400 m to 2400 m a.s.l. Five plots (20 m × 20 m) containing two or three selected structuring plant species were located at each altitude. (b) At the canopy limit of the structuring plant species, a 1 m × 1 m botanical survey was performed. (c) In the center of this quadrat, a soil monolith (0.25 m × 0.25 m x 0.15 m) was extracted.

species in the same plots and close to the monoliths, using gloves and ethanol sterilized material. Individuals of *V. myrtillus* were identified and root systems extracted manually. Soil attached to the youngest roots (comprising the root apices) was sampled by gently shaking the roots inside paper bags whilst still 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 (roots with a diameter $\leq$2 mm, whose primary function is resource uptake (Freschet and Roumet, 2017)), attached to this large root were collected and then shaken in paper bags. All 70 rhizosphere soil samples were taken to the laboratory and were air-dried for at least one week and stored at room temperature prior to catabolic activity and diversity analyses. We air-dried our soil samples to avoid logistical constraints linked to sampling, transport and storage (Gillespie et al., 2021).

2.3. Soil physical and chemical properties

Soil physical and chemical properties were measured in the same monoliths where we sampled bulk soil (Stokes et al., 2021). Analyses were performed on three replicates of 60–80 g of soil at the Laboratoire d'Analyses des Sols (INRAE, Arras, France), and included texture (clay, silt and sand content; pipette method, NF X 31–107), cation exchange capacity (CEC; Metson method, NF X 31–130), total soil organic C (SOC; dry combustion, NF ISO 10694), total N content (TN; dry combustion, NF ISO 13878), available phosphorus (P; Olsen method, NF ISO 11263) and pH (NF ISO 10390). Nitrate (NO_3^-) and ammonium (NH_4^+) were measured by extraction with potassium chloride solution on bulk soil samples (NF ISO 14256, CIRAD Montpellier, France). Aggregate stability, expressed by mean weight diameter (MWD), was determined on bulk soil samples taken from two depths (topsoil [MWD_{top}] at a depth of 0–0.25 m and subsoil [MWD_{sub}] at a depth of 0.25–0.50 m), using the fast wetting standard method (Le Bissonnais, 1996; ISO/CD 10930). Infiltration tests were carried out to measure soil hydraulic conductivity (Kfs), using a quasi-steady infiltration rate in a single ring infiltrometer (Marín-Castro et al., 2016; Wu et al., 1999) and the soil water volumetric content at a depth of 0.05–0.10 m beneath the soil surface was measured with soil moisture meter TDR100 (6440FS, from Fieldscout – Spectrum technologies).

2.4. Microbial global catabolic activity and diversity of soil microbiota: multiple substrate-induced respiration (MSIR; MicroResp™ analysis)

Multiple substrate-induced respiration (MSIR) is a method for characterising and assessing the activity and functional diversity of soil microbiota. This method is used to assess the soil microbial functional capacity in C cycling (Creamer et al., 2016; Fromin et al., 2020) and is therefore used as a proxy for global catabolic activity and diversity of soil microbial communities (i.e., the mineralization of organic C into carbon dioxide (CO_2), Beare et al., 1990 and Nannipieri et al., 1990). Here, we used the MicroResp™ system (Macaulay Scientific Consulting, Aberdeen, UK) to characterize the community level physiological profiles of the soil microbial communities and assess the capacity to use multiple substrates for respiration (i.e. catabolic diversity). Since the production of CO_2 is measured within 6 h after inducing respiration with the addition of substrates (Chapman et al., 2007), the advantage of this technique is that it gives immediate responses to C substrate decay instead of relying on microbial growth. Briefly, both bulk and rhizosphere soil samples were adjusted to 80% of their maximum water holding capacity and pre-incubated for 1 week at 25 °C in deepwell plates, that holds approximately 0.45 g $well^{-1}$ of soil (Baratella and Pinzari, 2019), before applying aqueous solutions of the different C substrates and assembling the deepwell-detection plate system. In this study, we used twelve substrates (with four replicates per substrate) of the three main families of organic compounds found in soil and root exudates; sugars (D-glucose, arabinose, N-acetyl-glucosamine), amino acids (L-alanine, L-lysine, L-asparagine, L-glycine) and organic acids (oxalic acid, malic acid, citric acid, vanillic acid) (Badri and Vivanco, 2009). Sugars and amino acids represent a main source of litterfall- and root-derived C compounds in the soil (Jones and Murphy, 2007) while organic acids are predominantly liberated in the form of root exudates (Farrar et al., 2003). All substrates were added at a concentration of 100 g L^{-1} except for L-asparagine (50 g L^{-1}) and vanillic acid (10 g L^{-1}) due to their low solubility. Additionally, water was used as a control to measure soil basal respiration that is defined as the steady rate of respiration in soil from the mineralization of organic matter. CO_2 emission was estimated using a colorimetric method. Detection plates containing 12.5 μg g^{-1} cresol red, 150 mM KCl and 2.5 mM $NaHCO_3$ set in 1% Noble agar were prepared and 150 μl of the solution were distributed per well. After pre-incubation in the dark, each deepwell plate was covered with a detection plate using a silicon seal. The system was secured with a clamp and incubated for 6 h at 28 °C. The optical density of each plate was determined using a microplate reader (Victor3 1420 Multilabel Counter, PerkinElmer, MA, USA) at 570 nm before and after incubation. The optical density was normalized and converted to multiple substrate-induced respiration rates (MSIR) expressed in μg CO_2 g^{-1} dry soil h^{-1} (Fromin et al., 2020). The respiration rates for the different C compounds were summed across all substrates as a proxy of the global catabolic activity. The catabolic diversity index (Shannon index, *H'*), an indicator of the soil microbial functional diversity (Sparling et al., 2000), was calculated for each sample using Equation (2):

$$H' = -\sum_{i=1}^{12} p_i * ln(p_i) \quad (2)$$

where p_i was the respiration rate for a given substrate divided by the sum of the total respiration rates of that particular substrate.

2.5. Measurements of community- and species-level root traits

2.5.1. Community level root traits

After the monoliths were extracted, the depth of the litter layer was noted and the litter removed. Half of the monolith was dissected to extract all roots (hereafter *community* level), that represented a mix of roots from one of the structuring species and from different plant species growing in the monoliths. This *community* level mixture of roots therefore comprised a large proportion of roots from one of the three structuring species. Within 1 day but sometimes up to 2 days after sampling, roots from the *community* level were sorted and fine, undamaged roots with a diameter <2 mm were removed and washed. Absorptive roots are lower root orders, typically the first, second and third root orders (defined as the most distal root orders) (Pregitzer et al., 2002; Freschet and Roumet, 2017). A subsample of absorptive roots were selected, scanned (Epson Expression 1680, Canada) in a tray of water and analysed (Winrhizo Pro version 2019, Regent Instruments, Canada), following Roumet et al. (2016). At the *community* level, root length (L_{com}), mean root diameter (RD_{com}), and root volume (V_{com}) were estimated in different diameter classes (from 0 to 2 mm diameter with a 0.1 mm bin size). 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}) as the ratio between L_{com} and RDM_{com}, root tissue density (RTD_{com}) as the ratio between RDM_{com} and V_{com}, root dry mass content ($RDMC_{com}$) as the ratio between RDM_{com} and RFM_{com}, root length density (RLD_{com}) and root mass density (RMD_{com}) were calculated for roots at the *community* level (Table S2). Root nitrogen (RNC_{com}) and carbon (RCC_{com}) content were measured on absorptive roots using an elemental analyser (CHN model EA 1108; Carlo Erba Instruments, Italy). The content of water-soluble compounds, cellulose, hemicellulose and lignin in absorptive *community* level roots was determined using the Van Soest method (Van Soest, 1990) in a fiber analyser (Fibersac 24; Ankom, Macedon, NJ, USA).

2.5.2. Species level root traits

Species level root trait data were obtained from a separate study by Weemstra et al. (2021) who examined root traits of 11 individual plant species in the same plots and at the same dates, including the three structuring species that we examined, along the same elevational gradient. In their study, roots from the three structuring plant species (*P. abies*, *V. myrtillus* and *J. communis*) were dug carefully from the top 0.15 m soil horizon and below the litter layer. Approximately 3–5 different roots were dug at a distance of 0.5 – 1.5 m from the base of the stem and traced back to the stem to verify that they belonged to the chosen plant individuals. Roots were stored in moist plastic bags and refrigerated until analysed in the laboratory. Within 1 day but sometimes up to 2 days after sampling, roots were washed and undamaged absorptive roots were scanned and SRL_{sp}, $RDMC_{sp}$, RTD_{sp} at the *species* level were calculated as described above for roots harvested at the *community* level.

2.6. Data analysis

We examined the variations in global catabolic activity and catabolic diversity (H') in bulk and rhizospheric soils with regard to elevation and structuring plant species.

Soil physical and chemical properties, *community*- and *species*-level root traits and global catabolic activity and diversity along the elevational gradient, were analysed as a function of elevational band using one-way analysis of variance (ANOVA) and Tukey's HSD to test significant differences among elevational bands. Unbalanced two-way ANOVA (Type-III sums of squares) was performed to assess the effect of elevation, structuring plant species and their interaction on the different soil physical and chemical properties and *community*- and *species*-level root traits.

Spearman's correlation coefficients were calculated to study the relationships between catabolic activity and diversity, climatic data, soil properties, plant diversity and *community*- and *species*-level root traits, along the elevational gradient for each one of the three structuring species.

Dissimilarity in global catabolic activity between bulk and rhizosphere soils, among elevation bands and structuring plant species, was examined using non-metric multidimensional scaling (NMDS) analysis with the Bray-Curtis distance. Permutational multivariate analysis of variance (PERMANOVA), implemented with the *adonis* function from the vegan R package (Oksanen et al., 2020), was used to assess the significance of the observed NMDS differences.

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 variations in global catabolic activity can be explained by environmental variables along the elevational gradient, followed by a stepwise model selection using Generalized Akaike Information Criterion (AIC, *ordistep* function of vegan R package with forward and backward direction). Finally, db-RDA analysis was performed only for the variables obtained from the model selection.

Variation partitioning analysis, using the function *varpart* of the R vegan package, 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 catabolic activity, which was later identified by partial redundancy analysis in Hellinger transformed data.

3. Results

3.1. Changes in soil physical and chemical properties along the elevation gradient

Beneath *V. myrtillus* and *P. abies*, soil nutrient content (SOC, TN and P), CEC and C/N ratio varied little along the elevational gradient except at 1600 m where they were significantly higher than at all other elevations (Table S3). Beneath *J. communis*, changes in these properties were negligible along the gradient (Table S3). In bulk soil beneath *P. abies*, NH_4^+ content was significantly higher at 1600 m than at all other elevations, but under *V. myrtillus* and *J. communis*, there were no significant differences among elevational bands (Table S3). With regard to NO_3^- content, there were no significant changes along the gradient beneath any structuring species (Table S3). Litter depth beneath *P. abies* decreased significantly from 1400 m to 2000 m while under *V. myrtillus* the main change was between 1400 and 1600 m. No trends beneath *J. communis* occurred along the gradient (Table S3). Soils beneath all three plant 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 (Table S3). Both elevation and structuring plant species were not significantly related to changes in the majority of soil physical and chemical properties (ANOVA analysis, Tables S4). However, elevation had a significant influence on silt and sand content ($p < 0.001$) and structuring species had a significant impact on MWD_{top} and soil water content ($p < 0.001$; Table S4).

3.2. Global catabolic activity and diversity (H') in bulk and rhizosphere soil along the elevation gradient

No significant differences were found between basal respiration and global catabolic activity, therefore, to simplify results, we present values for global catabolic activity only (Table S5).

In bulk soil beneath *V. myrtillus* and *P. abies*, global catabolic activity fluctuated along the gradient with a significant peak (pairwise comparisons between elevational bands, $p < 0.05$; Table S6) at 1600 m (Fig. 2 a,b). Catabolic activity was the same in bulk soil under *J. communis* in all elevation bands (Fig. 2 c; Table S6). Global catabolic activity in rhizosphere soil beneath *V. myrtillus* and *P. abies* gradually decreased with increasing elevation and had significantly greater values at 1400–1600 m compared to those at higher elevations (Fig. 2 a,b; Table S6). However, global catabolic activity in rhizosphere soil of *J. communis* did not show any significant differences among elevation bands (Fig. 2 c; Table S6).

Global catabolic activity in rhizosphere soil from *V. myrtillus* and *P. abies* at 1400, 1600 and 1800 m was greater compared to bulk soil (Fig. 2 a, b). Above 1800 m, bulk and rhizosphere catabolic activity values from *V. myrtillus* and *P. abies* converged and were not significantly different (Fig. 2 a,b; Table S6). In samples from beneath *J. communis*, although catabolic activity in rhizosphere soil was generally higher than in bulk soil, differences were not significant (Fig. 2 c; Table S6). The mean catabolic diversity index (H') of both bulk and rhizosphere soils was 2.4 and no significant differences were found (data not shown) either in bulk or rhizosphere soil, among plant species or along the elevation gradient.

No clear trend was detected between global microbial catabolic activity and elevational bands in bulk and rhizosphere soil (NMDS plot, Fig. S2), however the PERMANOVA test showed that elevation explained the majority of variation in catabolic activity in both rhizosphere soil ($p < 0.001$, $R^2 = 0.36$; Table 2) and bulk soil ($p = 0.01$, $R^2 = 0.10$; Table 2). In contrast, the variation in catabolic activity not explained by elevation and species was greater in bulk soil (residuals = 0.87; Table 2) than in rhizosphere soil (residuals = 0.52; Table 2). In addition, there was a significant influence of the interaction between plant species and elevation ($p = 0.006$, $R^2 = 0.08$) and of plant species ($p = 0.03$, $R^2 = 0.05$) on catabolic activity in rhizosphere soil. Finally, there was no significant influence of plant species ($p = 0.43$, $R^2 = 0.02$) nor of the interaction between plant species and elevation ($p = 0.87$, $R^2 = 0.01$; Table 2) on catabolic activity in bulk soil.

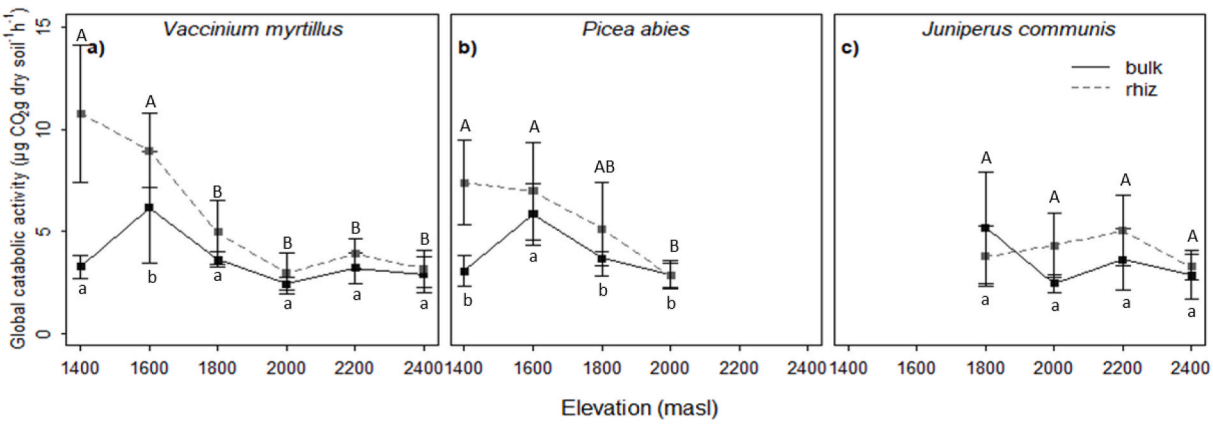

Fig. 2. Global catabolic activity of bulk soil (solid line) and rhizosphere soil (dotted line) beneath a) *Vaccinium myrtillus*, b) *Picea abies* and c) *Juniperus communis*. Data are means ± standard deviation. Significant differences ($p < 0.05$) assessed by Tukey's HSD test are shown in lowercase (bulk soil) and uppercase (rhizosphere soil) letters.

Table 2
Effect of structuring plant species and elevation on microbial global catabolic activity in bulk and rhizosphere soil assessed with PERMANOVA.

Factors	Bulk soil						Rhizosphere soil					
	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	0.05	0.02	0.84	0.02	0.43	2	0.19	0.09	3.20	0.05	0.03
Elevation	1	0.210	0.21	7.35	0.1	0.01	1	1.29	1.29	44.01	0.36	0.001
Plant species*Elevation	2	0.01	0.006	0.02	0.005	0.87	2	0.28	0.14	4.72	0.08	0.006
Residuals	64	1.83	0.03		0.87		64	1.87	0.03		0.52	
Total	69	2.11			1.0		69	3.63			1.0	

3.3. Relationships between abiotic factors and global catabolic activity and diversity (H') in bulk and rhizosphere soil

Climatic data did not explain global catabolic activity in either bulk ($p = 0.40$, adjusted $R^2 = 0.01$; Table 3) or rhizosphere soil ($p = 0.11$, adjusted $R^2 = 0.04$; Table 3). Moreover, no significant relationships were found between either MAP, MAT or MAR and catabolic activity or *H'* beneath *P. abies* or *J. communis* in bulk soil (Spearman correlation coefficients, Table 4). However, MAT was positively correlated with catabolic activity beneath *V. myrtillus*, whereas both MAP and MAR were negatively correlated (Table 4; Fig. S3). Bulk soil *H'* under *V. myrtillus* was negatively correlated with MAP (Table 4). In the rhizosphere of *V. myrtillus*, MAP and MAR were negatively correlated while MAT was positively correlated with catabolic activity (Table 4; Fig. S3). In the rhizosphere of *P. abies,* MAR was negatively correlated with catabolic activity (Table 4; Fig. S4). No climatic parameters were significantly correlated to global catabolic activity in the rhizosphere soil of *J. communis*.

The only significant relationships between *H'* and climatic variables were with MAP and MAT in the rhizosphere of *V. myrtillus*. MAP was positively correlated while MAT was negatively correlated with rhizosphere catabolic diversity (Table 4).

Along the elevation gradient, variations in soil physical and chemical properties were the main drivers explaining global catabolic activity in bulk soil even though the proportion of the variance was still limited ($p = 0.01$, adjusted $R^2 = 0.09$; Table 3). Soil texture, C, P and N contents, cation exchange capacity and pH were particularly important since catabolic activity was strongly and positively related to bulk soil SOC, soil TN, P, CEC and clay content but decreased with higher pH and sand content (Fig. 3, Table S7). Regarding catabolic diversity, it was significantly and positively correlated with silt content in bulk soil beneath *V. myrtillus* and *P. abies* (Table S7) but no relationships were found beneath *J. communis* (Table S7).

According to the partition of variance analysis, global catabolic activity in rhizosphere soil was not significantly explained by soil physical and chemical properties ($p = 0.71$, adjusted $R^2 = 0.02$; Table 3). However, when examining plant species individually, rhizosphere soil global catabolic activity was, to a lesser extent than bulk soil, positively related to SOC, TN, P, CEC and clay content, but decreased at higher pH and sand content, mostly in the rhizosphere of *V. myrtillus* (Table S7). Underneath *P. abies*, pH was the only soil property to be negatively and significantly correlated to rhizosphere soil catabolic activity (Table S7). Changes in *H'* were few, but occurred mostly in the rhizosphere of *V. myrtillus*, where they were strongly related to litter depth, soil water content, topsoil aggregate stability and ammonium content (Table S7).

Table 3
Partition of variance in constrained ordination distance-based redundancy analysis (db-RDA) for four environmental variables (soil physical and chemical properties, *community*- and *species*-level root traits and climatic data) influencing microbial catabolic activity and diversity for bulk and rhizosphere soil samples.

	Bulk soil				Rhizosphere soil			
	Df	R^2	R^2 adjusted	P	Df	R^2	R^2 adjusted	P
Climate	3	0.05	0.01	0.40	3	0.08	0.04	0.11
Soil properties	15	0.29	0.09	**0.01**	15	0.23	0.02	0.71
***Community* level root traits**	12	0.26	0.11	**0.02**	12	0.11	−0.07	0.64
***Species* level root traits**	3	0.08	0.04	*0.06*	3	0.02	−0.03	0.24

Values in bold are significant at $p < 0.05$; values in italics are significant at $p < 0.1$.

Table 4
Spearman correlations between global microbial catabolic activity and diversity (H') and climatic data of the three structuring plant species along the elevational gradient (significance levels p < 0.0001 ****; p < 0.001 ***, p < 0.01 **, p < 0.05 *).

Climate	Bulk soil						Rhizosphere soil					
	Vaccinium myrtillus		*Picea abies*		*Juniperus communis*		*Vaccinium myrtillus*		*Picea abies*		*Juniperus communis*	
	Catabolic Activity	H′	Catabolic Activity	H′	Catabolic Activity	H′	Catabolic Activity	H′	Catabolic Activity	H′	Catabolic Activity	H′
MAT	0.48**	0.25	−0.33	0.3	−0.16	−0.33	0.85****	−0.44*	0.41	0.21	0.04	−0.21
MAP	−0.44*	−0.37*	0.33	−0.3	0.16	0.33	−0.79****	0.38*	−0.41	−0.21	−0.04	0.21
MAR	−0.36*	0.07	−0.4	0.01	0.18	0.37	−0.60***	0.35	−0.59**	−0.37	0.01	0.2

MAT is mean annual temperature; MAP is mean annual precipitation; MAR is mean annual solar radiation.

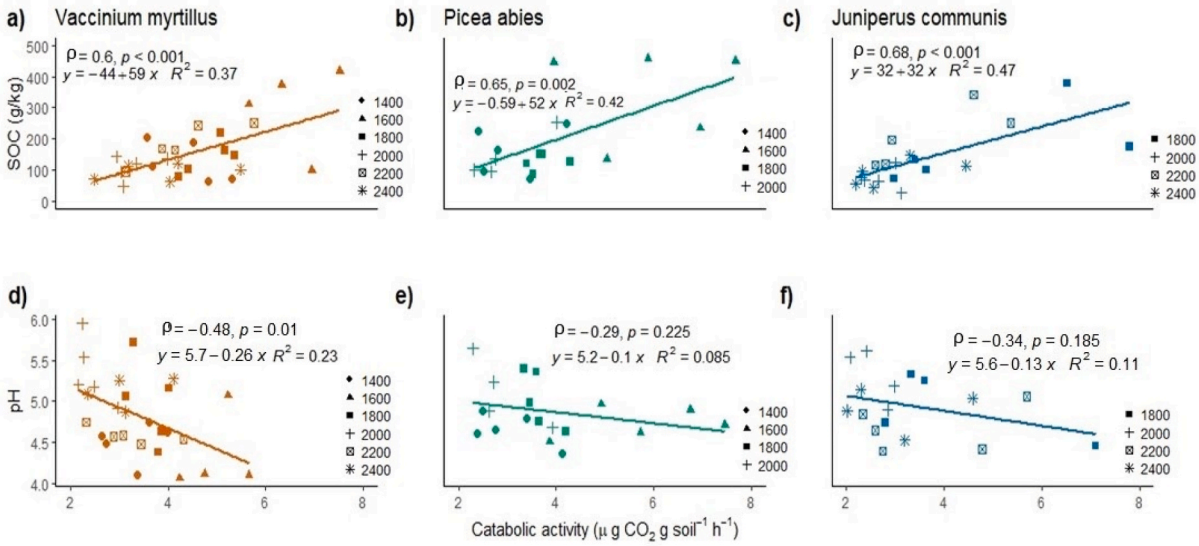

Fig. 3. Scatter plots for Spearman correlations between bulk soil global catabolic activity with soil organic carbon (SOC) and pH for *V. myrtillus* (orange), *P. abies* (green) and *J. communis* (blue) along the elevational gradient. Correlation coefficients (ρ), *p-values*, regression equations and R^2 are shown. Lines are linear regressions through data. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

3.4. Relationships between plant diversity and global catabolic activity and diversity (H') in bulk and rhizosphere soil

Along the elevational gradient (Table S8), vegetation cover and trees decreased whilst shrub cover increased at higher altitudes (Stokes et al., 2021; Weemstra et al., 2021). Graminoid and forb covers were greatest at 1800 m–2000 m. Simpson diversity in the quadrats containing *V. myrtillus* and *P. abies* changed significantly with elevation: at mid-elevations (1800 m–2000 m), plant diversity peaked and slowly decreased at 2200 m–2400 m (Table S8). However, plant diversity in the

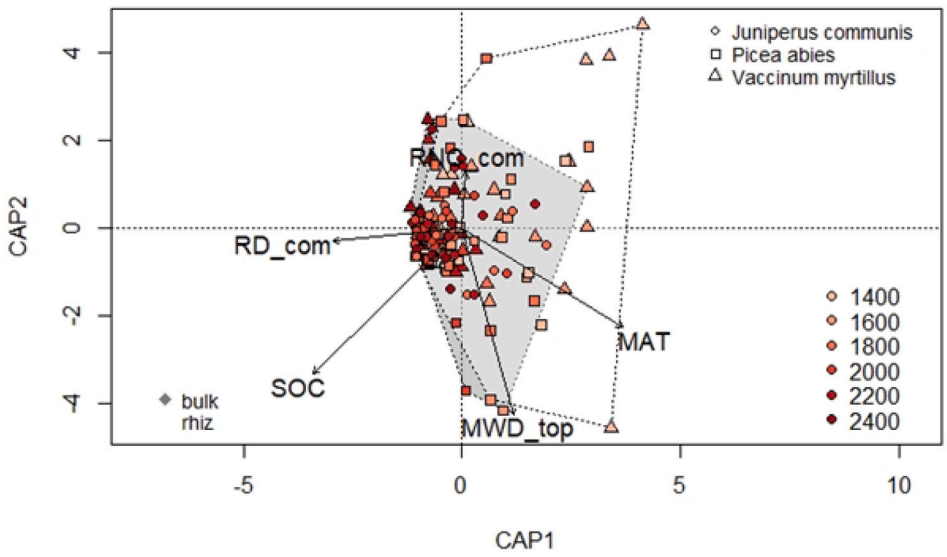

Fig. 4. Distance-based redundancy analysis ordination (db-RDA) of global microbial catabolic activity for six altitudinal bands from light red (lower elevations) to dark red (higher elevations) for bulk (convex hull polygon grey) and rhizosphere (convex hull polygon white) soils for *Vaccinium myrtillus* (triangles), *Picea abies* (squares) and *Juniperus communis* (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. MAT is mean annual temperature; MWD_{top} is mean weight diameter of topsoil aggregates; SOC is soil organic carbon; RD_{com} is root diameter and RNC is root nitrogen content. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

quadrats containing *J. communis* did not vary along the elevation gradient (Table S8).

Catabolic activity was negatively correlated with plant diversity in rhizosphere soil beneath *V. myrtillus* and bulk soil beneath *P. abies*, with no other significant relationships found among structuring plant species (Table S9). No significant relationships were found between *H'* and plant diversity in either bulk or rhizosphere soil (Table S9).

3.5. Relationships between plant root traits (community- and species-) and global catabolic activity and diversity (H') in bulk and rhizosphere soil

Overall, global catabolic activity was best explained by RD_{com}, SOC, MAT, MWD_{top} and RNC_{com} (db-RDA, Fig. 4).

In bulk soil, catabolic activity was best explained by *community* level root traits (partition of variance analysis: $p = 0.02$, adjusted $R^2 = 0.11$; Table 3) and to a lesser extent by *species* level root traits ($p = 0.06$, adjusted $R^2 = 0.04$; Table 3) although in both cases the proportion of variance was low.

Spearman correlation coefficients using *community* level root traits showed that *RD* and lignin content were always positively correlated with catabolic activity in bulk soil beneath all three plant species, whereas cellulose content was always negatively correlated (Fig. 5; Table S9). Hemicellulose was negatively correlated with bulk soil catabolic activity beneath *J. communis* only. SRL_{com} and RLD_{com} were both negatively correlated with bulk soil catabolic activity beneath *V. myrtillus* only (Table S9). With regard to *H'* in bulk soil, the only relationships between *community* level root traits were beneath *V. myrtillus*, whereby RNC_{com} was positively correlated and $RC{:}N_{com}$ was negatively correlated (Table S9).

At the *species* level, the only root trait that was significantly related to global catabolic activity in bulk soil was $RDMC_{sp}$ that was negatively correlated beneath *P. abies* only (Table S9). There were no other significant relationships between *H'* and root traits at the *species* level in bulk soil beneath all three plant species (Table S9).

Global catabolic activity in rhizosphere soil was not significantly explained by either *community*- (partition of variance analysis: $p = 0.64$, adjusted $R^2 = -0.07$; Table 3) or species- ($p = 0.24$, adjusted $R^2 = -0.03$; Table 3) level root traits.

In the rhizosphere, there were few significant relationships between global catabolic activity and *community* level root traits across the structuring species. Root N content was positively correlated with catabolic activity beneath *V. myrtillus* (Table S9) and $RC{:}N_{com}$ was negatively correlated beneath *P. abies* and *V. myrtillus* (Table S9). As in the bulk soil, root cellulose content was negatively correlated with catabolic activity, but in the rhizosphere of *V. myrtillus* only (Table S9). RLD_{com} was also negatively and significantly correlated with catabolic activity in the rhizosphere of *V. myrtillus* (Table S9). The only significant relationship between *H'* in the rhizosphere and *community* level root traits was beneath *V. myrtillus* and in contrast to that found in the bulk soil, RNC_{com} was negatively correlated with *H'* in the rhizosphere (Table S9).

With regard to *species*-level root traits, as in bulk soil, $RDMC_{sp}$ was negatively and significantly correlated with catabolic activity beneath *P. abies* only (Table S9). RTD_{sp} was negatively correlated with catabolic activity in the rhizosphere of *V. myrtillus* only (Table S9). However, no significant relationships were found between *H'* and *species* level root traits in the rhizosphere of any plant species.

4. Discussion

In agreement with our first hypothesis (H1), catabolic activity decreased at higher elevations (above 1800 m in the case of rhizosphere soil from *P. abies* and *V. myrtillus*). Along the elevation gradient, variations in soil physical and chemical properties were the main drivers of catabolic activity, especially texture, pH, cation exchange capacity, carbon and nitrogen content. Changes in catabolic diversity were few, but occurred mostly in the rhizosphere of *V. myrtillus*, where they were strongly related to litter depth and topsoil aggregate stability. Contrary

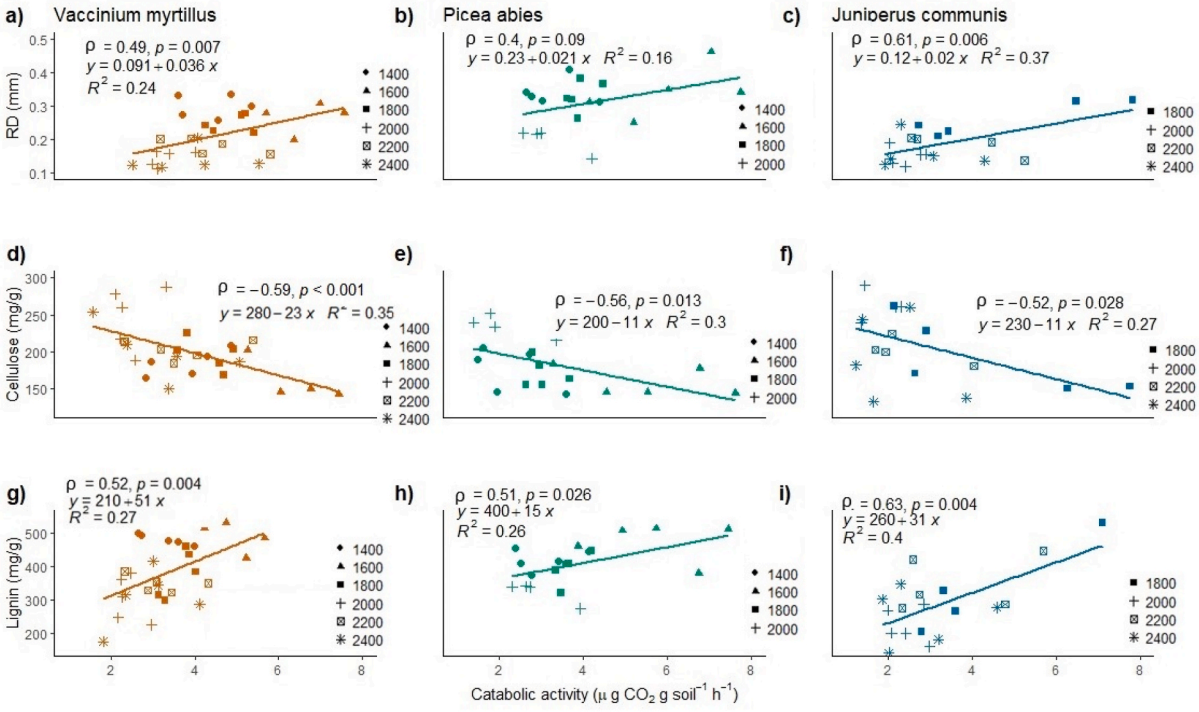

Fig. 5. Scatter plots for Spearman correlations between bulk soil global catabolic activity with root diameter (*RD*), cellulose and lignin root content at the *community*-level for *V. myrtillus* (orange), *P. abies* (green) and *J. communis* (blue) along the elevational gradient. Correlation coefficients (ρ), *p-values*, regression equations and R^2 are shown. Lines are linear regressions through data. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

to our H2, catabolic activity was negatively correlated with plant diversity in rhizosphere soil beneath *V. myrtillus* and bulk soil beneath *P. abies*, with no other significant relationships found among plant species. Also refuting our H2, we found no relationships between plant diversity and catabolic diversity in either bulk or rhizosphere soil. With regard to root traits, our H3 was not corroborated in that root traits that enhanced rhizosphere dimensions had little effect on global catabolic activity or diversity in the rhizosphere. Also contrary to our H3, we found that at the *community* level, root diameter (for *V. myrtillus* and *J. communis*) and lignin content (for *V. myrtillus*) were positively correlated with catabolic activity in bulk soil only, and had no effect on diversity. Also, root N and C contents had a negligible effect on catabolic activity and diversity, except for roots from *V. myrtillus*. Root cellulose content was negatively related to catabolic activity in bulk soil beneath all plant species and rhizosphere soil from *V. myrtillus* only, and had no effect on catabolic diversity. Trends in catabolic activity with root traits measured at the *species* level were only found for rhizosphere soil beneath *P. abies* and *V. myrtillus*, where more dense root tissue decreased catabolic activity, but had no effect on functional diversity.

4.1. Changes in catabolic activity and diversity along the elevation gradient and relationship with climatic factors

At elevations below 1800 m, global catabolic activity in the rhizosphere was 2–3 times greater than in bulk soil for *P. abies* and *V. myrtillus*, converging beyond 1800 m. *Juniperus communis* was not present at lower elevations, therefore biasing results for this species. As sampling was performed in early June, vegetation at lower elevations would have already commenced growth and photosynthesis several weeks previously (Wang et al., 2018a,b), thus stimulating the production of exudates during root elongation. At higher elevations, the growing season was only just beginning, and plants were commencing physiological processes after winter dormancy. Therefore, root exudate production and microbial activity in the rhizosphere would be less than at lower elevations. In line with this, changes in climate also partially explained trends in catabolic activity in the rhizosphere: increased MAP and MAR decreased catabolic activity in the rhizosphere of *V. myrtillus*, but activity increased with higher MAT. Similar relationships were found in bulk soil beneath this structuring species, but were less distinct. The only relationship found between a climatic variable and catabolic activity for another structuring species was in the rhizosphere beneath *P. abies*, where, as for *V. myrtillus*, high MAR decreased catabolic activity. Incident solar radiation can reach very high levels with increasing altitude, and causes photodegradation of plant litter (through the production of volatile compounds via photochemical mineralization) and photofacilitation (stimulation of biotic activity due to changes in litter chemistry) (Méndez et al., 2019). Therefore, at the treeline, where tree density is very sparse (stand basal area was only 18 m^2 ha^{-1} at our treeline site, Mao et al. (2015)), aboveground C input to mineral soil would be reduced via the biotic acceleration of C turnover in the litter layer (Méndez et al., 2019).

Unlike the study by Esch et al. (2017), where manipulated rainfall increased soil microbial activity, we found that high MAP decreased catabolic activity beneath *V. myrtillus* along the elevation gradient. As this species is an understory herb, with minimal buffering of precipitation by a large canopy, the effects of rainfall will be accentuated as precipitation reaches the soil surface directly. Similar results were found by Shi et al. (2018), who performed a rainfall manipulation experiment in a moist, temperate deciduous forest. In their study, increased precipitation rapidly decreased fungal biomass but with no effect on bacterial biomass, and it was suggested that high rainfall reduced sodium concentrations in the soil with a detrimental effect on fungal activity. However, it seems more likely that high MAP will impact photosynthesis via stomatal closure (Zhang et al., 2017; Li et al., 2019), decreasing the flow of photoassimilates and the subsequent production of C-rich root exudates into the rhizosphere. Also, heterotrophic respiration can be suppressed in extremely moist conditions (Horz et al., 2004; Zhao et al., 2016).

We also found a positive relationship between MAT and global catabolic activity in the rhizosphere of *V. myrtillus*, as several studies have shown that soil warming increases microbial activity (Grayston et al., 2001; Papatheodorou et al., 2004). Adamczyk et al. (2020) showed that increased microbial respiration rates at higher temperatures may be explained by additional C input to cold, nutrient-poor soils that are becoming warmer as a result of climate change. Additionally, increases in temperature stimulate microbial metabolism through changes in physiology or enzyme functioning involved in organic matter decomposition (Tang et al., 2018; Nottingham et al., 2019). Warmer temperatures also increase plant growth and biomass, stimulating photosynthesis and hence the production of C-rich root exudates (Rossi et al., 2020). Enhanced root exudation with warmer temperatures has also been shown to increase microbial catabolic diversity (Papatheodorou et al., 2004). Nevertheless, Klimek et al. (2020) found a decrease in catabolic diversity at higher temperatures because less functionally diverse microbial communities were unable to effectively degrade various organic substrates. Our results show that catabolic diversity decreased with higher MAT in rhizosphere soil, possibly indicating that colder temperatures lead to specialised microorganisms in these alpine soils (Donhauser and Frey, 2018; Collins et al., 2020).

4.2. Changes in catabolic activity and diversity along the elevation gradient and relationship with soil physical and chemical properties

Along the elevation gradient, bulk soil physical and chemical properties were major drivers of catabolic activity and to a lesser extent, catabolic diversity. SOC, TN, P, CEC, NH_4^+, sand and clay content were all strongly related to each other and generally decreased at higher altitudes (except for sand content that increased). Several authors report similar findings regardless of climate type (e.g., Xu et al., 2014; Kotas et al., 2018; Hofmann et al., 2016; Praeg et al., 2019). Therefore, we show that along an elevation gradient, nutrient content and texture had a greater influence on microbial activity in bulk soil than climate and vegetation.

The influence of soil properties on catabolic activity in the rhizosphere was less evident. Soil water content and pH had the strongest influence on catabolic activity in rhizosphere soil of *V. myrtillus*, with more activity in drier, acidic soils. Catabolic activity in the rhizosphere of *P. abies* was also greatest in the most acidic soils, suggesting that they were dominated by acid-tolerant microbial species (Shen et al., 2013). The respiratory activity of fungi increases under acidic conditions while that of bacteria decreases (Blagodatskaya and Anderson 1998). Therefore, if soil pH increases, the fungal contribution to respiration and decreasing overall catabolic activity could decrease, as observed in our results. A strong negative relationship did exist between catabolic diversity, litter depth and NH_4^+ in the rhizosphere of *V. myrtillus*. NH_4^+ is the nitrogen source on which *V. myrtillus* typically relies (Roth et al., 2021) but Morvan et al. (2020) found a high proportion of di-nitrogen (N_2) fixers (Rhizobiales) in the rhizosphere of *Vaccinium angustifolium*. Therefore, the availability of NH_4^+ in soil beneath *Vaccinium* sp. could reduce catabolic diversity if N_2-fixing bacteria dominate the rhizosphere, reflected in the negative relationship that we found between root nitrogen content and catabolic diversity in the rhizosphere of *V. myrtillus*. Alternatively, nutrient-poor soils could moderate competition among microbial species. Eldridge et al. (2017) found that altered levels of soil C changed the competitive abilities of different microbial phyla, with reduced carbon promoting bacterial diversity, but decreasing fungal diversity. Carbon input to soil and litter depth are strongly associated, therefore, although litter depth increases catabolic activity in the rhizosphere of *V. myrtillus*, competition between microbial phyla may be increased, leading to less catabolic diversity with increasing litter depth.

An increase in aggregate stability in topsoil was negatively related to

catabolic activity in the rhizosphere of *V. myrtillus*, but catabolic diversity increased. However, catabolic activity increased in the more stable subsoil aggregates. The decrease of catabolic activity with soil aggregate stability is likely due to physical protection of C in more stable aggregates (Chevallier, 2011), that may induce a reduction of catabolic activity. Many studies have highlighted the prominent role of microbial communities promoting aggregate stability (e.g. Bossuyt et al., 2001; Cosentino et al., 2006). Therefore, although Aspiras et al. (1971) postulated that the status of soil aggregation is determined by the cumulated effects of synthesis and degradation of binding materials by microbial populations, rather than the activity of specific microorganisms, our results suggest that species-specific microbial activity can have diverse effects on aggregate stability.

4.3. Relationships between plant diversity and catabolic activity and diversity

Generally, the more diverse a plant species assemblage, the more diverse the chemistry of the root exudates and litter produced (Klimek et al., 2015). Therefore, when plant diversity indices are high, more ecological niches should be available for soil microorganisms, as the spatial heterogeneity of soil resources and increased availability of labile C sources should promote catabolic activity (Creamer et al., 2016; Bongiorno et al., 2020). However, the relationship between species assemblage and the microbial communities of any given plant species is not necessarily direct. Individual plant species may be positively or negatively affected by increased plant diversity (Losapio et al.,2021), also affecting the production of root exudates and microbial communities closely related to them. Here, we found that contrary to our second hypothesis, catabolic activity was negatively correlated with plant diversity in rhizosphere soil of *V. myrtillus* and bulk soil beneath *P. abies*, with no other significant relationships found among structuring species or sampling location. Unlike Klimek et al. (2015), we did not discriminate between bacterial and fungal catabolic diversity in our study. It is therefore possible that the relative contribution of fungi and bacteria to global catabolic activity varied depending on the soil under study. The presence of various exudates may also impact catabolic activity differently, for example, Yuan et al. (2017) found that the addition of three common components of root exudates (oxalic acid, glucose and glycine) altered considerably microbial communities, activities and related processes. Hence, this disparity could explain the observed lower catabolic diversity in the rhizosphere of *V. myrtillus* and in the bulk soil under *P. abies*.

4.4. Influence of plant root traits on catabolic activity and diversity

Along this complex environmental gradient, where multiple properties simultaneously change, root traits of different species varied in diverse ways, leading to species-specific patterns in intraspecific root trait variation (described in detail by Weemstra et al., 2021) and associated microbial responses. We expected that in bulk soil, where litter is a major C source and microbial decomposers dominate, an increase in root N and C content should enhance global catabolic activity and diversity, but this hypothesis was only true for *V. myrtillus*, where catabolic diversity was positively related to root N content. Additionally, our hypothesis that plant root traits that increase rhizosphere dimensions or fungal colonisation (e.g., longer, thinner roots with large exchange surface areas), would increase global catabolic activity and diversity, was not corroborated in either bulk or rhizosphere soil. However, at the *community* level, thicker roots did increase catabolic activity in bulk soil beneath *V. myrtillus* and *J. communis*. Root thickness is partially linked to root age, and so reflects chemical composition. Thin, woody roots are usually young and possess a greater quantity of cellulose (Genet et al., 2005; Hales et al., 2009), that is negatively related to lignin content (Thomas et al., 2014). Our results showed that at the *community* level, root diameter and cellulose content were negatively related in *P. abies* and *V. myrtillus*, and lignin content was positively correlated with root diameter beneath all three plant species (Figure S4). Therefore, relationships between catabolic activity and root diameter may not be causal, but rather due to root age and chemical composition. We found a strong positive relationship between catabolic activity and root lignin content (at the *community* level) in all bulk soils. Lignin is considered difficult to degrade (Roumet et al., 2016; Poirier et al., 2018), but within microbial fungal and bacterial phyla, diverse strategies exist to facilitate the decomposition of lignin, allowing microbial specialists to thrive in different ecological niches (Janusz et al., 2017). Therefore, lignin-degrading fungal and bacterial specialists are likely to be dominant in these root- and leaf-litter rich soils.

Trends in catabolic activity with root traits measured at the *species* level were only found for rhizosphere soil beneath *P. abies* and *V. myrtillus*, where more dense root tissue decreased catabolic activity. The changes in root chemical composition that we observed will be reflected in tissue density: cellulose increases wood density and lignin decreases density (Nuopponen et al., 2006), thus explaining why dense tissue decreases catabolic activity.

Our results demonstrate that relationships between microbial functioning and abiotic and biotic variables are complex in a highly heterogeneous environment. Nevertheless, we show that the main drivers of microbial activity and functioning are soil physical and chemical properties, but the presence of vegetation enhances these relationships through belowground characteristics such as root exudation, root chemical composition and changes in litter production that affect soil C. Therefore, in this environment, shifts in functional diversity of soil microbes under future climate scenarios could be mediated primarily by plant species, because soil properties will be slower to change. To better understand these relationships, future studies should examine temporal fungal and bacterial community taxonomic diversity and functioning, and how they relate to plant growth and root demography in a wide range of plant species.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We thank our very helpful students and colleagues for their huge contribution in the field and in the laboratory: Isabelle Barois, Nereyda Cruz-Maldonado, Mathieu Fontaine, Stephane Fourtier, Leonor Jiménez, Beatriz Marín-Castro, Zhun Mao, Awaz Mohamed, Hans Reijnen, Hervé Rey and Katrin Sieron. Funding was provided by the French and Mexican governments (ECOPICS project, ANR–16-CE03-0009 and CONACYT–2 73659). DHC was funded by a doctoral bursary from CONACYT (471700). Thanks are due to the Mairie de Chamrousse for access to field sites. Partial data were presented in the session "Forest Management Practices and the Responses of Soil Carbon, Water, Nutrients and Their Interactions" organised at the XXV IUFRO World Congress 2019, "Forest Research and Cooperation for Sustainable Development," held at Curitiba, Brazil; 29 September - October 5, 2019.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2021.108485 and data for microbial community level physiological profiles can be downloaded at https://doi.org/10.15454/KFBNR8.

References

Abbott, R.J., Brennan, A.C., 2014. Altitudinal gradients, plant hybrid zones and evolutionary novelty. Philosophical Transactions of the Royal Society B: Biological Sciences 369 (1648), 20130346. https://doi.org/10.1098/rstb.2013.0346.

Adamczyk, M., Perez-Mon, C., Gunz, S., Frey, B., 2020. Strong shifts in microbial community structure are associated with increased litter input rather than temperature in High Arctic soils. Soil Biology and Biochemistry 151, 108054. https://doi.org/10.1016/j.soilbio.2020.108054.

Aislabie, J., Deslippe, J.R., Dymond, J.R., 2013. Soil microbes and their contribution to soil services. In: J, A., JR, D. (Eds.), Ecosystem Services in New Zealand - Conditions and Trends, pp. 143–161.

Aspiras, R.B., Allen, O.N., Harris, R.F., Chesters, G., 1971. The role of microorganisms in the stabilization of soil aggregates. Soil Biology and Biochemistry 3 (4), 347–353. https://doi.org/10.1016/0038-0717(71)90045-9.

Badri, D.V., Vivanco, J.M., 2009. Regulation and function of root exudates. Plant, Cell and Environment 32 (6), 666–681. https://doi.org/10.1111/j.1365-3040.2009.01926.x.

Baratella, Valentina, Pinzari, Flavia, 2019. Soil basal respiration and substrate induced respiration (SIR) by MicroResp™ Importance and applications. In: Jorge Álvaro-Fuentes, Dénes Lóczy, Sören Thiele-Bruhn, & Raúl Zornoza. https://doi.org/10.5281/zenodo.2553444 (2019). Handbook of plant and soil analysis for agricultural systems.

Bardgett, R.D., Caruso, T., 2020. Soil Microbial Community Responses to Climate Extremes: Resistance, Resilience and Transitions to Alternative States, vol. 375. Philosophical Transactions of the Royal Society B: Biological Sciences. https://doi.org/10.1098/rstb.2019.0112, 1794.

Baudoin, E., Benizri, E., Guckert, A., 2002. Impact of growth stage on the bacterial community structure along maize roots, as determined by metabolic and genetic fingerprinting. Applied Soil Ecology 19 (2), 135–145. https://doi.org/10.1016/S0929-1393(01)00185-8.

Beare, M.H., Neely, C.L., Coleman, D.C., Hargrove, W.L., 1990. A substrate-induced respiration (SIR) method for measurement of fungal and bacterial biomass on plant residues. Soil Biology and Biochemistry 22 (5), 585–594. https://doi.org/10.1016/0038-0717(90)90002-H.

Bjune, A.E., Ohlson, M., Birks, H.J.B., Bradshaw, R.H.W., 2009. The development and local stand-scale dynamics of a *Picea abies* forest in southeastern Norway. The Holocene 19 (7), 1073–1082. https://doi.org/10.1177/0959683609341004.

Blagodatskaya, E.V., Anderson, T.-H., 1998. Interactive effects of pH and substrate quality on the fungal-to-bacterial ratio and qCO2 of microbial communities in forest soils. *Soil Biology and Biochemistry* 30 (10–11), 1269–1274. https://doi.org/10.1016/S0038-0717(98)00050-9.

Bongiorno, G., Bünemann, E.K., Brussaard, L., Mäder, P., Oguejiofor, C.U., de Goede, R. G.M., 2020. Soil management intensity shifts microbial catabolic profiles across a range of European long-term field experiments. Applied Soil Ecology 154, 103596. https://doi.org/10.1016/j.apsoil.2020.103596.

Bossuyt, H., Denef, K., Six, J., Frey, S.D., Merckx, R., Paustian, K., 2001. Influence of microbial populations and residue quality on aggregate stability. Applied Soil Ecology 16 (3), 195–208. https://doi.org/10.1016/S0929-1393(00)00116-5.

Burke, D.J., Weintraub, M.N., Hewins, C.R., Kalisz, S., 2011. Relationship between soil enzyme activities, nutrient cycling and soil fungal communities in a northern hardwood forest. Soil Biology and Biochemistry 43 (4), 795–803. https://doi.org/10.1016/j.soilbio.2010.12.014.

Cavicchioli, R., Ripple, W.J., Timmis, K.N., Azam, F., Bakken, L.R., Baylis, M., et al., 2019. Scientists' warning to humanity: microorganisms and climate change. Nature Reviews Microbiology 17 (9), 569–586. https://doi.org/10.1038/s41579-019-0222-5.

Chapman, S.J., Campbell, C.D., Artz, R.R.E., 2007. Assessing CLPPs using MicroResp™ - a comparison with Biolog and multi-SIR. Journal of Soils and Sediments 7 (6), 406–410. https://doi.org/10.1065/jss2007.10.259.

Chen, X., Ding, Z., Tang, M., Zhu, B., 2018. Greater variations of rhizosphere effects within mycorrhizal group than between mycorrhizal group in a temperate forest. Soil Biology and Biochemistry 126, 237–246. https://doi.org/10.1016/j.soilbio.2018.08.026.

Chevallier, T., 2011. Physical protection of organic carbon in soil aggregates. In: Gliński, J., Horabik, J., Lipiec, J. (Eds.), Encyclopedia of Agrophysics. Encyclopedia of Earth Sciences Series. Springer, Dordrecht. https://doi.org/10.1007/978-90-481-3585-1_197.

Collins, C.G., Spasojevic, M.J., Alados, C.L., Aronson, E.L., Benavides, J.C., Cannone, N., Caviezel, C., Grau, O., Guo, H., Kudo, G., Kuhn, N.J., Müllerová, J., Phillips, M.L., Pombubpa, N., Reverchon, F., Shulman, H.B., Stajich, J.E., Stokes, A., Weber, S.E., Diez, J.M., 2020. Belowground impacts of alpine woody encroachment are determined by plant traits, local climate and soil conditions. Global Change Biology (May), 1–16. https://doi.org/10.1111/gcb.15340.

Creamer, R.E., Stone, D., Berry, P., Kuiper, I., 2016. Measuring respiration profiles of soil microbial communities across Europe using MicroResp™ method. Applied Soil Ecology 97, 36–43. https://doi.org/10.1016/j.apsoil.2015.08.004.

Cosentino, D., Chenu, C., Le Bissonnais, Y., 2006. Aggregate stability and microbial community dynamics under drying-wetting cycles in a silt loam soil. Soil Biology and Biochemistry 38 (8), 2053–2062. https://doi.org/10.1016/j.soilbio.2005.12.022.

Cui, Y., Bing, H., Fang, L., Wu, Y., Yu, J., Shen, G., Jiang, M., Wang, X., Zhang, X., 2019. Diversity patterns of the rhizosphere and bulk soil microbial communities along an altitudinal gradient in an alpine ecosystem of the eastern Tibetan Plateau. Geoderma 338, 118–127. https://doi.org/10.1016/j.geoderma.2018.11.047.

D'Alò, F., Odriozola, I., Baldrian, P., Zucconi, L., Ripa, C., Cannone, N., MAlfasi, F., Brancaleoni, L., Onofri, S., 2021. Microbial activity in alpine soils under climate change. The Science of the Total Environment 783, 147012. https://doi.org/10.1016/j.scitotenv.2021.147012.

Degens, B.P., Harris, J.A., 1997. Development of a physiological approach to measuring the catabolic diversity of soil microbial communities. Soil Biology and Biochemistry 29 (9–10), 1309–1320. https://doi.org/10.1016/S0038-0717(97)00076-X.

Degens, B.P., Schipper, L.A., Sparling, G.P., Duncan, L.C., 2001. Is the microbial community in a soil with reduced catabolic diversity less resistant to stress or disturbance? Soil Biology and Biochemistry 33 (9), 1143–1153. https://doi.org/10.1016/S0038-0717(01)00018-9.

Degens, B.P., Schipper, L.A., Sparling, G.P., Vojvodic-Vukovic, M., 2000. Decreases in organic C reserves in soils can reduce the catabolic diversity of soil microbial communities. Soil Biology and Biochemistry 32 (2), 189–196. https://doi.org/10.1016/S0038-0717(99)00141-8.

Donhauser, J., Frey, B., 2018. Alpine soil microbial ecology in a changing world. FEMS Microbiology Ecology 94 (9), 1–31. https://doi.org/10.1093/femsec/fiy099.

Eldridge, D.J., Delgado-Baquerizo, M., Travers, S.K., Val, J., Oliver, I., Hamonts, K., Singh, B.K., 2017. Competition drives the response of soil microbial diversity to increased grazing by vertebrate herbivores. Ecology 98 (7), 1922–1931. https://doi.org/10.1002/ecy.1879.

Esch, E.H., Lipson, D., Cleland, E.E., 2017. Direct and indirect effects of shifting rainfall on soil microbial respiration and enzyme activity in a semi-arid system. Plant and Soil 411 (1–2), 333–346. https://doi.org/10.1007/s11104-016-3027-6.

Fanin, N., Hättenschwiler, S., Fromin, N., 2014. Litter fingerprint on microbial biomass, activity, and community structure in the underlying soil. Plant and Soil 379 (1–2), 79–91. https://doi.org/10.1007/s11104-014-2051-7.

Farrar, J., Hawes, M., Jones, D., Lindow, S., 2003. How roots control the flux of carbon to the rhizosphere. Ecology 84 (4), 827–837. https://doi.org/10.1890/0012-9658(2003)084[0827:HRCTFO]2.0.CO;2.

Freschet, G.T., Roumet, C., 2017. Sampling roots to capture plant and soil functions. Functional Ecology 31 (8), 1506–1518. https://doi.org/10.1111/1365-2435.12883.

Fromin, N., Shihan, A., Santonja, M., Baldy, V., Hättenschwiler, S., 2020. Soil microbial activity in a Mediterranean garrigue responds more to changing shrub community than to reduced rainfall. Plant and Soil 449 (1–2), 405–421. https://doi.org/10.1007/s11104-020-04501-8.

Genet, M., Stokes, A., Salin, F., Mickovski, S.B., Fourcaud, T., Dumail, J.F., Van Beek, R., 2005. The influence of cellulose content on tensile strength in tree roots. Plant and Soil 278 (1–2), 1–9. https://doi.org/10.1007/s11104-005-8768-6.

Gillespie, L.M., Hättenschwiler, S., Milcu, A., Wambsganss, J., Shihan, A., Fromin, N., 2021. Tree species mixing affects soil microbial functioning indirectly via root and litter traits and soil parameters in European forests. Functional Ecology 1–15. https://doi.org/10.1111/1365-2435.13877, 00.

Grayston, S.J., Griffith, G.S., Mawdsley, J.L., Campbell, C.D., Bardgett, R.D., 2001. Accounting for variability in soil microbial communities of temperate upland grassland ecosystems. Soil Biology and Biochemistry 33 (4–5), 533–551. https://doi.org/10.1016/S0038-0717(00)00194-2.

Hagedorn, F., Gavazov, K., Alexander, J.M., 2019. Above-and belowground linkages shape responses of mountain vegetation to climate change. Science 365 (6458), 1119–1123. https://doi.org/10.1126/science.aax4737.

Hales, T.C., Ford, C.R., Hwang, T., Vose, J.M., Band, L.E., 2009. Topographic and ecologic controls on root reinforcement. Journal of Geophysical Research: Earth Surface 114 (3), 1–17. https://doi.org/10.1029/2008JF001168.

Han, M., Sun, L., Gan, D., Fu, L., Zhu, B., 2020. Root functional traits are key determinants of the rhizosphere effect on soil organic matter decomposition across 14 temperate hardwood species. Soil Biology and Biochemistry 151 (April), 108019. https://doi.org/10.1016/j.soilbio.2020.108019.

Hobbie, J.E., Hobbie, E.A., 2013. Microbes in nature are limited by carbon and energy: the starving-survival lifestyle in soil and consequences for estimating microbial rates. Frontiers in Microbiology 4 (NOV), 1–11. https://doi.org/10.3389/fmicb.2013.00324.

Hofmann, K., Lamprecht, A., Pauli, H., Illmer, P., 2016. Distribution of prokaryotic abundance and microbial nutrient cycling across a high-alpine altitudinal gradient in the austrian central alps is affected by vegetation, temperature, and soil nutrients. Microbial Ecology 72 (3), 704–716. https://doi.org/10.1007/s00248-016-0803-z.

Horz, H.-P., Barbrook, A., Field, C.B., Bohannan, B.J.M., 2004. Ammonia-oxidizing bacteria respond to multifactorial global change. Proceedings of the National Academy of Sciences 101 (42), 15136–15141. https://doi.org/10.1073/pnas.0406616101.

Janusz, G., Pawlik, A., Sulej, J., Świderska-Burek, U., Jarosz-Wilkolazka, A., Paszczyński, A., 2017. Lignin degradation: microorganisms, enzymes involved, genomes analysis and evolution. FEMS Microbiology Reviews 41 (6), 941–962. https://doi.org/10.1093/femsre/fux049.

Jones, D.L., Murphy, D.V., 2007. Microbial response time to sugar and amino acid additions to soil. Soil Biology and Biochemistry 39 (8), 2178–2182. https://doi.org/10.1016/j.soilbio.2007.03.017.

Kang, H., Kang, S., Lee, D., 2009. Variations of soil enzyme activities in a temperate forest soil. Ecological Research 24, 1137–1143. https://doi.org/10.1007/s11284-009-0594-5.

Khlifa, R., Paquette, A., Messier, C., Reich, P.B., Munson, A.D., 2017. Do temperate tree species diversity and identity influence soil microbial community function and composition? Ecology and Evolution 7 (19), 7965–7974. https://doi.org/10.1002/ece3.3313.

Klimek, B., Niklińska, M., Jaźwa, M., Tarasek, A., Tekielak, I., Musielok, Ł., 2015. Covariation of soil bacteria functional diversity and vegetation diversity along an altitudinal climatic gradient in the Western Carpathians. Pedobiologia 58 (2–3), 105–112. https://doi.org/10.1016/j.pedobi.2015.04.005.

Klimek, B., Chodak, M., Jaźwa, M., Azarbad, H., Niklińska, M., 2020. Soil physicochemical and microbial drivers of temperature sensitivity of soil organic matter decomposition under boreal forests. Pedosphere 30 (4), 528–534. https://doi.org/10.1016/S1002-0160(17)60400-4.

Körner, C., Paulsen, J., Spehn, E.M., 2011. A definition of mountains and their bioclimatic belts for global comparisons of biodiversity data. Alpine Botany 121 (2), 73–78. https://doi.org/10.1007/s00035-011-0094-4.

Kotas, P., Šantrůčková, H., Elster, J., Kaštovská, E., 2018. Soil microbial biomass, activity and community composition along altitudinal gradients in the High Arctic (Billefjorden, Svalbard). Biogeosciences Discussions 1–31. https://doi.org/10.5194/bg-2017-184.

Kuzyakov, Y., Razavi, B.S., 2019. Rhizosphere size and shape: temporal dynamics and spatial stationarity. Soil Biology and Biochemistry 135 (December 2018), 343–360. https://doi.org/10.1016/j.soilbio.2019.05.011.

Laufen, C.G., Chelsea, Galerie, 2001. Flora Helvetica.

Le Bissonnais, Y., 1996. Aggregate stability and assessment of soil crustability and erodibility: I. Theory and methodology. European Journal of Soil Science 67 (1), 11–21. https://doi.org/10.1111/ejss.4_12311.

Li, L., Zheng, Z., Biederman, J.A., Xu, C., Xu, Z., Che, R., Wang, Y., Cui, X., Hao, Y., 2019. Ecological responses to heavy rainfall depend on seasonal timing and multi-year recurrence. New Phytologist 223 (2), 647–660. https://doi.org/10.1111/nph.15832.

Liu, Z., Liu, G., Fu, B., Zheng, X., 2008. Relationship between plant species diversity and soil microbial functional diversity along a longitudinal gradient in temperate grasslands of Hulunbeir, Inner Mongolia, China. Ecological Research 23 (3), 511–518. https://doi.org/10.1007/s11284-007-0405-9.

Losapio G, Schöb C, Staniczenko PPA, Carrara F, Palamara GM, De Moraes CM, Mescher MC, Brooker RW, Butterfield BJ, Callaway RM, Cavieres LA, Kikvidze Z, Lortie CJ, Michalet R, Pugnaire FI, Bascompte J. Network motifs involving both competition and facilitation predict biodiversity in alpine plant communities. Proceedings of the National Academy of Sciences of the United States of America, 118(6). doi: 10.1073/pnas.2005759118.

McCormack, M.L., Iversen, C.M., 2019. Physical and functional constraints on viable belowground acquisition strategies. Frontiers of Plant Science 10 (October), 1–12. https://doi.org/10.3389/fpls.2019.01215.

Mao, Z., Wang, Y., Jourdan, C., Cécillon, L., Nespoulous, J., Rey, H., Saint-André, L., Stokes, A., 2015. Characterizing above- and belowground carbon partitioning in forest trees along an altitudinal gradient using area-based indicators. Arctic Antarctic and Alpine Research 47 (1), 59–69. https://doi.org/10.1657/AAAR0014-014.

Margesin, R., Jud, M., Tscherko, D., Schinner, F., 2009. Microbial communities and activities in alpine and subalpine soils. FEMS Microbiology Ecology 67, 208–218. https://doi.org/10.1111/j.1574-6941.2008.00620.x.

Marín-Castro, B.E., Geissert, D., Negrete-Yankelevich, S., Gómez-Tagle Chávez, A., 2016. Spatial distribution of hydraulic conductivity in soils of secondary tropical montane cloud forests and shade coffee agroecosystems. Geoderma 283, 57-67. https://doi.org/10.1016/j.geoderma.2016.08.002.

Maron, P.A., Mougel, C., Ranjard, L., 2011. Soil microbial diversity: methodological strategy, spatial overview and functional interest. Comptes Rendus Biologies 334 (5–6), 403–411. https://doi.org/10.1016/j.crvi.2010.12.003.

Méndez, M.S., Martinez, M.L., Araujo, P.I., Austin, A.T., 2019. Solar radiation exposure accelerates decomposition and biotic activity in surface litter but not soil in a semiarid woodland ecosystem in Patagonia, Argentina. Plant and Soil 445 (1–2), 483–496. https://doi.org/10.1007/s11104-019-04325-1.

Merino- Martín, L., Stokes, A., Gweon, H.S., Moragues-Saitua, L., Staunton, S., Plassard, C., Oliver, A., Le Bissonnais, Y., Griffiths, R.I., 2021. Interacting effects of land use type, microbes and plant traits on soil aggregate stability. Soil Biology and Biochemistry 154, 108072. https://doi.org/10.1016/j.soilbio.2020.108072.

Möhl, P., Mörsdorf, M.A., Dawes, M.A., Hagedorn, F., Bebi, P., Viglietti, D., Freppaz, M., Wipf, S., Körner, C., Thomas, F.M., Rixen, C., 2019. Twelve years of low nutrient input stimulates growth of trees and dwarf shrubs in the treeline ecotone. Journal of Ecology 107 (2), 768–780. https://doi.org/10.1111/1365-2745.13073.

Morvan, S., Meglouli, H., Lounès-Hadj Sahraoui, A., Hijri, M., 2020. Into the wild blueberry (Vaccinium angustifolium) rhizosphere microbiota. Environmental Microbiology 22 (9), 3803–3822. https://doi.org/10.1111/1462-2920.15151.

Moscatelli, M.C., Secondi, L., Marabottini, R., Papp, R., Stazi, S.R., Mania, E., Marinari, S., 2018. Assessment of soil microbial functional diversity: land use and soil properties affect CLPP-MicroResp and enzymes responses. Pedobiologia 66 (July 2017), 36–42. https://doi.org/10.1016/j.pedobi.2018.01.001.

Nannipieri, P., Grego, S., Ceccanti, B., 1990. Ecological significance of the biological activity in soil. In: Bollag, J., Stotzky, G. (Eds.), Soil Biochemistry. Marcel Dekker, New York, NY, USA, pp. 293–355.

Nottingham, A.T., Bååth, E., Reischke, S., Salinas, N., Meir, P., 2019. Adaptation of soil microbial growth to temperature: using a tropical elevation gradient to predict future changes. Global Change Biology 25 (3), 827–838. https://doi.org/10.1111/gcb.14502.

Nuccio, E.E., Starr, E., Karaoz, U., Brodie, E.L., Zhou, J., Tringe, S.G., Malmstrom, R.R., Woyke, T., Banfield, J.F., Firestone, M.K., Pett-Ridge, J., 2020. Niche differentiation is spatially and temporally regulated in the rhizosphere. The ISME Journal 14 (4), 999–1014.

Nuopponen, M.H., Birch, G.M., Sykes, R.J., Lee, S.J., Stewart, D., 2006. Estimation of wood density and chemical composition by means of diffuse reflectance mid-infrared Fourier transform (DRIFT-MIR) spectroscopy. Journal of Agricultural and Food Chemistry 54 (1), 34–40. https://doi.org/10.1021/jf051066m.

Nsabimana, D., Haynes, R.J., Wallis, F.M., 2004. Size, activity and catabolic diversity of the soil microbial biomass as affected by land use. Applied Soil Ecology 26 (2), 81–92. https://doi.org/10.1016/j.apsoil.2003.12.005.

Nybakken, L., Selås, V., Ohlson, M., 2013. Increased growth and phenolic compounds in bilberry (Vaccinium myrtillus L.) following forest clear-cutting. Scandinavian Journal of Forest Research 28 (4), 319–330. https://doi.org/10.1080/02827581.2012.749941.

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H., Szoecs, E., Wagner, H., 2020. vegan: community Ecology Package. R package version 2.5-7. https://CRAN.R-project.org/package=vegan.

Papatheodorou, E.M., Argyropoulou, M.D., Stamou, G.P., 2004. The effects of large- and small-scale differences in soil temperature and moisture on bacterial functional diversity and the community of bacterivorous nematodes. Applied Soil Ecology 25 (1), 37–49. https://doi.org/10.1016/S0929-1393(03)00100-8.

Piedallu, C., Gégout, J.C., Lebourgeois, F., Seynave, I., 2016. Soil aeration, water deficit, nitrogen availability, acidity and temperature all contribute to shaping tree species distribution in temperate forests. Journal of Vegetation Science 27 (2), 387–399. https://doi.org/10.1111/jvs.12370.

Piedallu, C., Gégout, J.C., Perez, V., Lebourgeois, F., 2013. Soil water balance performs better than climatic water variables in tree species distribution modelling. Global Ecology and Biogeography 22 (4), 470–482. https://doi.org/10.1111/geb.12012.

Praeg, N., Pauli, H., Illmer, P., 2019. Microbial diversity in bulk and rhizosphere soil of ranunculus glacialis along a high-alpine altitudinal gradient. Frontiers in Microbiology 10 (JULY). https://doi.org/10.3389/fmicb.2019.01429.

Pregitzer, K.S., DeForest, J.L., Burton, A.J., Allen, M.F., Ruess, R.W., Hendrick, R.L., 2002. Fine root architecture of nine north American trees. Ecological Monographs 72, 293–309. https://doi.org/10.1890/0012-9615(2002)072[0293:FRAONN]2.0.CO;2.

Price, L.W., Harden, C.P., 2013. Mountain soils. In: Price, L.W., Price, M.F., Byers, A.C., Friend, D.A., Kohler, T. (Eds.), Mountain Geography, first ed. University of California Press, pp. 167–182 http://www.jstor.org/stable/10.1525/j.ctt46n4cj.12.

Poirier, V., Roumet, C., Munson, A.D., 2018. The root of the matter: linking root traits and soil organic matter stabilization processes. Soil Biology and Biochemistry 120 (August 2017), 246–259. https://doi.org/10.1016/j.soilbio.2018.02.016.

Prosser, J.I., Bohannan, B.J.M., Curtis, T.P., Ellis, R.J., Firestone, M.K., Freckleton, R.P., Green, J.L., Green, L.E., Killham, K., Lennon, J.J., Osborn, A.M., Solan, M., van der Gast, C.J., Young, J.P.W., 2007. The role of ecological theory in microbial ecology. Nature Reviews Microbiology 384–392. https://doi.org/10.1038/nrmicro1643.

Rameau, J.C., Mansion, D., Dumé, G., 1999. Flore Forestière Française, Tome 2 Montagnes. Institut pour le Développement Forestier.

Reverchon, F., Bai, S.H., Liu, X., Blumfield, T.J., 2015. Tree plantation systems influence nitrogen retention and the abundance of nitrogen functional genes in the Solomon Islands. Frontiers in Microbiology 6 (DEC), 1–12. https://doi.org/10.3389/fmicb.2015.01439.

Rossi, L.M.W., Mao, Z., Merino-Martín, L., Roumet, C., Fort, F., Taugourdeau, O., Boukcim, H., Fourtier, S., Del Rey-Granado, M., Chevallier, T., Cardinael, R., Fromin, N., Stokes, A., 2020. Pathways to persistence: plant root traits alter carbon accumulation in different soil carbon pools. Plant and Soil 452 (1–2), 457–478. https://doi.org/10.1007/s11104-020-04469-5.

Roth, M., Günther, K., Michiels, H.G., Puhlmann, H., Sucker, C., Hauck, M., 2021. Nitrogen deposition is positively correlated to foliar nitrogen content in Vaccinium myrtillus and other understory species in temperate forests on acidic soil. Acta Oecologica 110 (December 2020), 103696. https://doi.org/10.1016/j.actao.2020.103696.

Roumet, C., Birouste, M., Picon-Cochard, C., Ghestem, M., Osman, N., Vrignon-Brenas, S., Cao, K. fang, Stokes, A., 2016. Root structure-function relationships in 74 species: evidence of a root economics spectrum related to carbon economy. New Phytologist 210 (3), 815–826. https://doi.org/10.1111/nph.13828.

Shen, C., Ni, Y., Liang, W., Wang, J., Chu, H., 2015. Distinct soil bacterial communities along a small-scale elevational gradient in alpine tundra. Frontiers in Microbiology 6 (JUN), 1–12. https://doi.org/10.3389/fmicb.2015.00582.

Shen, C., Xiong, J., Zhang, H., Feng, Y., Lin, X., Li, X., Liang, W., Chu, H., 2013. Soil pH drives the spatial distribution of bacterial communities along elevation on Changbai Mountain. Soil Biology and Biochemistry 57, 204–211. https://doi.org/10.1016/j.soilbio.2012.07.013.

Shi, L., Zhang, H., Liu, T., Mao, P., Zhang, W., Shao, Y., Fu, S., 2018. An increase in precipitation exacerbates negative effects of nitrogen deposition on soil cations and soil microbial communities in a temperate forest. Environmental Pollution 235, 293–301. https://doi.org/10.1016/j.envpol.2017.12.083.

Soest, P. J. Van, 1990. Use of detergents in the analysis of fibrous feeds. ii. a rapid method for the determination of fiber and lignin. Journal of AOAC International 73 (4), 491–497. https://doi.org/10.1093/jaoac/73.4.491.

Sparling, G.P., Schipper, L.A., Hewitt, A.E., Degens, B.P., 2000. Resistance to cropping pressure of two New Zealand soils with contrasting mineralogy. Australian Journal of Soil Research 38 (1), 85–100. https://doi.org/10.1071/SR99065.

Spitzer, C.M., Lindahl, B., Wardle, D.A., Sundqvist, M.K., Gundale, M.J., Fanin, N., Kardol, P., 2021. Root trait–microbial relationships across tundra plant species. New Phytologist 229, 1508–1520. https://doi.org/10.1111/nph.16982.

Stokes, A., Angeles, G., Anthelme, F., Aranda-Delgado, E., Barois, I., Bounous, M., et al., 2021. Shifts in soil and plant functional diversity along an altitudinal gradient in the French Alps. BMC Research Notes 14 (1), 1–4. https://doi.org/10.1186/s13104-021-05468-0.

Sweeney, C.J., de Vries, F.T., van Dongen, B.E., Bardgett, R.D., 2021. Root traits explain rhizosphere fungal community composition among temperate grassland plant species. New Phytologist 229, 1492–1507. https://doi.org/10.1111/nph.16976.

Tian, J., McCormack, L., Wang, J., Guo, D., Wang, Q., Zhang, X., et al., 2015. Linkages between the soil organic matter fractions and the microbial metabolic functional

diversity within a broad-leaved Korean pine forest. European Journal of Soil Biology 66, 57–64. https://doi.org/10.1016/j.ejsobi.2014.12.001.

Tang, Z., Sun, X., Luo, Z., He, N., Sun, O.J., 2018. Effects of temperature, soil substrate, and microbial community on carbon mineralization across three climatically contrasting forest sites. Ecology and Evolution 8 (2), 879–891. https://doi.org/10.1002/ece3.3708.

Thomas, F.M., Molitor, F., Werner, W., 2014. Lignin and cellulose concentrations in roots of Douglas fir and European beech of different diameter classes and soil depths. Trees 28, 309–315. https://doi.org/10.1007/s00468-013-0937-2.

Wang, Y., Mao, Z., Bakker, M.R., Bakker, Kim, J.H., 2018a. Linking conifer root growth and production to soil temperature and carbon supply in temperate forests. Plant and Soil 426, 33–50. https://doi.org/10.1007/s11104-018-3596-7.

Wang, Y., Kim, J.H., Mao, Z., Ramel, M., Pailler, F., Perez, J., et al., 2018b. Tree root dynamics in montane and sub-alpine mixed forest patches. Annals of Botany 122 (5), 861–872. https://doi.org/10.1093/aob/mcy021.

Weemstra, M., Freschet, G.T., Stokes, A., Roumet, C., 2021. Patterns in intraspecific variation in root traits are species-specific along an elevation gradient. Functional Ecology 35, 342–356. https://doi.org/10.1111/1365-2435.13723.

Williams, A., Langridge, H., Straathof, A.L., Muhamadali, H., Hollywood, K.A., Goodacre, R., de Vries, F.T., 2021. Root functional traits explain root exudation rate and composition across a range of grassland species. Journal of Ecology (September 2020), 1–13. https://doi.org/10.1111/1365-2745.13630.

Wu, L., Pan, L., Mitchell, J., Sanden, B., 1999. Measuring saturated hydraulic conductivity using a generalized solution for single-ring infiltrometers. Soil Science Society of America Journal 63 (4), 788–792. https://doi.org/10.2136/sssaj1999.634788x.

Xiao, L., Bi, Y., Du, S., Wang, Y., Guo, C., 2019. Effects of re-vegetation type and arbuscular mycorrhizal fungal inoculation on soil enzyme activities and microbial biomass in coal mining subsidence areas of Northern China. Catena 177 (February), 202–209. https://doi.org/10.1016/j.catena.2019.02.019.

Xu, M., Li, X., Cai, X., Gai, J., Li, X., Christie, P., Zhang, J., 2014. Soil microbial community structure and activity along a montane elevational gradient on the Tibetan Plateau. European Journal of Soil Biology 64, 6–14. https://doi.org/10.1016/j.ejsobi.2014.06.002.

Xu, Y., Seshadri, B., Bolan, N., Sarkar, B., Ok, Y.S., Zhang, W., Rumpel, C., Sparks, D., Farrell, M., Hall, T., Dong, Z., 2019. Microbial functional diversity and carbon use feedback in soils as affected by heavy metals. Environment International 125 (February), 478–488. https://doi.org/10.1016/j.envint.2019.01.071.

Xu, Z., Yu, G., Zhang, X., He, N., Wang, Q., Wang, S., Xu, X., Wang, R., Zhao, N., 2018. Divergence of dominant factors in soil microbial communities and functions in forest ecosystems along a climatic gradient. Biogeosciences 15 (4), 1217–1228. https://doi.org/10.5194/bg-15-1217-2018.

Yang, Q., Wang, X., Shen, Y., 2013. Comparison of soil microbial community catabolic diversity between Rhizosphere and bulk soil induced by tillage or residue retention. Journal of Soil Science and Plant Nutrition 13 (1), 187–199. https://doi.org/10.4067/s0718-95162013005000017.

Yuan, Y., Zhao, W., Xiao, J., Zhang, Z., Qiao, M., Liu, Q., Yin, H., 2017. Exudate components exert different influences on microbially mediated C losses in simulated rhizosphere soils of a spruce plantation. Plant and Soil 419 (1–2), 127–140. https://doi.org/10.1007/s11104-017-3334-6.

Zhang, Q., Huber, H., Beljaars, S.J.M., Birnbaum, D., De Best, S., De Kroon, H., Visser, E. J.W., 2017. Benefits of flooding-induced aquatic adventitious roots depend on the duration of submergence: linking plant performance to root functioning. Annals of Botany 120 (1), 171–180. https://doi.org/10.1093/aob/mcx049.

Zhao, C., Miao, Y., Yu, C., Zhu, L., Wang, F., Jiang, L., Hui, D., Wan, S., 2016. Soil microbial community composition and respiration along an experimental precipitation gradient in a semiarid steppe. Scientific Reports 6 (March), 1–9. https://doi.org/10.1038/srep24317.

Zhou, J., Xia, B., Treves, D.S., Wu, L., Marsh, T.L., Neill, R.V.O., Palumbo, A.V., Tiedje, J. M., 2002. Spatial and resource factors influencing high microbial diversity in soil spatial and resource factors influencing high microbial diversity in soil. Applied and Environmental Microbiology 68 (1), 326–334. https://doi.org/10.1128/AEM.68.1.326. N.