Environmental Drivers of Microbial Functioning in Mediterranean Forest Soils

Caroline Brunel, Anne Marie Farnet da Silva, Raphaël Gros

Research Paper

Title

Environmental drivers of microbial functioning in Mediterranean forest soils

Authors

Caroline Brunel[1,2], Anne-Marie Farnet Da Silva[1] and Raphael Gros[1]

[1] Mediterranean Institute of marine and continental Biodiversity and Ecology, IMBE, Aix Marseille Université, UMR CNRS 7263, IRD, Avignon Université, Campus l'Etoile, Av. Escadrille Normandie Niemen, 13397, Marseille, Cedex 20, France

[2] IRD, IPME; 911 avenue Agropolis, BP 64501, 34394, Montpellier, France

Corresponding Author: Caroline Brunel (caroline.brunel@ird.fr, tel: +33 658 483 326)

ORCID IDs:

Caroline BRUNEL : 0000-0002-0705-5165

Anne-Marie FARNET DA SILVA : 0000-0001-6603-1174

Raphael GROS : 0000-0001-6296-0558

Abstract

Mediterranean forests own distinct characteristics resulting from climate, soil, and vegetation that affect soil microbial communities' assembly and their associated functions. We initiated a multi-scalar analysis of environmental drivers of soil functioning to (1) identify pertinent factorial scales and (2) determine the relative importance of soil, vegetation, and geoclimate relative influences in shaping soil microbial functions across the French Mediterranean forests. Soil samples (0-15 cm) were collected from 60 forest sites and soil physico-chemical and microbiological properties were assessed across different factorial scales i.e., bioclimates, slope exposures, and forest stands. Patterns in microbial catabolic potential (i.e., extracellular enzymes and microbial respiration) and carbon (C) source utilisation (i.e., catabolic-level physiological profiling) were partitioned between vegetation cover, soil characteristics and geoclimate components. Our results reveal that the catabolic potential of soil microbes was strongly influenced by the forest stands, and mainly relied on ammonium and nitrate contents. In contrast, variation in C-source utilisation was mainly explained by vegetation cover. Soil metabolic capacities of microorganisms and resulting C dynamics were largely constrained by climate parameters, which suggests potentially important consequences for soil C storage. Our study revealed diverse structuration patterns between the catabolic potential and the carbon-source utilisation of soil microbial communities, and gives insights into the underlying mechanisms of soil microbial functioning in Mediterranean forests.

Key words

Ecological filters; Mediterranean forest stands; Microbial metabolism; Scale-dependent structuration; Soil microbial communities; Soil physico-chemical properties

Introduction

Studies of the microbial processes involved in the decomposition of soil organic carbon (C) have considerably increased in recent years owing to the major role soil C storage plays in the context of climate change [1]. In the C sink that forest soils represent [2], soil organic C stocks result largely from the balance between (i) inputs of C determined by the vegetation cover, (ii) processes of soil C humification by microbial communities and stabilization and (iii) outputs *via* soil erosion, CO_2 production from roots and microorganisms [3]. Thus, C dynamics in soils depend on complex interactions between climate conditions (e.g. long term mean annual precipitation and temperature) that shape forest structure and tree productivity, soil physico-chemical properties (such as calcium carbonate content and pH) influenced by the geological substratum and their combined effect on soil microbial communities and their metabolism [4].

Microbes are the primary decomposers of plant material due to their unique ability to produce enzymes to break down both simple molecules such as cellulose and more complex plant derived compounds such as lignin [5]. They mobilize and transform a broad range of soil C substrates, assimilating C in their biomass, emitting CO_2 or stabilizing C in soil [6]. There is a lot of debate on the potential drivers controlling soil microbiome assembly and the mechanisms maintaining community structure and composition, and we argue that these rules may be better understood if put in perspective (adapted to the needs and contexts). Soil pH, the chemical composition of organic matter (OM), and climate conditions have been pointed out as key driver of soil functioning at local to continental scales [7–11]. In a recent study from different geographical and climatic zones across Europe, variations in bacterial community diversity and structure were mainly driven by parameters corresponding to similar edaphic and climatic properties [12].

Although these findings provide essential information on soil communities structuration at large scale, the results cannot be transferred to more specific contexts.

In the Mediterranean context particularly, where forests are subjected to specific constrains such as drought stress, heat waves, low nutrient availability and recalcitrant organic compounds [13] we still lack a clear understanding of factors controlling microbial C metabolism and driving soil C dynamics. Experimental determination of drivers has helped to develop which drivers may be of importance on Mediterranean soils (i.e., soil OM quality and water availability [14, 15]), but a more accurate determination of drivers from measurements done *in situ* will give greater insight to the mechanisms maintaining microbial communities' structures and functions in these soils. Determining pertinent spatial scales to observe variation in the functioning of such specific soils is now required to clarify how this ecosystem compartment is driven and to assess microbial functioning and C cycling vulnerability to environmental changes.

Here we report on a study designed to (1) determine pertinent factorial scales for unraveling microbial functional patterns and (2) estimate the relative importance of environmental components (vegetation, geoclimate and soil properties) in shaping of soil microbial functioning and (3) identify specific drivers. To disentangle the patterns and drivers of soil microbial functioning in Mediterranean forest soils, we sampled 60 sites covering an area of about 10.000 km^2 in south-eastern France. We hypothesized that soil microbial metabolic capabilities and C substrate affinities varied according to bioclimates at a regional scale, side slope exposure at a landscape scale and forest stand at a local scale (Fig. 1) due to environmental variations specific to these scales.

Methods

Study sites and sampling design

The sampling was conducted in April 2013 in South-Eastern France (Provence-Alpes-Côte d'Azur), an area characterized by frequent and intense droughts and heat waves typical of the Mediterranean climate. Mean annual temperature and precipitation respectively reach 11.3°C and 799mm (from WorldClim [16], Appendix 2, Fig. S1). Soils are characterized by carbonatic pedofeatures (i.e., fine calcareous silt clay loam). We conducted our study in sub-humid and humid bioclimates [17] corresponding to meso-Mediterranean and supra-Mediterranean altitudinal arrangement of vegetation respectively covered with *Pinus halepensis and Quercus ilex*, and *Pinus sylvestris* and *Quercus pubescens*. Three forest stands were selected for each of the two bioclimates, and were a mono-specific stand of *Pinus* and a mono-specific stand of *Quercus,* and a mixed stand 60/40% of *Pinus*/*Quercus*. All forest stands were about 60 ±10 years-old and were without forest management for 35 years. For each of the six types of forests, five 20mX20m plots were selected on both south- and north-facing slopes. These plots were considered to be true replicates as the distance between them exceeded 2km. All sites were located at altitudes from 500 to 900m for meso-Mediterranean and from 900 to 1100m for supra-Mediterranean bioclimates. Plots were first randomly localized by crossing information from the French Geological Survey (for pedofeature), the Forest Property Regional Center (for forest stands informations) and the National Institute of Geography (for altitude and exposition). The sixty forest plots were visited for cross-validation and were then integrated to the survey (Fig. 1b).

Within each plot, bulk soil samples were collected after removing the litter layer and digging ten small pits over an approximately 20 cm × 20 cm area (the depth was ranging between 10 and 15 cm and was defined by the A horizon limits). The ten sub-samples where sieved (2 mm), pooled and mixed to form a composite sample and placed into a polyethylene bag. In the lab, one

aliquot of each composite sample was stored at 4°C for microbial analyses. A second aliquot was air dried in a warm room (48 hours at 30 degrees) for chemical analyses.

Soil chemical characteristics

Soil water holding capacity (WHC) was determined by the amount of water held in the soil sample vs. the dry weight of the sample. Soil C and N content were measured using a C/N elementary analyzer (Flash EA 1112 ThermoScientific series, Waltham, Massachusetts, USA). Determination of $CaCO_3$ in 10 g soils (dry weight equivalent) was based on the release of CO_2 after addition of HCl 4N [18]. The percentage of inorganic C (C in $CaCO_3$) was calculated as follows: $\%C\text{-}CaCO_3 = 11.991 / 100 \times \%CaCO_3$. Organic C was calculated as the difference between total C and inorganic C contents. Soil pH was determined in distilled water (5 g dry weight in 12.5 mL) after a 45 min equilibration [19]. A subsample of 10 g (dry weight equivalent) was analyzed after extraction in 100 ml of 1 M KCl with electrodes to determine inorganic NH_4^+ and NO_3^- concentration in soils (expressed per gram of dried soil).

Soils C biochemical forms were characterized with solid-state ^{13}C-NMR spectroscopy using cross-polarization and magic angle spinning (CP-MAS). Spectra were obtained on a Bruker Avance 400MHz NMR spectrometer (Rheinstetten, Germany) at a ^{13}C resonance frequency of 106 MHz and a Bruker double-bearing probe (further detailed in Appendix 1). The relative distribution of C groups with different structures was determined by integrating the signal intensities over defined chemical shift windows using Dmfit software [20]. Spectra were divided into four chemical regions (Mathers et al., 2003): alkyl C (0-45 ppm), O-alkyl C (45-112 ppm), aromatic C (112-160 ppm) and carbonyl C (160-185 ppm). To describe OM quality, the following ratios of

125 humification (HR_1 and HR_2) and aromaticity (AR) were calculated [21]: HR_1=alkyl C/ carboxyl C
126 ; HR_2= alkyl C/ O-alkyl C ; AR= aromatic C/(alkyl C+ O-alkyl C+ aromatic C)

127 ***Soil microbial functioning***

128 A full description of the methods used for microbial analyses is provided in the Appendix
129 1. Briefly, the determination of C source utilisation (i.e., catabolic-level physiological profiling,
130 CLPP) was performed using BIOLOG® Eco plate (BIOLOG Inc., Hayward, CA). Plates with an
131 average well colour development (AWCD) of 0.4 were used in the statistical calculations. Shannon
132 index and the mean growth on carbohydrates, carboxylic and ketonic acids, amines/amides,
133 polymers and amino-acids substrates were then determined. Microbial biomass (MB) was
134 estimated using Substrate-Induced Respiration (SIR). Ten grams (dry weight equivalent) of
135 standardized samples at 60% of WHC were placed in 117 ml flushed-air glass jars and amended
136 with a powder of talc and glucose (1000 µg C g-1 soil). After ninety minutes, 1 ml of air was
137 sampled with a syringe and injected into a gas chromatograph (Chrompack CHROM 3 – CP 9001,
138 Middelburg, The Netherlands) to determine CO_2 production. The gas chromatograph was equipped
139 with a thermal conductivity detector and a packed column (Porapack). The carrier gas helium flow
140 was regulated at 60 ml h^{-1}. The CO_2 concentration of flushed air was subtracted from the CO_2
141 concentration of each sample and resulting values were adjusted to 22°C according to Ideal Gas
142 Laws using Q10 = 2. Substrate-induced respiration rates were converted into MB using equations
143 given by [22]. Basal respiration was determined without adding glucose and talc powder and was
144 estimated to calculate the metabolic quotient qCO_2 (the ratio of basal respiration to microbial
145 biomass), which is a sensitive ecophysiological indicator of soil stress induced by environmental
146 conditions [23]. The activity of five extracellular enzymes (EEA) involved in soil C and N cycles
147 were assessed (n=3) for each soil sample to determine the catabolic potential of microbial

communities. Tyrosinase activity was assessed according to the modified method of Saiya-Cork et al. [24]. Two ml of 25 mM L-DOPA solution (L-3,4-dihydroxyphenylalanine) in potassium phosphate buffer (50 mM, pH 6.5) were added to 0.4 g of soil (fresh weight), mixed and incubated for 30 min, in darkness at 25°C. The mixture was centrifuged for 3 minutes at 12 000 g before absorption was measured at 590 nm. Transesterase activity of lipase was assessed according to the method of Goujard et al. [25]. Two ml of distilled water and 4 ml of 10 mM *p*-nitrophényl-caprylate in heptane were added to 1 g of soil (fresh weight) and incubated for 12 h at 30 °C. The reaction was stopped and colour revealed by adding 200µL of the mixture to 4 mL of 0.1 M NaOH, which was immediately centrifuged for 2 min at 12 000 g. The amount of *p*-nitrophenol released was measured at 412 nm. Cellulase activity was assayed using CarboxyMethylCellulose (CMC) 1% in 2 mL of sodium acetate buffer (50 mM, pH 6) added to 0.5g of soil (fresh weight) incubated for 4h at 50°C. Glucose was quantified according to the Somogyi-Nelson method [26] and absorption was read at 870 nm [27]. Protease activity was measured using 5 g of soil (fresh weight) in 5 mL of casein at 2% in Tris HCl buffer (50mM, pH 8.1). The mixture was incubated for 3 hours at 50°C and then the reaction was stopped with 5 mL of Trichloroacetic acid solution (at 15%) and the mixture centrifuged (2min, 12 000g). Aromatic amino acids were detected using Folin reagent (33%) at 700nm. Tyrosine was used as standard. Urease activity was assessed using 0.5g of soil (fresh weight) in 2mL of urea solution (80mM) in a sodium acetate buffer (50mM, pH 6). The mixture was incubated for 2h at 37°C and then centrifuged (2min, 12 000g). Ammonium was revealed in microplates using an adapted Mulvaney method (1996) [28]: to 30µL of the supernatant were added 15µL of EDTA solution, 60µL of Na-salicylate solution and 30µL of hypochlorite solution. After stabilization (45 min), mixture absorption was measured at 667 nm. Enzyme activities were expressed as µmoles of reaction products released per minute (U) per gram of dry

soil ($U.g^{-1}DS$). Another 10 g of soil was incubated for 30 days at 25 °C and maintained at initial moisture and the selective electrodes (Fisher BioBlock Scientific, Hampton, United-States) were used to determine net ammonification and nitrification rates. Ammonification and nitrification rates are here defined as the difference in concentrations of ammonium and nitrate respectively before and after incubation.

Geoclimate and vegetation variables

Mediterranean forests grow on a wide variety of sites defined by different geoclimatic properties that include climate (e.g., precipitation and temperatures) and physiographic characteristics (e.g., slope, distance to the sea, and elevation), here referred as geoclimate. Climatic data were collected from the WorldClim BioClim dataset (0.93km x 0.93km). The 19 collected variables (listed in supplementary Methods, Appendix 1, Table S1) represent temperature and precipitation annual trends, seasonality, and extreme or limiting environmental factors and results from interpolations of observed data representative of 1950-2000 [16]. Geographic data (Altitude, Exposure, Slope, Distance to the sea, Latitude and Longitude) were obtained from the National Institute of Geography BD ALTI® database (1mx1m). Values were extracted from each site locations using QGIS software (QGIS Development Team, 2.8 'Wien' 2015).

Vegetation cover surveys were based on species identification and overlapped among the different layers that were further analysed using an abundance community matrix (Braun-Blanquet approach [29]. The species composition was determined at each site for tree, shrub and herb layers. Vegetation measurements included the plant species richness, the angiosperm and gymnosperm abundances and their relative abundance, the vegetation evenness, the Shannon diversity indexes of the whole community, and of the herb, shrub and non-tree layers.

Statistical analyses

The different variables and matrix considered were summarized in Table S1 (Appendix 1). Two sets of microbiological data were obtained, and were a matrix of the catabolic potential based on the EEA and SIR analyses and a matrix of C source utilisation based on CLPP. Three environmental datasets (i.e., soil, geoclimate [hereafter geoclimate variables] and vegetation were constituted respectively from 12 soil variables, 25 geoclimate variables, and from the vegetation cover. Dissimilarity matrix were calculated based on Euclidian distances for geoclimate, soil, CLPP and EEA datasets and Manhattan distance for the vegetation cover.

Permutational analysis of variance (PERMANOVA), implemented in the 'adonis' function ('vegan' package [30]), were used to test for significant effects of the factorial scales and their interaction on the environmental components (distance matrixes).

The effects of factorial scales and their interaction on each microbial and soil variable were also addressed using analysis of variance (ANOVA) on linear models. Shapiro and Levene tests were respectively used to assess the normality and equality of variances. Variables that did not fit linear model requirements were transformed using the 'bestNormalize' function ('bestNormalize' package, Peterson, 2018). Used transformations are listed in the Table S1 (Appendix 1).

Variation partitioning was completed to rank the influence of environmental components on microbial functioning, their variations were examined as follows. First, non-metric multidimensional scaling (NMDS), which were best designed to represent the ordering relationships among objects in a small and specified number of axes [31], were used for variable reduction [33] of each distance matrixes and the 5 first axis were subsequently used. Then, variations in microbial functioning were partitioned into the pure effects of geo-climatic, soil and

vegetation components (i.e., variation of the model explained independently by each factor) and into their interactions (i.e., variation of the model explained simultaneously by two or three factors) using constrained and un-constrained distance-based redundancy analysis (db-RDA, function 'varpart', vegan package [30]). The significance of the testable fractions was determined by ANOVA of the partial db-RDA test using 999 permutations and results were represented by Venn diagrams.

Unconstrained RDA were then performed on models that considered a subset of the environmental variables. We first reduced the number of explanatory variables based on a variance inflation factor (VIF) analysis to account for multicollinearity among covariates [34]. Variance inflation factor values were calculated for a full model then for a reduced model obtained by sequentially deleting each variable for which the VIF was the highest until all remaining VIFs were below 2 [35]. Significance of covariates was tested by ANOVA using 999 permutations.

Finally, we observed relationships linking microbial variables to environmental variables by determining the Spearman correlation coefficient (rho).

All statistical analyses were performed using R version 3.5.0. To account for multiple comparisons Benjamini and Hochberg false discovery rate corrections were applied when required [36].

Results

Relevance of bioclimate, slope exposure and forest stand in shaping soil functioning patterns

First, PERMANOVA were performed to test the effects of the different factorial scales and their interaction on both the microbiological and environmental components (Fig. 2 and Table S1 in

Appendix 2). Bioclimates explained a low but significant part of variation in both C source utilisation (F=2.28, r^2=0.04, p=0.006) and in catabolic potential (F=4.15, r^2=0.06, p=0.005). PERMANOVA also revealed modification in enzymatic rates induced by forest stands (F=2.503, r^2=0.08, p=0.011). As expected, variation in geoclimate and vegetation cover were well described both across bioclimates (F=124.88, r^2=0.66, p=0.001 and F=42.831, r^2=0.37, p=0.001, respectively) and the interaction between bioclimate and forest stands (F=3.75, r^2=0.19, p=0.020 and F=4.11, r^2=0.07, p=0.001, respectively). Both bioclimate and forest stand defined pertinent scales that could be used to disentangle variations in soil microbial functioning. Bioclimate was the main factor driving microbial functioning. In contrast, variation due to slope exposure was imperceptible on soil, vegetation cover or microbial patterns. It is worth mentioning here that we observed differences in both mean annual temperatures and annual precipitation between north- and south-exposed sites in both meso- and supra-Mediterranean contexts (Appendix 2, Fig. S1), but did not capture the expected lower temperatures on north-facing slopes in the supra-Mediterranean context. Additionally, PERMANOVA revealed that soil properties were not structured through any of the considered scales, suggesting that soil physico-chemical characteristics were likely to be driven at larger or smaller scales.

Bioclimates had a substantial effect on both soil physico-chemical and microbial characteristics (Fig. 3 and Table S2 in Appendix 2). Total N (F=21.893, p_{adj}<0.001) and organic C content (F=27.761, p_{adj}<0.001, Fig. 3h) indicated a greater quantity of OM in soils from meso- than from supra-Mediterranean forests together with a higher water holding capacity of the soils (F=11.513, p_{adj}=0.008, Fig. 3g). On the other hand, certain characteristics of soil linked with the inorganic fraction, such as calcium carbonate and pH, did not vary between bioclimates. No differences in OM aromaticity (AR) were observed between the two bioclimates, while a higher amount of

organic acids with long alkyl-chains (HR_1, F=8.725, p_{adj}=0.0019, Fig. 3i) was found in supra-Mediterranean forest soils. This suggested a higher humification rate associated with production of organic acids with short-alkyl chains (F=16.104, p_{adj}=0.001, Fig. 3j) in meso-Mediterranean soils.

None of the enzyme potential or C source preferences was differently expressed between the two bioclimates although the H' index calculated from CLPP indicated slightly but not significantly higher functional diversity in meso-Mediterranean soils (F=5.160, p=0.028, p_{adj}=ns). Relating microbial-C to organic-C underlined a higher biomass content per unit of OM in supra-Mediterranean soils (F=23.517, p_{adj}<0.001, Fig. 3l). The catabolic quotient qCO_2 was also higher in the meso-Mediterranean context (F=20.499, p_{adj}<0.001, Fig. 3k).

Both slope exposure and forest stand had substantial influences on microbial parameters and soil characteristics. Cellulase activity was higher on south-facing slopes (F=14.132, p_{adj}=0.011, Fig. 3f), where we observed slightly higher carboxyl C and WHC (respectively, F=7.002, p=0.011, p_{adj}=ns and F=5.061, p=0.029, p_{adj}=ns). The influence of forest stand was expressed on measurements of OM recalcitrance, i.e N content and C/N ratio, aromatic C and AR ratio, and tyrosinase activity. Total N content was higher in soils of oak than pinewoods and mixed stands (F=20.872, p_{adj}=0.006, Fig. 3a), which resulted in the opposite C/N trend (F=20.368, p_{adj}<0.001, Fig. 3c). Aromatic content and Ar ratio (F=8.061, p_{adj}=0.006, Fig. 3d) were higher in pinewoods (F=9.557, p_{adj}=0.004). *Pinus* forests soils, characterized by lower N and higher pH (F=6.333, p_{adj}=0.020, Fig. 3e), were observed together with a weaker tyrosinase activity (F=8.141, p_{adj}=0.006, Fig. 3b).

Variation partitioning of soil microbial functioning

281 Because microbial patterns varied between the two bioclimates, variance partitioning analyses
282 were carried out at both regional and sub-regional scales to determine the effects of geoclimate,
283 soil and vegetation on both C source utilisation and catabolic potential patterns (Fig. 4 and Table
284 S3, Appendix 2).

285 Overall explanation by environmental components reached *ca.* 30% and 45% of the C source
286 utilisation (Fig. 4a) and of catabolic potential respectively (Fig. 4d) at the regional scale, and
287 increased to *ca.* 50% (Fig.4b, 4c) and 65% (Fig. 4e, 4f) at landscape and local scales respectively.
288 Carbon source utilisation patterns were mainly and significantly influenced by vegetation cover
289 ($F=1.352$, $r^2=0.11$, $p=0.030$, Fig. 4a) at the regional scale, but no significant influences of the three
290 components were observed at sub-regional meso- and supra-Mediterranean scales (Fig. 4b, 4c).
291 Soil physico-chemical prints explained a large part of variation in microbial catabolic potential at
292 both regional (shared effect: $F=5.111$, $r^2=0.32$, $p=0.001$; pure effect: $F=3.929$, $r^2=0.243$, $p=0.001$,
293 Fig. 4d) and sub-regional scales. In meso-Mediterranean forest, variation in catabolic potential was
294 due to the shared effect of soil properties ($F=3.288$, $r^2=0.406$, $p=0.001$, Fig. 4e) encompassing 16%
295 of vegetation-soil common explanation. In supra-Mediterranean forests, both soil (shared effect:
296 $F=2.747$, $r^2=0.364$, $p=0.003$; pure effect: $F=2.376$, $r^2=0.0255$, $p=0.004$, Fig. 4f) and vegetation
297 (pure effect: $F=1.902$, $r^2=0.204$, $p=0.029$, Fig. 4f) explained variation in catabolic potential.

298 ***Driving forces of soil microbial functioning***

299 To disentangle effects of specific drivers amongst the edaphic, geoclimate and vegetation
300 components, we tested the influence of the main environmental covariates on soil microbiological
301 patterns. Collinear factors were first removed sequentially based on their VIF scores. Remaining
302 covariates included site exposition, slope, longitude, mean temperature of the wettest quarter,

303 $CaCO_3$, total N, carboxyl C, O-alkyl C, nitrate, ammonium, bulk density, and vegetation richness
304 (Table S4, Appendix 2). Only edaphic parameters stand out as driving forces of microbial
305 functioning patterns. Carbon source utilisation was related to total N content at regional scale
306 (F=1.825, p=0.019), and to soil bulk density in supra-Mediterranean forests (F=1.698, p=0.035).
307 Catabolic potential was structured by $CaCO_3$ (F=9.49, p=0.001), total N (F=7.389, p=0.001),
308 nitrate (F=5.320, p=0.002) and ammonium (F=3.157, p=0.009) content at regional scale, and by
309 $CaCO_3$ content (F=5.780, p=0.001) in meso-Mediterranean context.

310 To further describe underlying mechanisms of microbial functioning, we examined relationships
311 linking microbial assemblages to environmental parameters (Fig. 5 and supplementary Fig. 2 in
312 Appendix 2). Carbon source preferences and associated functional diversity index were unrelated
313 to environmental variables. Five edaphic parameters were favoring soil microbial properties: the
314 BR, qCO_2, urease, tyrosinase, and cellulase increased with soil WHC; BR, MB and urease activity
315 increased with the soil alkyl C fraction; BR and qCO_2 increased with soil organic C content, MB
316 and MB per unit of organic C increased with HR1. Soil pH and $CaCO_3$ both negatively affected
317 BR, MB and tyrosinase activity. The metabolic quotient (qCO_2) was particularly sensitive to
318 several climate parameters as it was upregulated by temperature indexes (MTWaQ, min TCM,
319 MTDQ, MTCQ, annual MT, max MTWaM) and downregulated by precipitation indexes (annual
320 P, PWaQ, PDQ and PDM). Interestingly, tyrosinase activity was restrained by gymnosperm total
321 and relative abundances together with plant species richness. Although vegetation has been
322 previously shown to influence overall soil microbial patterns, vegetation diversity indexes were
323 not directly related to any specific microbial markers, suggesting that forest community
324 composition rather that forest community structure plays an important role in microbial
325 functioning.

Discussion

Multiple scaling of soil microbial functioning

Comparison between C utilisation and microbial catabolic potential patterns highlighted different structuration scales (i.e., a strong influence of bioclimates on C source utilization *vs.* a finer effect of forest stands on catabolic potentials), and testified to scale-dependent variations in microbial functioning. While limitations in the BIOLOG methodology for the characterization of whole communities are well known, CLPP remain a useful tool to detect culturable copiotrophic bacteria living in forest soil [37]. Fungi have broader enzymatic capabilities [38] and potentially greater C use efficiency [39] than bacteria. Shift in the respective functional capabilities of fungi and bacteria could partly explain the observed patterns (Fig. 2d and 2e), with substantial implications relevant to C and N cycling as these taxa own distinct C use efficiencies [40].

Variation in C source utilisation was detected between the two bioclimates considered, and was mainly explained by vegetation cover (Fig. 4). However, C use patterns did not differ between forest stands and no substrate preference has been related with descriptor of vegetation (Fig. 5). This suggested that some plant species associated with bioclimates influenced C-substrate affinity over the dominant tree species considered here. Previous studies in grasslands showed modification in soil microbial communities in response to plant community composition [41, 42] but fewer considered treed ecosystems [43, 44], and to our knowledge, only one considered CLPP profiles. In this study [45], both tree diversity and identity affected CLPP. Tree species and even tree genotypes have been previously shown to influence litter chemistry and to control C and nutrient dynamics [46, 47]. Although not further tested here, it is likely that the observed modifications in CLPP reveal a shift in community composition of copiotrophic bacteria [37], that preferentially consume the labile pool of organic C (e.g., freshly fallen litter) [48]. The main driver

of C use patterns was the total N content, which has previously been revealed as a driver of microbial functional and taxonomic diversity. For instance, Fierer et al. [10] observed shifts in C use utilisation across N gradients together with bacterial phylogenetic and metagenomic responses. Moreover, in our study, functional diversity decreased with soil pH, which has often been pointed out as an important driver of bacterial diversity [49] and of functional diversity approached *via* CLPP [50].

Unlike C source utilisation, catabolic potential patterns were shaped by forest stands beyond the influence of bioclimates, and more strongly relied on N-related markers (i.e., nitrate, ammonium and total N content). This is likely because decomposer N-requirements are not completely fulfilled by N plant litter concentration [51]. Nitrogen has to be immobilized by decomposers from their resources until the elements reach a critical threshold that allows N conversion into microbial biomass or production of EEA [52]. Nitrogen content significantly changed across the six forest stands considered in our study with particularly exacerbated differences between oak and pine forests. Such differences in OM quality between these Mediterranean tree species have been previously shown together with significant consequences in the resulting soil organic C stocks [53]. These results together indicate that the functional composition of microbial communities (estimated through the C uses) is shaped at larger scales than their actual catabolic potential, which relies on marker of OM quality defined at the forest stand scale.

Differential microbial functioning across bioclimates

It is a central principle of biogeography that climate exerts a dominant control over the natural distribution of species [54]. In our study, we revealed that the vegetation stage was not a suitable

predictor of soil chemical properties. According to Pons and Quézel (1998) [55], the meso-Mediterranean bioclimate corresponds to a warm bioclimate with drought waves, while supra-Mediterranean winters are harsher and summer more temperate. In the Mediterranean context, vegetation has acquired morphological traits to face the deficit of water, e.g. slow growth, thick cuticle, allelopathic compounds and low nutrient content [56]. These particular climate conditions, exacerbated in meso-Mediterranean forests, select sclerophyllous vegetal species (such as *Quercus ilex, Quercus coccifera* etc.) that we expected to detect in soil physico-chemical prints. We did not detect such general pattern. However, a higher carboxyl C contents in meso-Mediterranean soils could relate to a larger sclerophyllous plant community in meso-Mediterranean forests (as carboxyl C reflects the cutin polymer of leaves cuticle [57]).

Meso-Mediterranean soils were characterized by higher organic C and WHC. Water holding capacity in soil relies on its OM content [58]. Differences in C content can be driven by higher C inputs (i.e., primary production) and/or by lower microbial mineralization rates. Calcium carbonate content had a strong influence in shaping microbial catabolic potential, even more pronounced in meso-Mediterranean soils. Exchangeable calcium positively correlates with SOC concentration and its resistance to microbial degradation [59]. This is in line with our results that point out higher organic C in meso-Mediterranean soils where the $CaCO_3$ content had higher influence on the microbial catabolic potential. As water molecules prevent the direct approach or sorption of an organic substance to a mineral's surface [59], lower precipitation in meso-Mediterranean forests might have increase an inhibitor effect of $CaCO_3$ on microbial catabolic potential.

Slightly higher functional microbial diversity under recalcitrant vegetation and more arid conditions from meso-Mediterranean could suggest a shift of functional communities [60] since

394 harsher conditions tend to select resistant and specialist populations corresponding to an
395 oligotrophic lifestyle [61]. Tardy et al. [62] showed that a higher microbial diversity improved the
396 functional stability of microbial communities after heat stresses, which are common in the meso-
397 Mediterranean bioclimate. We also found respectively a small and a significant increase in
398 microbial basal respiration and in qCO_2 of meso-Mediterranean forest soils that suggests a better
399 potential for mineralization in meso-Mediterranean when water is available (60 % WHC). Thus,
400 in meso-Mediterranean forest soils, when hydric conditions are suitable, the mineralization
401 potential is likely to be high and to lead to CO_2 production (for instance in spring and fall), while
402 during summer drought, OM decomposition is probably drastically inhibited. This suggests that
403 the observed higher C contents in the meso-Mediterranean context then result from climate
404 constraints.

405 ***Detection of variations in microbial functioning at smaller scales***

406 We found that soil chemical and microbial characteristics (total N, aromatic C and
407 tyrosinase activity) related to OM recalcitrance varied among forest stands (Fig. 3 a-e). In a
408 previous study, Brunel et al. [53] investigated the effect of relative oak/pine tree composition using
409 the same tree species and bioclimates and determined that relative aromatic content increased
410 linearly with pine abundance. Recalcitrant OM, mainly aromatic compounds, limits microbial
411 growth by toxic effects on microbial cells (for instance on membranes), limiting bioavailability of
412 nutrients, and can also bind to microbial enzymes and inhibit their activities [63, 64]. As soil N is
413 tightly bound to recalcitrant humic acids, a limited availability and/or quantity of N under
414 pinewood is likely to enhance aromatic C accumulation in pine stands [65].

Side slope exposure has previously been reported to affect forest soils directly through its influence on radiation, temperature and moisture [66], and indirectly by affecting vegetation cover and structures [67]. Variations in geoclimate were not perceptible between south- and north facing slopes. This can point out two mutually non-exclusive scenarios: (i) that the used climate data are unsuitable to unravel differences at the slope scale (pixel of 0.86 km^2 for the BioClim modeled variables [16]), and/or (ii) that microclimatic variations between sites were consistently larger than the variations caused by differences induced by slope exposure. We detected higher cellulase activities in soils of south-exposed forests, and slight modifications in carboxyl C content. In contrast with our results, differences in vegetation cover between north- and south-facing slopes have been previously reported in Mediterranean areas; overall vegetation cover and species richness were shown to be reduced on north-facing slopes and a greater number of evergreen species have been observed on south slopes [68, 69]. In their study on forest soils, Måren et al [70] showed that, in arid environments, slope aspect is less important in driving soil properties than in moister environments where more microclimatic contrasts are observed.

Environmental drivers and their potential implications in the context of climate change

While Mediterranean forests have the potential to strongly contribute to global C sink [71], their SOC stocks are known to be lower than other forest types. This pattern is commonly explained by factors such as landuse legacy (abandoned pastures) and fire frequency, and also by the low productivity of Mediterranean tree species [72]. Here we also observed that soil microbial functioning is largely explained by climate parameters. We reported above that C stocks in meso-Mediterranean forest soils resulted from climate constraints. Two other observations tend to reveal that soil microbial functioning in Mediterranean forest soils is controlled by climate variation. First, qCO_2, which indicates the efficiency by which soil microorganisms use C-resources in soil

[73], was strongly associated to climate variation (up-regulated by 6 indexes of temperature and down-regulated by 5 indexes of precipitation). Then, 5 microbial markers (i.e., basal respiration, metabolic quotient, urease, tyrosinase, cellulase activities and ammonium mineralization rate) were strongly related to the water holding capacity, which reflects the water-balance fluctuations in soils. As in the Mediterranean region, average annual temperatures are well above current global warming trends [74], our results suggest that soil microbial functioning and resulting C dynamics will be drastically affected in the coming years. Our results support recent findings of Diamond et al. [75] who reveal that climate change can have a direct impact on the relative abundance and metabolic capacities of microorganisms in Mediterranean soil ecosystems, with potentially important impacts for both soil C storage and gas release.

Conclusion

The use of a multi-scalar sampling design allowed us to show the prevailing influence of bioclimates and forest stands in controlling soil functioning over slope exposure and provide insights into the underlying mechanisms. Because climate is responsible for vegetation type, which in turn provides different soil C substrates that are metabolized under different conditions (e.g., temperature or moisture), the bioclimate effect *per se* is hard to disentangle. However, by describing variations associated with the bioclimate in soil properties, climate and vegetation cover, this study provides clues on which facet of the bioclimatic context (i.e., geo-climate, soil or vegetation) are the most structuring. We found that vegetation cover better explained variations in C source utilization, whereas edaphic characteristics better explained variations in catabolic potential of microbial communities. These results, therefore, contribute to a better understanding of the environmental drivers of microbial functioning across the French Mediterranean landscape. Further studies focusing on soil metabarcoding and transcriptomic would be required to shed light

on the environmental filters of microbial composition in relation to their expressed metabolism, and on the drivers of bacterial and fungal communities respectively.

Acknowledgments

The project received funding from the French Environment and Energy Management Agency (ADEME) and Region Provence Alpes Côte d'Azur (France). We thank Florence Ruaudel, Fabio Ziarelli, and Sebastien Milanesio for their technical assistance and are grateful to the Forest Property Regional Center (CRPF) for their contribution, especially to Olivier Martineau for his valuable support in determining the selected sites.

References

1. Bradford MA, Wieder WR, Bonan GB, et al (2016) Managing uncertainty in soil carbon feedbacks to climate change. Nat Clim Change 6:751

2. Lal R (2005) Forest soils and carbon sequestration. For Soils Res Theory Real Its Role Technol Sel Ed Pap 10th North Am For Soils Conf Held Saulte Ste Marie Ont Can 20-24 July 2003 220:242–258. https://doi.org/10.1016/j.foreco.2005.08.015

3. Tian H, Lu C, Yang J, et al (2015) Global patterns and controls of soil organic carbon dynamics as simulated by multiple terrestrial biosphere models: Current status and future directions. Glob Biogeochem Cycles 29:775–792. https://doi.org/10.1002/2014GB005021

4. Pan Y, Birdsey RA, Fang J, et al (2011) A Large and Persistent Carbon Sink in the World's Forests. Science 333:988–993. https://doi.org/10.1126/science.1201609

5. Romaní AM, Fischer H, Mille-Lindblom C, Tranvik LJ (2006) Interactions of bacteria and fungi on decomposing litter: differential extracellular enzyme activities. Ecology 87:2559–2569

6. Bardgett RD, Freeman C, Ostle NJ (2008) Microbial contributions to climate change through carbon cycle feedbacks. ISME J 2:805–814

7. Davidson EA, Janssens IA (2006) Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. Nature 440:165–173

8. Rousk J, Baaath E, Brookes PC, et al (2010) Soil bacterial and fungal communities across a pH gradient in an arable soil. ISME J 4:1340–1351

9. Rousk J, Brookes PC, Baath E (2009) Contrasting soil pH effects on fungal and bacterial growth suggest functional redundancy in carbon mineralization. Appl Env Microb 75:. https://doi.org/10.1128/AEM.02775-08

10. Fierer N, Lauber CL, Ramirez KS, et al (2012) Comparative metagenomic, phylogenetic and physiological analyses of soil microbial communities across nitrogen gradients. ISME J 6:1007–1017. https://doi.org/10.1038/ismej.2011.159

11. Bonanomi G, Capodilupo M, Incerti G, Mazzoleni S (2014) Nitrogen transfer in litter mixture enhances decomposition rate, temperature sensitivity, and C quality changes. Plant Soil 381:307–321. https://doi.org/10.1007/s11104-014-2119-4

12. Plassart P, Prévost-Bouré NC, Uroz S, et al (2019) Soil parameters, land use, and geographical distance drive soil bacterial communities along a European transect. Sci Rep 9:1–17. https://doi.org/10.1038/s41598-018-36867-2

13. Castaño C, Lindahl BD, Alday JG, et al (2018) Soil microclimate changes affect soil fungal communities in a Mediterranean pine forest. New Phytol 220:1211–1221. https://doi.org/10.1111/nph.15205

14. Brunel C, Gros R, Lerch TZ, Da Silva AMF (2020) Changes in soil organic matter and microbial communities after fine and coarse residues inputs from Mediterranean tree species. Appl Soil Ecol 149:103516

15. Boukhdoud N, Silva AMFD, Darwish T, Gros R (2017) Olive mill waste and glyphosate-based herbicide addition to olive grove soils: effects on microbial activities and their responses to drying–rewetting cycles. Soil Use Manag 33:499–510. https://doi.org/10.1111/sum.12367

16. Hijmans RJ, Cameron SE, Parra JL, et al (2005) Very high resolution interpolated climate surfaces for global land areas. Int J Climatol 25:1965–1978. https://doi.org/10.1002/joc.1276

17. Medail F, Quezel P (2003) Que faut-il entendre par" forêts méditerranéennes"? XXIV:

18. Chaney RC, Slonim SM, Slonim SS (1982) Determination of calcium carbonate content in soils. In: Geotechnical properties, behavior, and performance of calcareous soils. ASTM International, 3-15, https://doi.org/101520/STP28907S

19. Aubert G (1978) Méthodes d'analyses des sols. Centre national de documentation pédagogique, Centre régional de documentation pédagogique de Marseille

20. Massiot D, Fayon F, Capron M, et al (2002) Modelling one-and two-dimensional solid-state NMR spectra. Magn Reson Chem 40:70–76

21. Baldock J, Preston CM (1995) Chemistry of carbon decomposition processes in forests as revealed by solid-state 13C NMR. 89–117, https://doi.org/10.2136/1995.carbonforms.c6

22. Beare MH, Neely CL, Coleman DC, Hargrove WL (1990) A substrate-induced respiration (SIR) method for measurement of fungal and bacterial biomass on plant residues. Soil Biol Biochem 22:585–594. http://dx.doi.org/10.1016/0038-0717(90)90002-H

23. Anderson T-H (2003) Microbial eco-physiological indicators to asses soil quality. Agric Ecosyst Environ 98:285–293. http://dx.doi.org/10.1016/S0167-8809(03)00088-4

24. Saiya-Cork K, Sinsabaugh R, Zak D (2002) The effects of long term nitrogen deposition on extracellular enzyme activity in an *Acer saccharum* forest soil. Soil Biol Biochem 34:1309–1315

25. Goujard L, Villeneuve P, Barea B, et al (2009) A spectrophotometric transesterification-based assay for lipases in organic solvent. Anal Biochem 385:161–167

26. Ladd J, Butler J (1972) Short-term assays of soil proteolytic enzyme activities using proteins and dipeptide derivatives as substrates. Soil Biol Biochem 4:19–30

27. Farnet Da Silva AM, Qasemian L, Guiral D, Ferré E (2010) A modified method based on arsenomolybdate complex to quantify cellulase activities: Application to litters. Pedobiologia 53:159–160. http://dx.doi.org/10.1016/j.pedobi.2009.09.001

28. Mulvaney DL, America SSS of, Agronomy AS of (1996) Methods of Soil Analysis Part 3: Chemical Methods. Amer Society of Agronomy, Madison, Wis

29. Braun-Blanquet J (1932) Plant sociology. The study of plant communities. First ed. Plant Sociol Study Plant Communities First Ed

30. Dixon P (2003) VEGAN, a package of R functions for community ecology. J Veg Sci 14:927–930. https://doi.org/10.1111/j.1654-1103.2003.tb02228.x

31. Peterson RA (2018) bestNormalize: Normalizing Transformation Functions, R package version 1.2. 0

32. Borcard D, Gillet F, Legendre P (2018) Unconstrained Ordination. In: Borcard D, Gillet F, Legendre P (eds) Numerical Ecology with R. Springer International Publishing, Cham, pp 151–201

33. Quinn GP, Keough MJ (2002) Experimental design and data analysis for biologists. Cambridge University Press

34. James G, Witten D, Hastie T, Tibshirani R (2013) An introduction to statistical learning. Springer

35. Zuur AF, Ieno EN, Elphick CS (2010) A protocol for data exploration to avoid common statistical problems. Methods Ecol Evol 1:3–14

36. Benjamini Y, Hochberg Y (1995) Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. J R Stat Soc Ser B Methodol 57:289–300

37. Lladó S, Baldrian P (2017) Community-level physiological profiling analyses show potential to identify the copiotrophic bacteria present in soil environments. PLOS ONE 12:e0171638. https://doi.org/10.1371/journal.pone.0171638

38. Waring BG, Averill C, Hawkes CV (2013) Differences in fungal and bacterial physiology alter soil carbon and nitrogen cycling: insights from meta-analysis and theoretical models. Ecol Lett 16:887–894. https://doi.org/10.1111/ele.12125

39. Six J, Frey SD, Thiet RK, Batten KM (2006) Bacterial and Fungal Contributions to Carbon Sequestration in Agroecosystems. Soil Sci Soc Am J 70:555–569. https://doi.org/10.2136/sssaj2004.0347

40. McGuire KL, Treseder KK (2010) Microbial communities and their relevance for ecosystem models: Decomposition as a case study. Soil Biol Biochem 42:529–535. https://doi.org/10.1016/j.soilbio.2009.11.016

41. Bardgett RD, Shine A (1999) Linkages between plant litter diversity, soil microbial biomass and ecosystem function in temperate grasslands. Soil Biol Biochem 31:317–321

42. Eisenhauer N, Beßler H, Engels C, et al (2010) Plant diversity effects on soil microorganisms support the singular hypothesis. Ecology 91:485–496. https://doi.org/10.1890/08-2338.1

43. Scheibe A, Steffens C, Seven J, et al (2015) Effects of tree identity dominate over tree diversity on the soil microbial community structure. Soil Biol Biochem 81:219–227. https://doi.org/10.1016/j.soilbio.2014.11.020

44. Thoms C, Gattinger A, Jacob M, et al (2010) Direct and indirect effects of tree diversity drive soil microbial diversity in temperate deciduous forest. Soil Biol Biochem 42:1558–1565. https://doi.org/10.1016/j.soilbio.2010.05.030

45. Khlifa R, Paquette A, Messier C, et al (2017) Do temperate tree species diversity and identity influence soil microbial community function and composition? Ecol Evol 7:7965–7974. https://doi.org/10.1002/ece3.3313

46. Chavez-Vergara B, Merino A, Vázquez-Marrufo G, García-Oliva F (2014) Organic matter dynamics and microbial activity during decomposition of forest floor under two native neotropical oak species in a temperate deciduous forest in Mexico. Geoderma 235–236:133–145. https://doi.org/10.1016/j.geoderma.2014.07.005

47. Pérez- Izquierdo L, Saint- André L, Santenoise P, et al (2018) Tree genotype and seasonal effects on soil properties and biogeochemical functioning in Mediterranean pine forests. Eur J Soil Sci 69:1087–1097. https://doi.org/10.1111/ejss.12712

48. Fierer N, Bradford MA, Jackson RB (2007) Toward an ecological classification of soil bacteria. Ecology 88:1354–1364

49. Lauber CL, Hamady M, Knight R, Fierer N (2009) Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. Appl Environ Microbiol 75:5111–5120

50. Rutgers M, Wouterse M, Drost SM, et al (2016) Monitoring soil bacteria with community-level physiological profiles using Biolog^{TM} ECO-plates in the Netherlands and Europe. Appl Soil Ecol 97:23–35. https://doi.org/10.1016/j.apsoil.2015.06.007

51. Zechmeister-Boltenstern S, Keiblinger KM, Mooshammer M, et al (2015) The application of ecological stoichiometry to plant-microbial-soil organic matter transformations. Ecol Monogr 85:133–155. https://doi.org/10.1890/14-0777.1

52. Parton W, Silver WL, Burke IC, et al (2007) Global-Scale Similarities in Nitrogen Release Patterns During Long-Term Decomposition. Science 315:361–364. https://doi.org/10.1126/science.1134853

53. Brunel C, Gros R, Ziarelli F, Da Silva AMF (2017) Additive or non-additive effect of mixing oak in pine stands on soil properties depends on the tree species in Mediterranean forests. Sci Total Environ 590:676–685

609 54. Pearson RG, Dawson TP (2003) Predicting the impacts of climate change on the
610 distribution of species: are bioclimate envelope models useful? Glob Ecol Biogeogr
611 12:361–371. https://doi.org/10.1046/j.1466-822X.2003.00042.x

612 55. Pons A, Quézel P (1998) À propos de la mise en place du climat méditerranéen. Comptes
613 Rendus Académie Sci - Ser IIA - Earth Planet Sci 327:755–760.
614 http://dx.doi.org/10.1016/S1251-8050(99)80047-0

615 56. Sardans J, Peñuelas J (2013) Plant-soil interactions in Mediterranean forest and shrublands:
616 impacts of climatic change. Plant Soil 365:1–33. https://doi.org/10.1007/s11104-013-1591-
617 6

618 57. Bhunia RK, Showman LJ, Jose A, Nikolau BJ (2018) Combined use of cutinase and high-
619 resolution mass-spectrometry to query the molecular architecture of cutin. Plant Methods
620 14:117. https://doi.org/10.1186/s13007-018-0384-6

621 58. Lal R (2018) Digging deeper: A holistic perspective of factors affecting soil organic carbon
622 sequestration in agroecosystems. Glob Change Biol 24:3285–3301.
623 https://doi.org/10.1111/gcb.14054

624 59. Rowley MC, Grand S, Verrecchia ÉP (2018) Calcium-mediated stabilisation of soil organic
625 carbon. Biogeochemistry 137:27–49. https://doi.org/10.1007/s10533-017-0410-1

626 60. Yuste JC, Fernandez-Gonzalez AJ, Fernandez-Lopez M, et al (2014) Strong functional
627 stability of soil microbial communities under semiarid Mediterranean conditions and
628 subjected to long-term shifts in baseline precipitation. Soil Biol Biochem 69:223–233

629 61. Paul EA (2014) Soil microbiology, ecology and biochemistry. Academic press

630 62. Tardy V, Mathieu O, Lévêque J, et al (2014) Stability of soil microbial structure and
631 activity depends on microbial diversity. Environ Microbiol Rep 6:173–183

632 63. Chomel M, Guittonny-Larchevêque M, Fernandez C, et al (2016) Plant secondary
633 metabolites: a key driver of litter decomposition and soil nutrient cycling. J Ecol 104:1527-
634 1541

635 64. Mutabaruka R, Hairiah K, Cadisch G (2007) Microbial degradation of hydrolysable and
636 condensed tannin polyphenol–protein complexes in soils from different land-use histories.
637 Soil Biol Biochem 39:1479–1492

638 65. Rovira P, Vallejo VR (2002) Labile and recalcitrant pools of carbon and nitrogen in organic
639 matter decomposing at different depths in soil: an acid hydrolysis approach. Geoderma
640 107:109–141. http://dx.doi.org/10.1016/S0016-7061(01)00143-4

641 66. Pook EW, others (1966) The influence of aspect on the composition and structure of dry
642 sclerophyll forest on Black Mountain, Canberra. ACT. Aust J Bot 14:223–242

643 67. Cantlon JE (1953) Vegetation and microclimates on north and south slopes of Cushetunk
644 Mountain, New Jersey. Ecol Monogr 241–270

645 68. Martínez-Murillo JF, Gabarrón-Galeote MA, Ruiz-Sinoga JD (2013) Soil water repellency
646 in Mediterranean rangelands under contrasted climatic, slope and patch conditions in
647 southern Spain. Catena 110:196–206

648 69. Nadal-Romero E, Petrlic K, Verachtert E, et al (2014) Effects of slope angle and aspect on
649 plant cover and species richness in a humid Mediterranean badland. Earth Surf Process
650 Landf 39:1705–1716

651 70. Maaaren IE, Karki S, Prajapati C, et al (2015) Facing north or south: Does slope aspect
652 impact forest stand characteristics and soil properties in a semiarid trans-Himalayan valley?
653 J Arid Environ 121:112–123

654 71. Allard V, Ourcival JM, Rambal S, et al (2008) Seasonal and annual variation of carbon
655 exchange in an evergreen Mediterranean forest in southern France. Glob Change Biol
656 14:714–725. https://doi.org/10.1111/j.1365-2486.2008.01539.x

657 72. Muñoz- Rojas M, Jordán A, Zavala LM, et al (2015) Impact of Land Use and Land Cover
658 Changes on Organic Carbon Stocks in Mediterranean Soils (1956–2007). Land Degrad Dev
659 26:168–179. https://doi.org/10.1002/ldr.2194

660 73. Insam H, Haselwandter K (1989) Metabolic quotient of the soil microflora in relation to
661 plant succession. Oecologia 79:174–178. https://doi.org/10.1007/BF00388474

662 74. Vicente Serrano SM, López-Moreno JI, Beguería S, et al (2014) Evidence of increasing
663 drought severity caused by temperature rise in southern Europe, Environ. Res Lett,
664 https://doi.org/10.1088/1748-9326/9/4/044001

665 75. Diamond S, Andeer PF, Li Z, et al (2019) Mediterranean grassland soil C–N compound
666 turnover is dependent on rainfall and depth, and is mediated by genomically divergent
667 microorganisms. Nat Microbiol 4:1356–1367. https://doi.org/10.1038/s41564-019-0449-y

Figures

Fig. 1 | The overview of experimental design; (a) the schematic representation of the study design. The variables assessed and matrix used to describe geoclimate, vegetation cover, soil characteristics, carbon source utilisation and catabolic potential are fully detailed in the supplementary methods (Appendix 1, Table S1) ; (b) Sampling sites map. The colors of the symbols (*n*=60) refer to the different forest stands: red and blue tones correspond to meso- and supra-Mediterranean bioclimates, respectively; filled and open symbols correspond to north- and south-exposed plots, respectively.

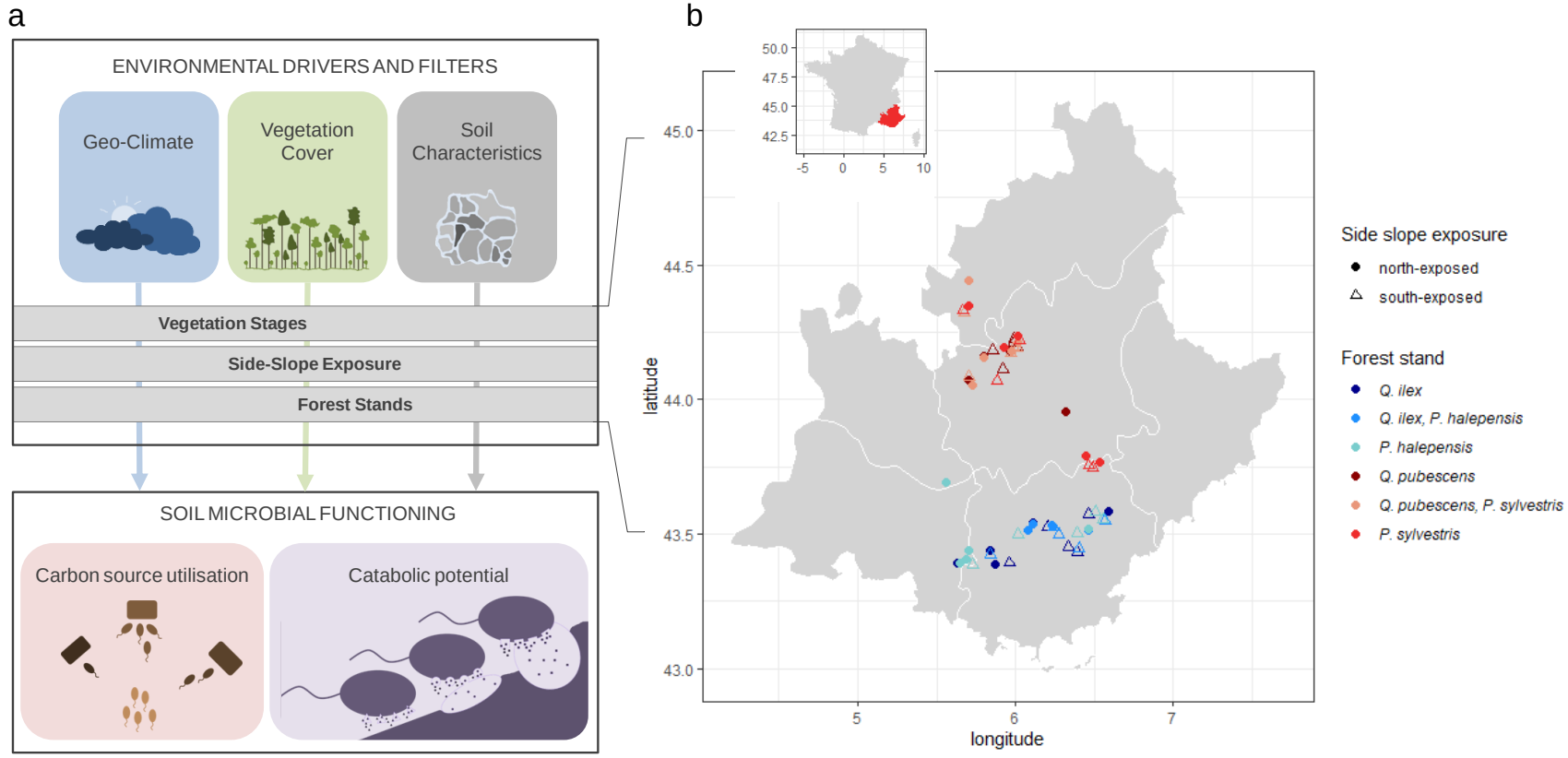
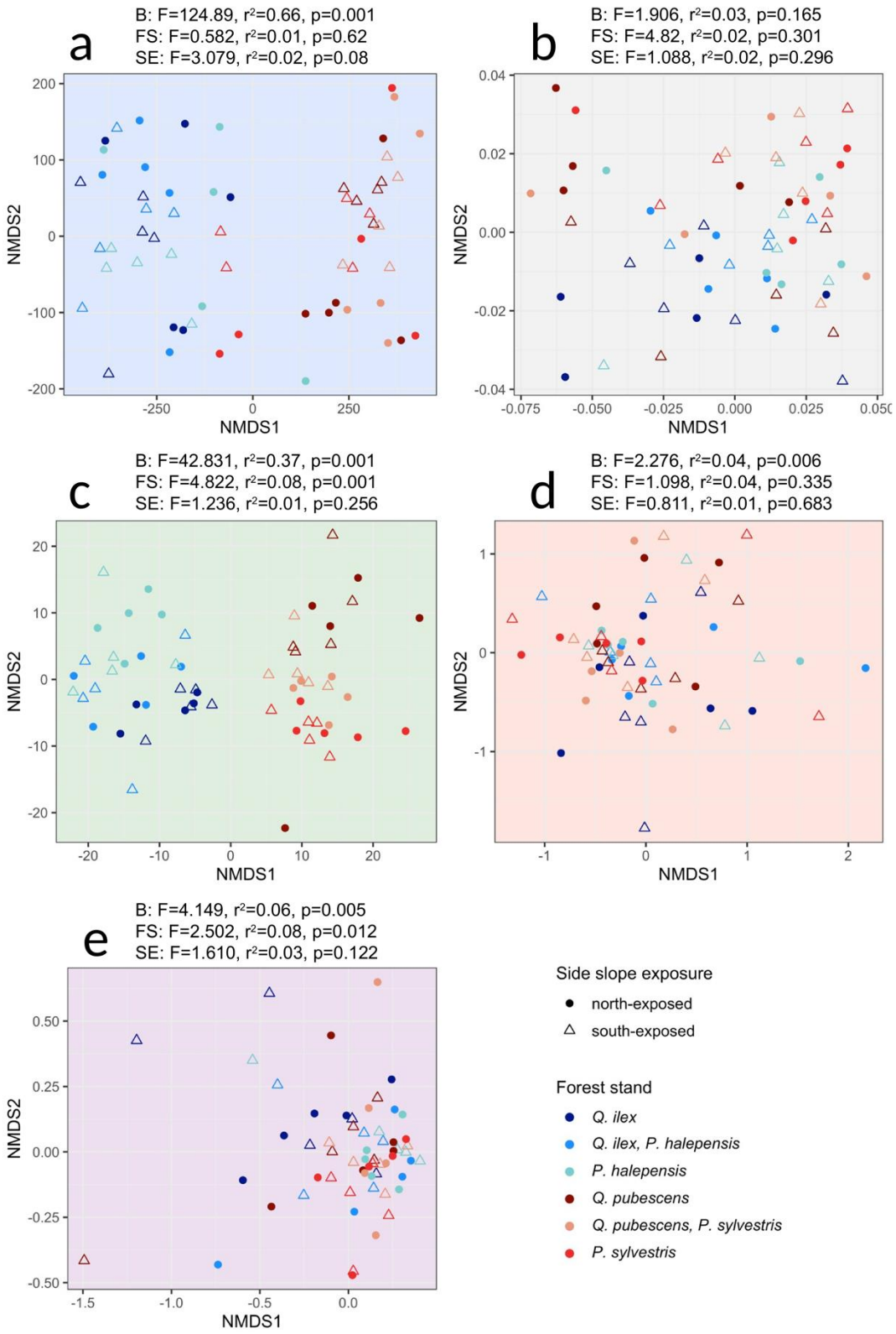


Fig. 2 | Nonmetric multi-dimensional scaling (NMDS) of (a) geoclimate, (b) soil, (c) vegetation, (d) carbon source utilisation, and (e) microbial catabolic potential patterns based on Euclidian (a, b, d, e) and Manhattan (c) dissimilarity distances. The colors of the symbols refer to the different forest stands: red and blue tones correspond to meso- and supra-Mediterranean bioclimates, respectively; filled and open symbols correspond to north- and south-exposed plots, respectively. PERMANOVA results of main factors (B for bioclimate, FS for forest stand and SE for slope exposure) are provided above each panel. Interaction effects (all not significant) are provided in Appendix 2, Table S1.


a
B: F=124.89, r²=0.66, p=0.001
FS: F=0.582, r²=0.01, p=0.62
SE: F=3.079, r²=0.02, p=0.08
b
B: F=1.906, r²=0.03, p=0.165
FS: F=4.82, r²=0.02, p=0.301
SE: F=1.088, r²=0.02, p=0.296
c
B: F=42.831, r²=0.37, p=0.001
FS: F=4.822, r²=0.08, p=0.001
SE: F=1.236, r²=0.01, p=0.256
d
B: F=2.276, r²=0.04, p=0.006
FS: F=1.098, r²=0.04, p=0.335
SE: F=0.811, r²=0.01, p=0.683
e
B: F=4.149, r²=0.06, p=0.005
FS: F=2.502, r²=0.08, p=0.012
SE: F=1.610, r²=0.03, p=0.122
NMDS1
NMDS2
Side slope exposure
north-exposed
south-exposed
Forest stand
Q. ilex
Q. ilex, P. halepensis
P. halepensis
Q. pubescens
Q. pubescens, P. sylvestris
P. sylvestris

682

Fig. 3 | Shaping of soil biological and chemical characteristics across the considered factorial scales (B for bioclimate, FS for forest stand and SE for slope exposure). Panel a, b, c, d and e respectively stand for the total N, the microbial tyrosinase activities, the C/N ratio, the aromaticity ratio and pH as affected by forest stands (Q/P stands for mixed Quercus/Pinus stands). Panel f stands for the microbial the slope exposure effect on cellulase activity. Panels g to l respectively stand for the water holding capacity, the soil organic carbon, the HR1 ratio, the Carboxyl C fraction, the microbial metabolic quotient and the microbial biomass per unit of organic C respectively, as affected by the meso- or supra- mediterranmean bioclimates. Only significant effects, i.e., $p \leq 0.05$ after Benjamini and Hochberg (1995) post-hoc correction, are shown.

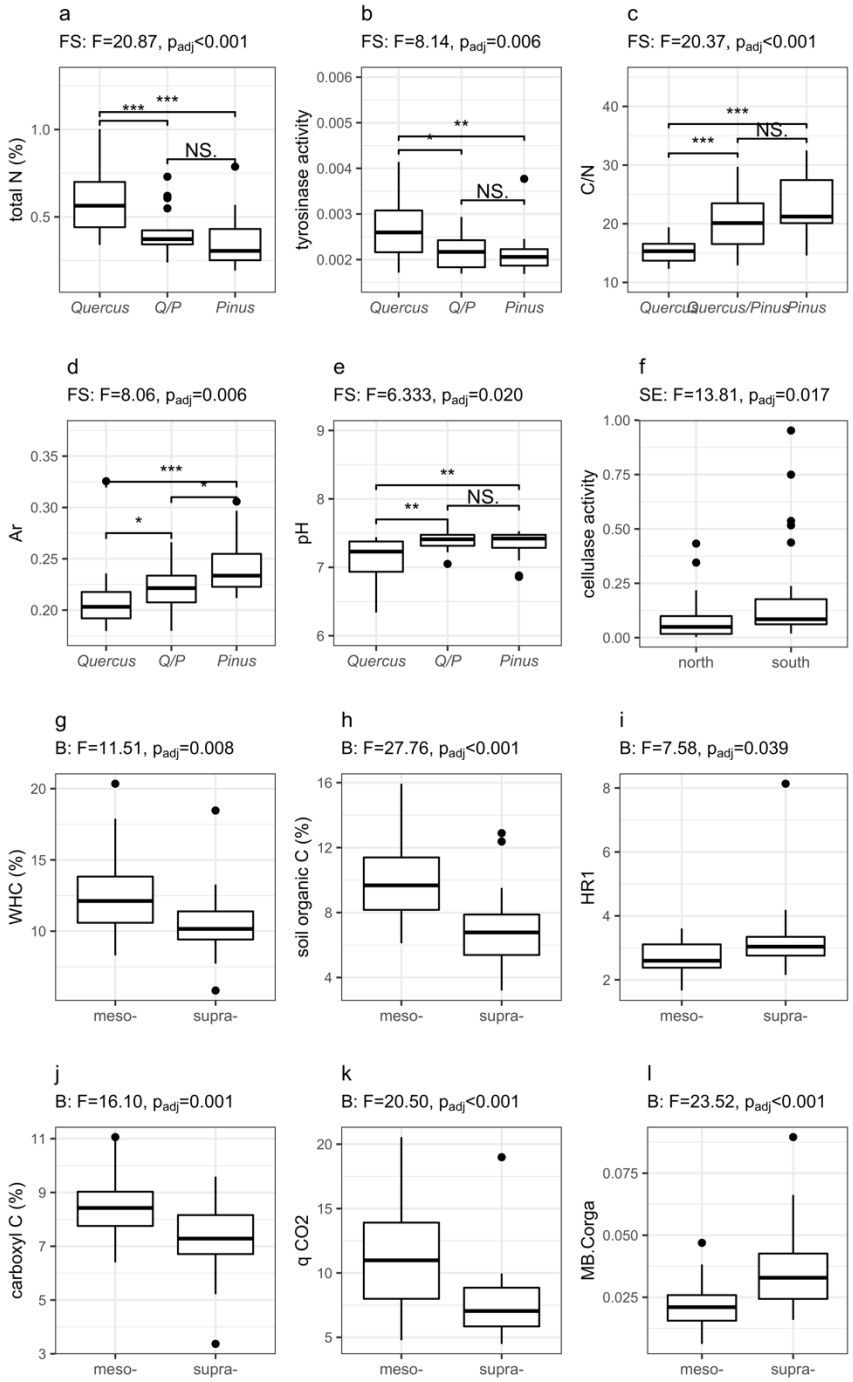


Fig. 4 | Variation partitioning of soil microbial carbon source utilisation (a, b and c) and catabolic potential (d, e and f) into soil, vegetation and geoclimate components at different spatial scales (a and d at the regional-scale (*n=60)*; b and e at the sub-regional meso-Mediterranean scale (*n=30*); and c and f at the sub-regional supra-Mediterranean scale (*n=30*)). Each Venn diagram represents a given biological variation (r^2) partitioned into the relative effects of each components or combination of components. Pure and shared effects of the different fractions were analyzed by constrained and un-constrained db-rda tests respectively, and are reported in the Table S3. Significant component are underlined when implying a pure effect and are enclosed by a bold line when implying a whole component.

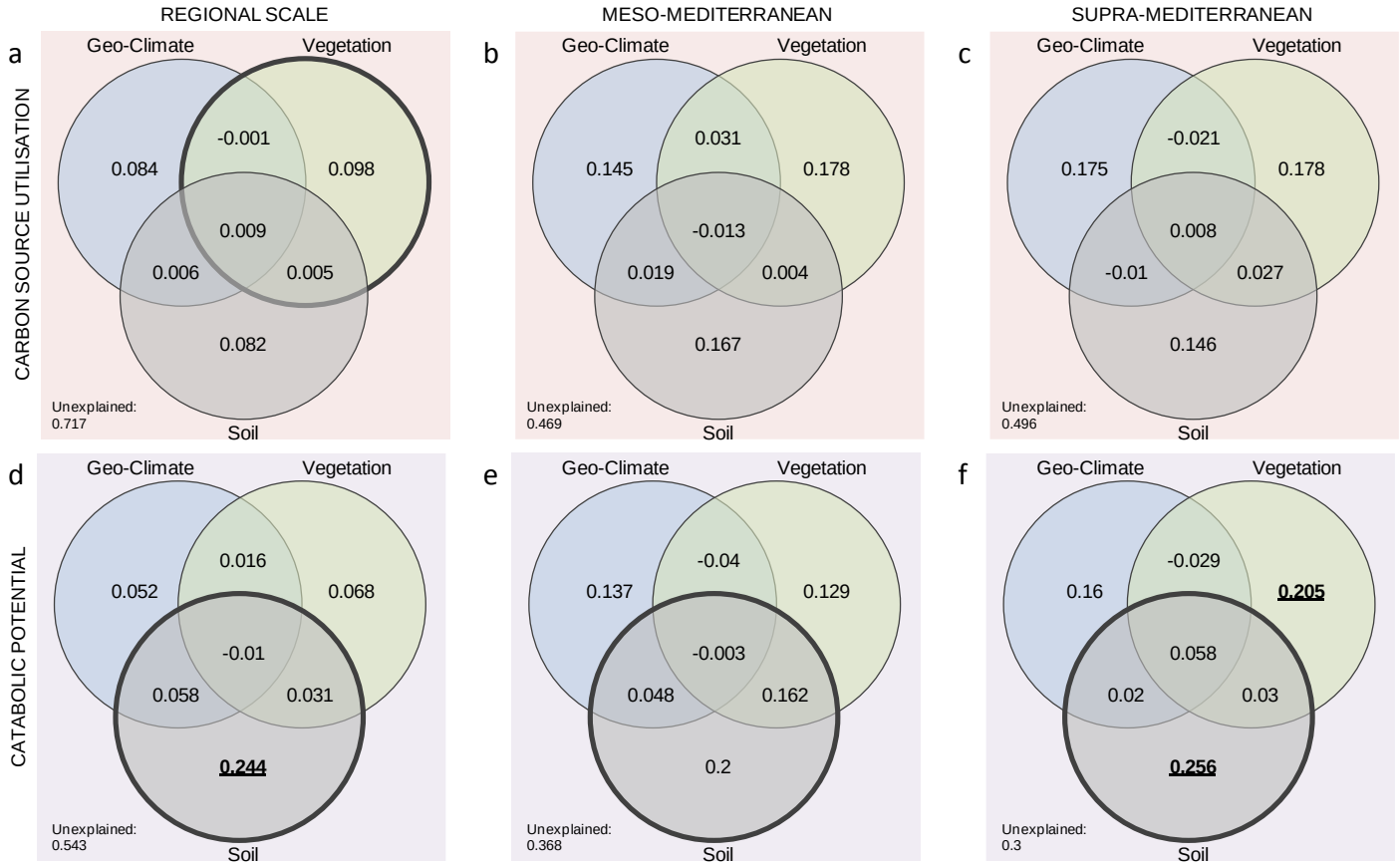


Fig. 5 | Heat map of Spearman correlation coefficient (rho) between microbial and environmental variables. The color ramp refers to the Spearman correlation coefficient, blue tones stand for positive relations and red tones for negative relations, and the point size refers to the correlation strength (abs(rho)). Top annotations refer to environmental variables and left annotations to microbial variables (D_{sea}: distance to the sea; P seasonality: precipitation seasonality; TAR: temperature annual range; T seasonality : temperature seasonality; MTWaQ: maximum temperature of the warmest quarter; minTCM: minimum temperature of the coldest month; MTDQ: mean temperature of the driest quarter ; MTCQ: mean temperature of the coldest quarter; annual P: annual precipitation; PWaQ: precipitation of the warmest quarter; annual MT: annual mean temperature; max TWaM: maximum temperature of the warmest month; MDR: mean diurnal range; PDQ: precipitation of the driest quarter; PDM: precipitation of the driest month; WHC: water holding capacity; HR1: humification ratio 1; A/G: angiosperm to gymnosperm ratio; BR: basal respiration; MB: microbial biomass; qCO_2 : metabolic quotient; MB.Corga: microbial biomas per unit of organic carbon). Only variables that show significant correlations are shown, i.e., $p \leq 0.05$ after Benjamini and Hochberg (1995) post-hoc correction.