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Forest soil microbial functional patterns and response to a drought and warming event: key role of climate-plant-soil interactions at a regional scale

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Abstract

Little is known about how spatial and environmental patterns structure soil microbial activities. We investigated, on 47 soil samples collected in Mediterranean forests, the net and interaction effects of climatic-geographic and edaphic variables as well as vegetation cover and composition on soil microbial community-level physiological profiles (CLPPs) assessed by MicrorespTM. The effects of these variables were also analyzed on CLPP response to an experimental drought treatment. CLPPs were shown to be mainly driven by climate-plant-soil and plant-soil interactions; even after drought treatment, there was a decrease in microbial activity but no change in CLPPs. Our findings highlight the robustness of these relationships, which need to be assessed within different ecosystems considering various spatial scales to reliably predict climate change effects on terrestrial ecosystems.

Keywords: CLPP; MicrorespTM; Mediterranean soils; aboveground-belowground interactions.

It remains difficult to predict the responses of plant and microbial community relationships to climate change (Bardgett et al., 2008), partly due to lack of knowledge about the deterministic factors of the soil microbial functional patterns directly linked to ecosystem processes (Green et al., 2008; Griffiths et al., 2011). Focusing on Mediterranean forest ecosystems, particularly vulnerable to increased length of summer drought and frequency of heatwaves (IPCC, 2007), the aims of our study were first to assess the environmental surrogates driving soil microbial community-level physiological profiles (CLPPs), and then to determine the robustness of their relationships with environmental surrogates after an experimental *ex situ* hard “drought” event, like those that occur in Mediterranean regions.

The study area, about 7000 km² (long 4°5'-6°2' E, lat 43°4', 43°5'N), is situated in an area of limestone-based soil in Provence, Southeastern France, with a Mediterranean climate (severe summer drought and mild humid winters). Forests are mixed stands of *Pinus halepensis* Mill., *Quercus ilex* L. and *Quercus pubescens* Willd. 47 soils were sampled across the area, covering a bioclimatic gradient (Figure S1) during the 2010 summer drought period, when extreme heatwave events are likely to occur. On each plot (20m x 20m), 12 subsamples from the upper soil horizon (0-5 cm) were systematically cored along 3 transects (5, 10 and 15 m from the border), with 4 subsampling points on each transect at 4, 8, 12 and 16 m. All subsamples of the same plot were pooled to obtain a composite sample. Samples were then sieved at 2 mm, air-dried (due to the length of the sampling period, one month) and stored until analysis.

Soils were rewetted to 70% water holding capacity (WHC) (identified in pre-testing as optimal value to increase basal respiration in our 47 soils while conserving their variability, as against 30% and 50% WHC, *data not shown*) and incubated at 25°C for eight days to standardize and equilibrate them before Time 0 (T0) analysis (Goberna et al., 2005). T0 CLPPs were determined by MicrorespTM measuring substrate-induced respirations (SIR) on eight substrates, glucose (gluc), sucrose (suc), trehalose (treha), D+ cellobiose (cello), glycine (gly), caffeic acid (caff), ellagic acid (ella) and catechol

(cat), following the adapted protocol of Campbell et al. (2003). Briefly, our aim being to compare SIRs of soils subjected to the same solution of substrate instead of their absolute rate of mineralization, we used the lowest water content among our samples to determine concentration of C substrate solutions; solutions were adjusted to pH=7, a mean value of soil pH (Table 1), both to minimize chemical artifacts due to carbonate-derived CO₂ release and to avoid any substrate-pH effect on microbial communities (Bérard et al., 2011). After T0 measurements, samples were dried for ten days at 50°C, to obtain “stressed” samples (ST), rewetted and maintained at 70% WHC, 25°C for eight days. Simultaneously, 47 “unstressed” samples (NS), already subjected to the standardization conditions, were maintained at 70% WHC, 25°C throughout. SIRs on both “NS” and “ST” samples were measured in the same way as at T0.

Organic carbon (Corg) and total nitrogen (Ntot) contents, Corg_N ratio, pH and water holding capacity (WHC), variables constitutive of the EDA compartment, were determined via the usual procedure for soil physicochemical analyses (Forster, 1995). Climatic and geographic variables (CG compartment) presented in Table 1, vegetation composition and structure of each plot (VEG compartment, list of species given Table S1), as well as other EDA variables (Table 1) are based on data from Vennetier et al. (2008) and Vennetier and Ripert (2009).

Table 1: Descriptive statistics of the climatic-geographic (CG) and edaphic (EDA) variables. Soil texture is presented for informative purposes as a percentage of soil samples.

CG variables	Unit	Minimum	Maximum	Mean	Median	Std	Abbr
Elevation	m	30	770	387	370	189	elev
Becker light-climate index	(-)	0.24	1.34	0.93	1.00	0.28	ikr
Mean annual rainfall	mm	530	1088	736	700	117	annrain
Summer rainfall	mm	58	175	109	110	29	sumrain
Mean annual temperature	°C	9.30	14.80	12.38	12.30	1.34	anntemp
Cumulated elevation direction south south east	hm	0	3600	1516	1200	1098	elevcumsse
Distance to the sea direction south south east	km	1	86	44	50	24	distsea
Cumulated elevation direction west south west	hm	0	1700	589	500	437	elevcumswsw
Distance to the sea direction west south west	km	1	75	37	38	22	distseawsw
Distance to the ridge	m	0	3050	276	75	598	distridge
EDA variables	Unit	Minimum	Maximum	Mean	Std	Abbr	
Parent rock outcrops on the plot	%	0.00	65.00	4.90	13.40	proc	
Total soil depth	cm	20.00	150.00	83.94	36.05	depth	
Stones on litter ratio	(-)	0.01	0.15	0.05	0.06	st/lit	
Coarse fragments in the topsoil	%	0.00	62.50	26.78	23.37	cofr	
Number of fine roots (<2mm) in the topsoil	/dm2	1.50	15.00	13.82	2.80	roots	
pH value	(-)	6.43	7.58	7.16	0.29	pH	
Calcium Carbonate content	g/100g dry matter	0.00	45.73	13.82	12.63	CaCO3	
Organic Carbon content	g/100g dry matter	3.20	21.53	11.31	4.46	Corg	
Total Nitrogen content	g/100g dry matter	0.10	0.93	0.48	0.19	Ntot	
Organic Carbon on total Nitrogen ratio	(-)	14.48	32.19	24.16	4.65	Corg_N	
Water holding capacity of sieved soil	g/100g dry matter	39.31	161.60	96.35	25.99	WHC	
Water holding capacity based on soil texture	mm/cm	1.30	1.95	1.71	0.19	whcst	
Soil texture		silty-clayey			51.06		
(based on the silt, sand, clay fractions of soil samples)	% of soil samples	sandy-silty			19.15		
		sandy-siltv-clayey			29.79		

Abbr: abbreviation; Std: standard deviation.

Before statistical analyses, SIRs on each substrate and each sample were standardized by scaling (subtracting the mean SIR of all soils on all substrates, then dividing by the standard deviation). RDA sets combining selected variables from the various compartments and derived adjusted R² values, followed by both variance partitioning analysis and Monte Carlo permutation tests, were used to assess both their relative impact and their interactions on T0 CLPPs and on their responses to drought. Effects of each compartment (individual effect) were thus broken down into real individual effect (net effect) and effect through their interactions (interaction effect), and synthesized through Venn diagrams. It was not possible to statistically test the significance of the interaction effect. T0, NS and ST CLPPs were compared through PCA. Two-way ANOVA followed by Tukey LSD *post hoc* tests were performed to assess the effects of interaction between drought treatment (T0, NS, ST) and type of substrate. CLPP response to drought was assessed by computation of the arithmetic difference between ST and NS (R Development Core Team 2012).

RDA results and Venn diagrams showed that although the CG, EDA and VEG compartments all had significant individual effects on initial CLPPs (adjusted R^2 (%): CG 20.64, EDA 42.44, VEG 43.47; $p < 0.01$; Figure 1a), their main impact resulted from their interactions (adjusted R^2 (%): $CG \cap EDA \cap VEG = 18.13$; $EDA \cap VEG = 17.55$), always including EDA and VEG. Although soil-plant-climate interactions are well documented, their effect on CLPP has not previously been shown so clearly at a regional scale, within a single type of ecosystem (Singh et al., 2009; Liu et al., 2010). This may be because our sampling strategy focusing on forest ecosystems excludes *de facto* any effects of different land use (Drenovsky et al., 2010) and different geological substratum (Fierer and Jackson, 2006).

CLPPs appeared to be mainly discriminated by their SIR on carbohydrates and glycine (axis 1) and complex phenolic compounds (axis 2), depending on forest habitat type (four types described; Figure 2), which resulted in a strong dissimilarity between CLPPs in stands dominated by broadleaved trees (high respiration rates on carbohydrates) and those dominated by coniferous trees (high respiration rates on phenolic compounds). The litter of coniferous species is known to be very rich in recalcitrant polymeric phenolic compounds (Zechmeister-Boltenstern et al., 2011), whose inputs may have selected microbial community with the adequate enzymes to oxidize them. It is also known to decrease soil N availability and mineralization processes (Hättenschwiler and Vitousek, 2000), which is consistent with our observations. These results highlight the influence of vegetation cover and composition on microbial activity through its influence on soil organic matter quality and quantity, and thereby soil physicochemical properties (Wardle, 2006).

Figure 1 a), b): Venn diagram of the individual, interaction and net effects of
a) the various environmental compartments Climatic-geographic (CG), Edaphic (EDA) and Vegetation (VEG) on initial CLPPs (T0); Adjusted R -squared for each part of the circles are indicated with result of Monte –Carlo permutation test p value significance (p values are not shown; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$).

b) the various environmental compartments Climatic-geographic (CG), Edaphic (EDA) and Vegetation (VEG), and initial CLPP (T0) on CLPP responses to drought; adjusted R -squared of individual effects are indicated on the diagram with their significance level **: $p < 0.01$; net effects and interaction effects are indicated near the diagram with their significance level (*; $p < 0.05$).

The squares represent 100% of variance explained. Significance of the impact due to interactions could not be tested.

Figure 2: Redundancy analysis (RDA) of the initial CLPP (T0) with respect to Climatic–geographic compartment (CG), Edaphic compartment (EDA) selected variables and Vegetation (VEG) selected plant species. On right is the synthesis of the different habitat types defined according to the associations of the selected variables. Microbial utilization rates of each substrate are in grey; CG selected variables are in bold (*elev*: elevation; *elevcumsw*: cumulated elevation direction west south west; *distseawsw*: distance to the sea direction west south west); EDA selected variables are in italics; VEG selected species are not in bold and not in italics (*cyse*: *Cytisus sessilifolius*; *soto*: *Sorbus torminalis*; *pece*: *Peucedanum cervaria*; *juco*: *Juniperus communis*; *dala*: *Daphne laureola*; *phla*: *Phyllirea latifolia*; *sodo*: *Sorbus domestica*; *pile*: *Pistacia lentiscus*; *cial*: *Cistus albidus*; *phan*: *Phyllirea angustifolia*; *buse*: *Buxus sempervirens*; *rhal*: *Rhamnus alaternus*; *piha*: *Pinus halepensis*; *brre*: *Brachypodium retusum*; *spju*: *Spartium junceum*; *himu*: *Hieracium murorum*; *pisy*: *Pinus sylvestris*).

Drought stress induced a decrease in SIR, significant for all substrates except for both catechol, which might have a toxic inhibitory effect on microbial activity (very low respiration rates at T0, NS and ST) (Chen et al., 2009), as well as ellagic acid (Figure 3, $p < 0.001$). The release of C and nutrients from dead biomass after drought might have been used by surviving microorganisms to enable attack on recalcitrant compounds (Fontaine et al., 2004), which could explain the higher respiration rates observed after drought on the two phenolic acids compared to those on simple compounds. However, there was no major change in CLPP between the ST and the NS or T0 treatments as analyzed by PCA (Figure 3). In addition, CLPP responses to drought were driven by all compartments (adjusted R^2 (%): T0=29.22; CG=12.97; EDA=27.74, VEG=23.56; $p < 0.01$), and mainly by T0 CLPPs and their interactions with CG, VEG and EDA, explaining 13 to 26% of variance, neither of which had any net effect (Figure 1b). Moreover, there was no major difference in ranking or numbers among the RDA selected variables after drought treatment compared to T0 (*data not shown*). More studies would be necessary both, to confirm these trends, and to discriminate the relevant environmental variables driving microbial functional patterns, depending on the type of ecosystem considered.

Figure 3: PCA of initial time (T0), unstressed (NS) and stressed (ST) CLPPs with on top left ANOVA results testing interaction effects between drought treatment and substrate used, on SIR of soils at initial time (T0), unstressed soils (NS) and stressed soils (ST). *F* value and level of significance are indicated on top left. Cat: catechol; gly: glycine; caff: caffeic acid; ella: ellagic acid; suc: sucrose; cello: cellobiose; treha: trehalose; gluc: glucose.

These results illustrate the strength and the robustness of soil microbial community - plant relationships, which may thus mitigate climate change effect on both belowground and aboveground communities, at least as long as vegetation patterns are maintained. Their robustness after such treatment supports the results of Waldrop and Firestone (2006), Ge et al. (2008), Lau and Lennon (2012) and thereby highlights the need to further inquire relationships between soil microbial communities and their environment by assessing the importance of their life history in the face of contemporary disturbance events, so as to reliably predict climate change effects on ecosystem processes.

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Figure S1 (supporting information): Study area with sampling sites (black circles).

Table S1 (supporting information)
constitutive species and abbreviations (in bold) of the Vegetation compartment (VEG) used to explain soil microbial functional profiles: Tree species (TREES), Shrub species (SHRUBS) and Herbaceous species (HERBS).

TREES	<i>Ilex aquifolium</i> ilaq	HERBS	<i>Dorycnium hirsutum</i> dohi	<i>Lonicera implexa</i> loim
<i>Acer monspessulanum</i> acmo	<i>Juniperus communis</i> juco	<i>Aphyllanthes monspelliensis</i> apmo	<i>Dorycnium pentaphyllum</i> dope	<i>Onobrychis saxatilis</i> onsa
<i>Pinus halepensis</i> piha	<i>Juniperus oxycedrus</i> juox	<i>Arabis hirsuta</i> arhi	<i>Euphorbia characias</i> euch	<i>Ononis minutissima</i> onmi
<i>Pinus sylvestris</i> pisi	<i>Juniperus phoenicea</i> juph	<i>Argyrolobium zanonii</i> arza	<i>Festuca ovina</i> feov	<i>Osyris alba</i> osal
<i>Quercus ilex</i> quil	<i>Olea europaea</i> oleu	<i>Asparagus acutifolius</i> asac	<i>Filipendula vulgare</i> fivu	<i>Peucedanum cervaria</i> pece
<i>Quercus pubescens</i> qupu	<i>Phillyrea angustifolia</i> phan	<i>Avena bromoides</i> avbr	<i>Galium verum</i> gave	<i>Potentilla hirsuta</i> pohi
<i>Sorbus aria</i> soar	<i>Phillyrea latifolia</i> phla	<i>Brachypodium phoenicoides</i> brph	<i>Genista hispanica</i> gehi	<i>Psoralea bituminosa</i> psbi
<i>Sorbus domestica</i> sodo	<i>Pistacia lentiscus</i> pile	<i>Brachypodium pinnatum</i> brpi	<i>Genista pilosa</i> gepi	<i>Rubia peregrina</i> rupe
<i>Sorbus torminalis</i> soto	<i>Pistacia terebinthus</i> pite	<i>Brachypodium retusum</i> brre	<i>Geranium robertianum</i> gero	<i>Sedum anopetalum</i> sean
<i>Taxus baccata</i> taba	<i>Quercus coccifera</i> quco	<i>Bupleurum rigidum</i> buri	<i>Hedera helix</i> hehe	<i>Silene italica</i> siit
SHRUBS	<i>Rhamnus alaternus</i> rhal	<i>Carex halleriana</i> caha	<i>Helianthemum hirtum</i> hehi	<i>Smilax aspera</i> smas
<i>Amelanchier ovalis</i> amov	<i>Rhamnus saxatilis</i> rhas	<i>Carex humilis</i> cahu	<i>Helianthemum italicum</i> heit	<i>Stachelina dubia</i> stdu
<i>Arbutus unedo</i> arun	<i>Rosa canina</i> roca	<i>Clematis flammula</i> cflf	<i>Hieracium murorum</i> himu	<i>Stipa offneri</i> stof
<i>Buxus sempervirens</i> buse	<i>Rosmarinus officinalis</i> roof	<i>Coronilla emerus</i> coem	<i>Hieracium pilosella</i> hipi	<i>Teucrium chamaedrys</i> tech
<i>Cistus albidus</i> cial	<i>Rubus ulmifolius</i> ruul	<i>Coronilla juncea</i> coju	<i>Lavandula latifolia</i> lala	<i>Teucrium montanum</i> temo
<i>Cornus sanguinea</i> cosa	<i>Ruscus aculeatus</i> ruac	<i>Coronilla minima</i> comi	<i>Lavandula angustifolia</i> laan	<i>Teucrium polium</i> tepo
<i>Crataegus monogyna</i> crmo	<i>Spartium junceum</i> spju	<i>Dactylis glomerata</i> dagl	<i>Leuzea conifera</i> leco	<i>Thymus vulgaris</i> thvu
<i>Cytisus sessifolius</i> cyse	<i>Ulex parviflorus</i> ulpa	<i>Daphne gnidium</i> dagn	<i>Ligustrum vulgare</i> livu	<i>Viola sp</i> visp
<i>Fragaria vesca</i> frve	<i>Viburnum tinus</i> viti	<i>Daphne laureola</i> dala	<i>Lonicera etrusca</i> loet	