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Drought-resistant fungi control soil organic matter decomposition and its response to temperature

J. C. YUSTE*, J. PEÑUELAS*, M. ESTIARTE*, J. GARCIA-MAS†, S. MATTANA*, R. OGAYA*, M. PUJOL† and J. SARDANS*

Global Ecology Unit CREAF-CEAB-CSIC, CREAF (Centre de Recerca Ecológica i Aplicacions Forestals), Edifici C, Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona, Spain, †IRTA, Centre de Recerca en Agrigenòmica CSIC-IRTA-UAB, Carretera de Cabrils Km 2, 08348 Cabrils, Barcelona, Spain

Abstract

Microbial-mediated decomposition of soil organic matter (SOM) ultimately makes a considerable contribution to soil respiration, which is typically the main source of CO_2 arising from terrestrial ecosystems. Despite this central role in the decomposition of SOM, few studies have been conducted on how climate change may affect the soil microbial community and, furthermore, on how possible climate-change induced alterations in the ecology of microbial communities may affect soil CO_2 emissions. Here we present the results of a seasonal study on soil microbial community structure, SOM decomposition and its temperature sensitivity in two representative Mediterranean ecosystems where precipitation/throughfall exclusion has taken place during the last 10 years. Bacterial and fungal diversity was estimated using the terminal restriction fragment length polymorphism technique. Our results show that fungal diversity was less sensitive to seasonal changes in moisture, temperature and plant activity than bacterial diversity. On the other hand, fungal communities showed the ability to dynamically adapt throughout the seasons. Fungi also coped better with the 10 years of precipitation/throughfall exclusion compared with bacteria. The high resistance of fungal diversity to changes with respect to bacteria may open the controversy as to whether future 'drier conditions' for Mediterranean regions might favor fungal dominated microbial communities. Finally, our results indicate that the fungal community exerted a strong influence over the temporal and spatial variability of SOM decomposition and its sensitivity to temperature. The results, therefore, highlight the important role of fungi in the decomposition of terrestrial SOM, especially under the harsh environmental conditions of Mediterranean ecosystems, for which models predict even drier conditions in the future.

Keywords: bacteria, climate change, diversity, drought, fungi, Q_{10}, soil organic matter decomposition

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Introduction

Soil organic matter (SOM) decomposition, involving oxidation of SOM by heterotrophs and transport of the resulting CO_2 through the soil, ultimately contributes very significantly to soil CO_2 efflux (Bond-Lamberty *et al.*, 2004), and thus to global CO_2 emissions. How climate change will perturb SOM decomposition and hence the soil organic carbon (C) reservoir remains, however, controversial (Kirschbaum, 2006). While current models predict that global warming will increase the net CO_2 emissions from terrestrial ecosystems due to the strong sensitivity of heterotrophic respiration to temperature (Cox *et al.*, 2000; Friedlingstein *et al.*, 2003), other studies also highlight the large uncertainties associated to predicting the soil emissions component (Meir *et al.*, 2006). These uncertainties are primarily due to the limited knowledge about the mechanisms underlying soil respiration (Davidson & Janssens, 2006).

Among the questions that remain unresolved, the role of microbial diversity in SOM decomposition could be of paramount importance. Although the microbial community responsible for decomposing SOM is rarely considered explicitly in models (Moorhead & Sinsabaugh, 2006; Allison & Martiny, 2008) the structure and functioning of soil microbial communities is affected by seasonality and site characteristics (Lipson *et al.*, 2002, 2009; Waldrop & Firestone, 2006a, b; Schmidt *et al.*, 2007; Williams, 2007; Williams & Rice, 2007). Moreover, although some evidence suggests that the complexity (functional or specific) of the soil microbial community may positively affect SOM decomposition (Deacon, 1985; Robinson, 1993; Wall, 1999; Hulot, 2000; Aerts, 2006; Strickland *et al.*, 2009) or the activity of the microflora (Degens, 1998; Griffiths *et al.*, 2000), the relation between the diversity and the system function remains in question (Deacon, 1985; Rayner, 1994; Cox, 2001).

Correspondence: J. C. Yuste, tel. + 34 93 581 4677, fax + 34 93 581 4151, e-mail: j.curiel@creaf.uab.es

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Another source of controversy is deciphering the relative contribution of the different microbial functional groups such as fungi and bacteria to SOM decomposition and these groups' direct effect on C terrestrial C cycling (e.g. Bardgett *et al*., 1996). In terrestrial ecosystems, where soil pores are filled with air and SOM is highly protected (physically, chemically or biochemically), fungi have an adaptive advantage with respect to bacteria, which have dominated aquatic ecosystems (De Boer *et al*., 2005). The inability of the bacterial unicellular body form to bridge these air-filled voids has been overcome by the hyphal/mycelial growth form, especially present in fungi (Griffin, 1985), which facilitates organisms to cross water-poor and/or nutrient-poor spots in soil in searching for the heterogeneously distributed water/nutrient resources (Jennings, 1987). Although actinomycetes, have also developed the hyphal growth form (Griffin, 1985), this has not enabled bacteria to monopolize terrestrial heterotrophic processes. A shift to a fungal world could have consequences for C cycling for different reasons: firstly, because fungal decomposers have developed pathways to degrade recalcitrant structural compounds unique to terrestrial vascular plants (e.g. lignin) and these pathways are not present in bacteria (Ewbank *et al*., 1996; Robinson, 1996; Taylor & Osborne, 1996), and secondly, because various studies have suggested that bacteria and fungi cycle C very distinctly due to different growth efficiencies (Holland & Coleman, 1987), a different quality of by-products generated by their activity (Martin & Haider, 1979) and/or a different involvement in the physical stabilization of organic matter (Six *et al*., 2006).

Mediterranean ecosystems are an exceptional playground to test how microbial communities would evolve under more arid conditions. In Mediterranean-type ecosystems water availability is already the most important environmental constraint, due to the combination of high summer temperatures and low rainfall (Specht *et al*., 1983; Larcher, 2000). This region is therefore expected to be extremely vulnerable to the effects of climate change (Schröter *et al*., 2005). Drought limits the physiological performance of microbes and the diffusion of nutrients in the soil pore space (Harris, 1981; Papendick & Campbell, 1981; Robinson *et al*., 1993). It therefore submits the microbial community to an important adaptive force. Furthermore, current global circulation and regional models predict an increase in temperatures in the Mediterranean Basin during the present century, while rainfall is predicted to decrease and become more irregular (Gibelin & Deque, 2003; IPCC, 2007). Given that soil microbial communities exhibit seasonal changes, the impact of increasing drought levels is likely to depend on changes in rainfall seasonality, especially if rainfall decreases at times when there normally is some rainfall. It is therefore important to assess whether the ongoing global changes in climate and the subsequent effects on soil moisture, vegetation and soil chemical and biochemical properties may affect the distinct role of fungi and bacteria.

Here we present the results of a study designed to explore the role of certain ecological aspects of the soil microbial community (e.g. biomass and diversity) in the variability of SOM decomposition. Ecological attributes of the microbial community such as microbial biomass, fungal biomass, bacterial and fungal diversity as well as other soil biogeochemical attributes [total organic carbon (TOC)] were studied seasonally at two Mediterranean sites where precipitation exclusion has been taking place during the last 10 years, reducing soil moisture up to 20% with respect to controls (Ogaya & Peñuelas, 2007; Peñuelas *et al*., 2007). These results were compared with parallel measurements of SOM decomposition and its response to temperature. Our hypotheses were (1) site and seasonality exert a strong influence over the microbial community structure and its diversity; (2) drought simulations will affect the drought-sensitive bacterial community more than the drought-tolerant fungal community and (3) the drought-tolerant fungal community exerts a strong influence over the temporal and spatial variability of SOM decomposition and its response to temperature changes.

Materials and methods

Experimental sites

Soil samples were collected from two different experimental sites, a holm-oak forest (Prades) and a schrubland (Garraf), where precipitation/throughfall exclusion has been maintained over the last 10 years.

The natural holm-oak (*Quercus ilex*) forest of the Prades Mountains is located in Southern Catalonia (North-Eastern Iberian Peninsula) (418130N, 08550E), on a south-facing slope (25%). The soil is a stony Dystric Xerochrept (Soil Survey Staff, 1998) on a bedrock of metamorphic sandstone. Its depth ranges between 35 and 100 cm. Depth of Horizon A ranges between 25 and 30 cm. The average annual temperature is 12.8 °C and the average annual rainfall is 658 mm. Summer drought is pronounced and usually lasts for 3 months. The vegetation is a dense forest dominated by *Q. ilex* L. as a dominant tree with abundant presence of *Phillyrea latifolia* and *Arbutus unedo* L. and other evergreen species well adapted to drought conditions (*Erica arborea* L., *Junniperus oxycedrus* L. and *Cistus albidus* L.), and occasional individuals of deciduous species (*Sorbus torminalis* L. Crantz and *Acer monspessulanum* L.). Eight 15 m × 10 m plots were established at the same altitude (930 m above sea level) along the slope in 1999. Four of the plots received the drought treatment and the other half

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were control plots. The drought treatment consisted of partial throughfall exclusion by suspending PVC strips and funnels at a height of 0.5–0.8 m above the soil surface. Strips and funnels covered approximately 30% of the total plot surface. Two plastic strips of 14 m long and 1 m wide were placed along the drought treatment plots from the top until the bottom part, and 30 plastic funnels of 1 m^2 each one were randomly placed in each drought treatment plot. A 0.8–1 m deep ditch was excavated along the entire top edge of the upper part of the treatment plots to intercept runoff water supply. The water intercepted by strips, funnels and ditches was conducted outside the plots, below their bottom edge. Within the drought treatment we further distinguished two treatments: mild drought, corresponding to soils of the drought treatment only affected by the intercept runoff (trenching) and severe drought, which corresponded to soils affected by both, water runoff (trenching) and throughfall exclusion (plastics strips). Therefore, the study area consisted of three treatments (control, mild drought and severe drought) with four replicates per treatment.

The Mediterranean calcareous shrubland on a south-facing slope in the Garraf Mountains is also located in southern Catalonia (NE Spain) (418180N, 18490E) at 210 m above sea level. The site is located on formerly cultivated terraces – abandoned approximately a century ago – with a Petrocalcic calcixerepts (Soil Survey Staff, 1998) soil lying on bedrock of sedimentary limestone. The average annual temperature is 15.1 °C and the average annual rainfall was 580 mm (Period 1999–2004). The summer drought is pronounced and usually lasts for 3 months. The total vegetation cover is 70% and consists of a calcareous shrubland with plants about 1 m high, dominated by the shrubs *Globularia alypum*, *Erica multiflora*, *Dorycnium pentaphyllum*, *Rosmarinus officinalis*, *Ulex parviflorus* and *Pistacia lentiscus*. Field-scale drought, warming and control treatments were applied in three plots/replicates per treatment. The warming treatment consisted in increasing nighttime temperatures by covering the vegetation with an aluminum curtain coiled on a beam and connected to a motor controlled by light sensors that automatically covered the vegetation at night. This curtain reflected long-wave infrared radiation back into the vegetation, resulting in a temperature increase in relation to untreated plots. In order to avoid interfering with the hydrological cycle, roofs were automatically removed when it rained. The drought treatment reduced spring and autumn rainfall input. This was achieved by automatically covering vegetation with a transparent plastic curtain during periods of rain by means of automatic rain sensors. Once the rain stopped, the curtain was automatically removed. Roofs were removed if wind speed exceeded 1 m s^{-1}. Given that plots were located on a slope, pipes were laid along the upper edges of the drought plots to minimize runoff water entering. The control plots were equipped with the same scaffolding as the treatment plots, without the roof. Each plot occupied an area of 4 m × 5 m, and to avoid the effect of edge disturbance, samples were only taken from an internal area of 3–4 m. Manipulation started in March 1999 and has continued up to the present day.

For more information about the experimental sites and drought treatments see Peñuelas *et al*. (2007) and Ogaya & Peñuelas (2007).

SOM decomposition and temperature sensitivity

Soil cores, 4.5 cm diameter and 10 cm depth, were collected using a stainless steel core soil sampler. Samples were collected during three different periods of the year, winter (cold and moist with little vegetation activity), spring (warm and wet with maximum vegetation activity) and summer (very warm and very dry, with minimum vegetation activity).

In Prades, three samples were collected per replicate (four replicates) for each treatment (control, mild drought and severe drought) and period (winter, spring and summer). In total 36 samples per period, 12 for control, 12 for mild drought (trenched but not covered by PVC strips) and 12 for severe drought (trenched and covered by PVC strips). A total number of 108 soil samples were collected in Prades for this experiment. In Garraf, three samples were collected per replicate (three replicates) for each treatment (ctrl, drought and warming) and period (winter, spring and summer). In total 27 samples per period, nine for control, nine for drought and nine for warming and a total number of 81 soil samples were collected in Garraf.

Soils samples were sieved (2 mm) and stored in plastic jars. To assess the temperature sensitivity of soil decomposition, soils were subjected to temperature cycles following a similar procedure to that followed by Yuste *et al*. (2007) and Yuste *et al*. (2010). Before the temperature cycles, sieved soils were maintained at room temperature (approximately 23 °C) 3 days. Soil moisture was kept as it was at soil collection by maintaining the plastic jars closed during the whole process, avoiding evaporation. During each cycle respiration rates were measured at three temperatures: 10, 25 and 45 °C. These temperatures are experienced at the site from winter to summer. Incubation temperature was not gradually increased and soil cores went straight from one temperature to the other. Soil CO_2 efflux from the soil samples was measured at each of the three temperatures 24 h after the given temperature was applied. Thirty minutes before measurement jars were opened to equilibrate the air CO_2 concentrations of the jar and the room. After the 30 min we used a modified soil chamber connected to an EGM-4 portable system (PP-systems, Hitchin, UK) to measure soil CO_2 efflux. Commercial soil chamber of PP-systems, the SRC-1, was enlarged with a 1 L, 13 cm large PVC tube of the same diameter as the SRC-1 chamber and with a rubber rim at the end. Plastic jars containing the incubated soils were then introduced into the enlarged chamber, sealing the inner volume as a closed system. The modified chamber produced readings similar to those obtained with the commercial SRC-1.

In addition we used the decomposition rates obtained at each of the incubation temperatures to assess the relative increase in soil decomposition with temperature. Q_{10} computes the relative increase in decomposition rate per 10 °C difference.

$$F_a = F_{a10} Q_{10}^{T-10/10}, \quad (1)$$

where F_a is the measured soil CO_2 efflux, F_{a10} is the basal respiration rate at 10 °C, Q_{10} is the relative change in F_a with 10 °C increases and T is the temperature of soil at measurement time. We fitted this exponential function for each of the four soils studied.

We used the soil CO_2 efflux averaged over all the temperatures as the rate of SOM decomposition per site and treatment.

Microbial biomass and TOC

Microbial biomass in the sample was measured by the chloroform-fumigation-extraction method (Vance *et al.*, 1987) after incubation for 4 days in the dark at 25 °C to eliminate the effects of sampling, sieving and storage. Unfortunately, analyses of microbial biomass were only performed on soils collected in the winter and spring. TOC was measured by oxidation with potassium dichromate and the assessment of excess chromic acid not consumed by an iron salt (salt Mhor).

Fungal biomass

Fungal biomass was estimated using the ergosterol method (Grant & West, 1986). For the measurement 2 g (wet weight) of freeze dried soil was extracted with 16 mL methanol, vortexed and then submitted to an ultrasonic bath for 30 min and centrifuged for 10 min at 1600 g. The ergosterol material extracted was analyzed by HPLC (WATERS 2965 Separations Module, Milford, MA, USA) with a PDA detector (WATERS 2996). Ergosterol was confirmed by comparing retention times and absorption spectrum with an external standard (Fluka, Buchs, Switzerland) and by coinjection of samples plus standard ergosterol. Thus,

$$\text{g Ergosterol}^{-1}\,\text{soil (DM)} = \text{A} \times \text{S} \times 2/R \times \text{DM}, \quad (2)$$

where A is the peak area of ergosterol in the sample; S is the slope of the standard curve (μg ergosterol per unit of peak area); 2 is the sample volume; DM is dry matter content of the soil sample analyzed and R is the added standard recovery.

DNA extraction

Soil DNA was extracted from Prades soil samples using the PowerSoil™ DNA Isolation Kit (MoBio Laboratories Inc., Carlsbad, CA, USA) as recommended by the manufacturer. The MoBio ultraclean DNA soil kit (MoBio Laboratories Inc.) was used to extract DNA from Garraf soil. The PowerSoil Isolation Kit differs from the MOBIO UltraClean Soil DNA isolation kit (Solana Beach, CA, USA) because of a different humic substance removal procedure. Briefly, the DNA extraction methods involved chemical lysis of microbial cells with gentle bead-beating, released DNA was bound to a silica spin filter which was subsequently washed, and the DNA was recovered in elution buffer solution. DNA yields and quality were checked after electrophoresis in 0.8% (w/v) agarose gel stained with ethidium bromide under UV light (Sambrook *et al.*, 1989).

Bacterial and fungal community fingerprinting by terminal restriction fragment length polymorphism (TRFLP) analysis

The 16S rRNA from the extracted DNA samples was amplified using the universal eubacterial primers 8F, a fluorescently labelled forward primer (AGAGTTTGATCCTGGCTCAG) and primer 1398R (ACGGGCGGTGTGTACAAG). Fungal DNA was amplified using the highly conserved fungal rRNA gene primers ITS1-F (CTTGGTCATTTAGAGGAAGTAA) and ITSF4 (TCCTCCGCTTATTGATATGC). The forward primer ITS 1-F was labelled with fluorescent dye 6 FAM (Applied BioSystem, Warrington, UK). PCR reactions were performed in 25 μL PCR Master Mix by Promega (Madison, WI, USA) (containing 50 units mL^{-1} Taq DNA polymerase in a proprietary reaction buffer, 400 μL each dNTP and 3 mM $MgCl_2$) 10 μM of each primer and 1 μL of template DNA.

The thermocycling conditions for bacterial DNA were as follows, a hot start at 95 °C for 3 min (one cycle); 95 °C for 30 s, 53 °C for 30 s, 72 °C for 1 min (35 cycles); with a final extension at 72 °C for 7 min. The thermocycling conditions for fungal DNA were as follows, a hot start at 94 °C for 4 min (one cycle); 94 °C for 1 min, 55 °C for 1 min, 72 °C for 2 min (40 cycles); with a final extension at 72 °C for 7 min.

PCR products of the correct size were confirmed by 1% (w/v) agarose gel electrophoresis and subsequently purified using UltraClean® PCR Clean-Up Kit (MoBio Laboratories Inc.) according to manufacturer's instructions (50 μL final volume). The purified PCR product (30 μL) was then digested with 2 U of *Msp*I restriction enzyme (Biolabs) in the manufacturer's buffer (total reaction volume 50 μL) overnight at 37 °C. Samples were analyzed using an automated sequencer ABI Prism 3100 Genetic Analyzer (Applied Biosystems).

TRFLP profiles were produced using the GeneMapper software (version 3.7; ABI, United Kingdom). Terminal restriction fragments (TRFs) were quantified using the advanced mode and second-order algorithm. Only peaks at positions between 50 and 700 bp were considered. The relative abundance of a TRF in a TRFLP profile was calculated by dividing the peak height of the TRF by the total peak height of all TRFs in the profile. All peaks with heights that were $<0.5\%$ of the total peak height were not included in further analyses. This approach minimized the effect of variations in the TRFLP profiles caused by the quantity of DNA analyzed. TRF profiles were aligned to prevent incorrectly identifying one TRF peak as two separate peaks. The TRF peaks that differed by <0.5 bp were then considered identical, and were clustered together.

Statistical methods

The effects of season and treatment on bacterial and fungal community structure were assessed using data on both TRF fragment proportional area and in presence/absence of TRF's. Principal component analyses (PCA) were conducted to reduce the *n*-dimensional data of TRF's obtained for each sample into a series of linear axes that explain the maximum amount of variance in the data. The relative position of each sample along the principal-component axes can then be used to describe the degree of community-level similarity between samples.

Additionally, number and area of TRF peaks was used to describe the diversity of TRF's in the samples. Soil TRF richness was calculated as the total number of distinct TRF sizes (between 50 and 700 bp) in a profile. The Shannon–Weaver diversity index was calculated as follows:

$$\text{Shannon–Weaver}\,(H) = \frac{C}{N}\left(N \times \log_{10} N - \sum n_i \log_{10} n_i\right), \quad (3)$$

where C is 2.3, N the sum of peak areas in a given TRFLP profile, n_i the area of TRF i and i the number of TRFs in each TRFLP profile.

We used repeated measures analysis of variance (ANOVA) to study the effect of site, season and treatment over the variables and indexes generated. For treatment we only tested the effect of drought treatment (Garraf) and severe drought (Prades) because previous analyses suggested only a slight effect of warming (Garraf) and mild drought (Prades) on the variables studied (data not shown). We, therefore, removed the 'warming' treatment of Garraf and the 'mild drought' treatment of Prades from this analysis. We also applied a repeated measure ANOVA to investigate the effect of site, season and treatment on factors 1 and 2 generated by the PCA.

The correlation of different environmental variables with SOM decomposition and its sensitivity to temperature was studied by averaging the data per treatment and season. We averaged the value of the three samples collected per plot (replicates) before analysis. Additionally, statistics were performed on the average of the three replicates in Garraf and the four replicates in Prades. Therefore statistics were performed on nine means per site corresponding to three treatments and three seasons. We performed two different correlation tests (1) Pearson's correlations to investigate the correlation of each environmental variable with SOM decomposition and Q_{10}; and (2) stepwise multiple regressions were additionally applied to study the relative contribution of environmental variables to the variability of SOM decomposition and Q_{10}. We reduced the number of explicative variables to five to reduce redundancy. The criteria to choose those five environmental variables were that they generated high Pearson's correlation coefficients and that they were representative of different environmental factors, e.g. climate (moisture), soil biogeochemistry (soil organic C), microbial biomass (fungal biomass) and microbial diversity (fungal and bacterial TRF richness).

All analyses were conducted using STATISTICA 6.0 (StatSoft Inc., OK, USA).

Results

Values for microbial and fungal biomass, as well as diversity indexes for bacteria and fungi derived from the analyses of TRF profiles are reported in Table 1. Results from the repeated measures ANOVA showed considerable effects of site, season and treatment over the structure of the soil microbial community (Table 2). In general, the holm-oak forest site showed values for biomass (microbial and fungal) and fungal diversity significantly higher than those found in the Mediterranean shrubland (Table 2). Bacterial diversity, on the other hand, did not differ significantly between sites (Table 2). Fungal and bacterial community structure was also strongly influenced by site, as illustrated by the ordination plots of the first and second axis of the PCA analyses (Fig. 1, Table 2). Within-sites, season strongly affected fungal biomass, which peaked during winter at both sites (Table 1) especially in Prades. Treatment did not affect significantly microbial and/or fungal biomass (Table 2) although at both sites microbial biomass was proportionally more affected than fungal biomass alone (Table 1).

The study of the diversity indexes (richness and/or Shannon–Weaver diversity index) further revealed that bacterial diversity was, in general, more affected by season and drought than fungal diversity (Table 2). Season affected bacterial diversity especially in Garraf, where both diversity indexes (richness and Shannon–Weaver) showed strong sensitivity to seasonality (Table 2). In this Mediterranean shrubland, the lowest bacterial diversity values were obtained during winter, when soils have the lowest temperatures. In Prades, on the other hand, the lowest bacterial diversity values were obtained under the driest summer conditions (Table 1), but only the Shannon–Weaver diversity index showed some sensitivity to seasonality (Table 2). The 10 years precipitation exclusion exerted, in general, a stronger effect over bacterial diversity than did seasonality (Table 2). While in Prades (holm-oak forest) drought affected equally bacterial diversity at all seasons (Table 1), at Garraf the main effect was observed in spring, when bacterial diversity was significantly higher in the control with respect to the precipitation exclusion plots (Table 1; see effect of season $\times$ treatment in Table 2). Neither seasonality nor treatment affected fungal diversity significantly at any of the sites (Table 2).

Results of the PCAs indicate that season strongly influenced the structure of the microbial community, but treatment did not (Fig. 1, Table 2). The effect of seasonality was strong in the Mediterranean shrubland (Garraf) where ordinates generated by the first and second dimensions clearly separated both bacterial and fungal communities by season rather than treatment (Fig. 1a and b). Treatment only exerted a significant effect in the microbial community structure of spring soils (Fig. 1, Table 2), when plant activity was typically maximal at this Mediterranean site (Peñuelas *et al*., 2007). Although season influenced significantly the microbial community structure at both experimental sites, the effect on both the fungal and bacterial community structure appeared to be, in general, stronger in Garraf than in Prades (Fig. 1).

We wanted to further test how microbial community structure may affect SOM decomposition and its sensitivity to temperature. Variability of soil CO_2 effluxes (SOM decomposition) measured under laboratory conditions was best explained by variations in soil fungal biomass, both within and between ecosystems (Fig. 2a, see Table 3). Values of fungal biomass and values of SOM decomposition were both generally

Table 1 Measured variables averaged per treatment/season

Site	Season	Treat	SWC	SE	R_h	SE	Q_{10}	SE	MB	SE	FB	SE	BR	SE	BH	SE	FR	SE	F H	SE
	Winter	Control	0.15	0.02	0.7	0.1	2.7	0.3	529	71	4.9	0.3	7.7	0.9	1.9	0.1	21	3.2	1.6	0.2
	Spring	Control	0.19	0.01	0.4	0.1	0.9	0.0	308	71	3.6	2.4	28.7	6.2	3.1	0.2	14.3	8.9	1.4	0.6
	Summer	Control	0.07	0	0.3	0.1	1.7	0.1			2.0	0.3	22.3	3.8	2.8	0.2	26.5	6.4	2.2	0.4
Garraf																				
	Winter	Drought	0.16	0.01	0.4	0.0	2.5	0.3	451	67	3.8	0.9	7	1	1.8	0.1	26.7	8	1.9	0.5
	Spring	Drought	0.17	0.02	0.6	0.2	1.2	0.1	266	53	2.9	0.8	5.3	2	1.4	0.3	20.3	3.6	1.5	0.2
	Summer	Drought	0.06	0.01	0.3	0.1	1.9	0.2			1.8	0.2	21.6	5.1	2.6	0.3	12.6	3.8	1.4	0.2
	Winter	Control	0.16	0	4.5	1.2	4.7	0.6	737	273	13.4	1.8	25.3	6.2	2.7	0.2	53.1	6.2	2.7	0.2
	Spring	Control	0.2	0.02	0.9	0.4	1.9	0.3	843	169	4.8	1.2	25.6	10.2	2.4	0.3	45.9	4.3	2.7	0.1
	Summer	Control	0.09	0.01	1.2	0.5	8.8	1.2			1.8	0.5	13.5	3.1	2.3	0.3	71	5.5	3.2	0.1
Prades																				
	Winter	Drought	0.1	0	2.5	1.1	3.8	0.4	673	224	10.2	1.2	14.6	4	2.3	0.2	50.1	5.7	2.7	0.1
	Spring	Drought	0.18	0.01	0.9	0.3	2.5	1.4	578	250	7.1	2.3	8.9	2.5	1.8	0.3	48.1	6.4	2.6	0.2
	Summer	Drought	0.05	0.01	0.2	0.1	4.3	0.8			2.4	0.7	6.1	1.5	1.5	0.2	52.4	7.8	2.6	0.2

SWC: soil moisture, g H_2O g^{-1} dry soil; Rh: SOM decomposition; mg CO_2 g^{-1} soil h^{-1}; MB: microbial biomass; μg C g^{-1} dry soil; FB: fungal biomass; μg erg g^{-1} dry soil.
BR, bacterial richness; BH, bacterial Shannon–Weaver; FR, fungal richness; FH, fungal Shannon–Weaver; SE, standard error of the mean; SOM, soil organic matter.

Table 2 *F* coefficient and *P* values of the repeated measures ANOVA

	MB		FB		BR		BH		FR		FH		B PCA		F PCA	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
All																
Site	7.317	0.009*	14.261	0.000***	0.016	0.900	1.076	0.303	71.367	0.000***	59.147	0.000***	51.194	0.000***	9.626	0.000***
Season	0.616	0.436	16.986	0.000***	0.466	0.629	0.066	0.936	1.823	0.169	2.252	0.112	4.804	0.000***	5.936	0.000***
Treat	0.340	0.562	0.451	0.504	10.850	0.002**	20.672	0.000***	0.843	0.362	1.231	0.271	2.253	0.111	0.287	0.751
Garraf																
Season	7.637	0.011*	6.703	0.004**	8.438	0.001**	5.900	0.007*	0.196	0.823	1.184	0.322	6.345	0.004**	6.033	0.000***
Treat	0.022	0.883	0.000	0.994	10.437	0.003**	16.085	0.000***	0.070	0.793	0.011	0.917	0.876	0.355	0.637	0.534
$S \times T$	0.117	0.736	0.040	0.961	5.301	0.0114**	5.651	0.009 **	2.832	0.076	2.900	0.072	3.533	0.04*	1.229	0.307
Prades																
Season	0.001	0.981	19.143	0.000***	1.739	0.187	3.812	0.029*	2.744	0.074	1.602	0.212	5.367	0.001**	4.449	0.002**
Treat	0.529	0.473	0.802	0.375	5.630	0.022*	8.468	0.005**	2.450	0.124	4.162	0.047*	0.836	0.440	1.437	0.246
$S \times T$	0.196	0.661	0.100	0.905	0.496	0.612	0.384	0.683	1.049	0.358	0.716	0.494	0.421	0.659	0.313	0.733

Categorical variables : site, season, treat (treatment; ctrl-drought) and $S \times T$ (season × treatment).
*Significant at 0.05; **0.005; and ***0.0005 confidence interval.
MB, microbial biomass; FB, fungal biomass; ANOVA, analysis of variance; BR, bacterial richness; BH, bacterial Shannon–Weaver; FR, fungal richness; FH, fungal Shannon–Weaver; B PCA, bacterial principal component analyses; F PCA, fungal principal component analyses.

higher for the holm-oak forest than for Mediterranean shrubland soils (Fig. 2a). Results from a stepwise multiple regression further confirmed that soil fungal biomass was the best single factor explaining variance in SOM decomposition (semipartial correlation = 0.46, $P < 0.01$), outstripping fungal diversity, bacterial diversity, soil moisture, or soil organic C (semipartial correlations 0.22, 0.16, 0.15 and 0.05 respectively) (Table 4).

Surprisingly, the response of soil CO_2 effluxes to temperature was best explained by soil diversity (richness and/or the Shannon–Weaver diversity index) in fungal TRFs (Fig. 2b, Table 3). Again, values of Q_{10} and values of fungal diversity were generally higher for the holm-oak forest than for Mediterranean shrubland soils (Fig. 2b). Results from a stepwise multiple regression analysis indicated that fungal TRF richness was the best single factor explaining

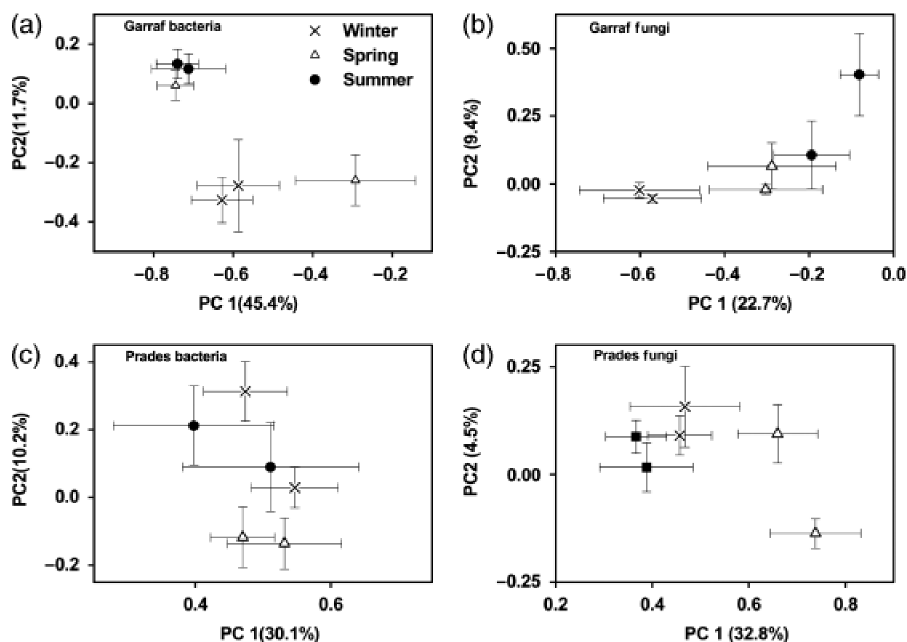

Fig. 1 Ordination plot of the first (X-axis) and the second dimensions (Y-axis) of principal coordinate (PCA) scores for the soil bacterial and fungal community at the two study sites. Symbols represent different seasons. Error bars represent the standard error of the mean. PCA, principal component analyses.

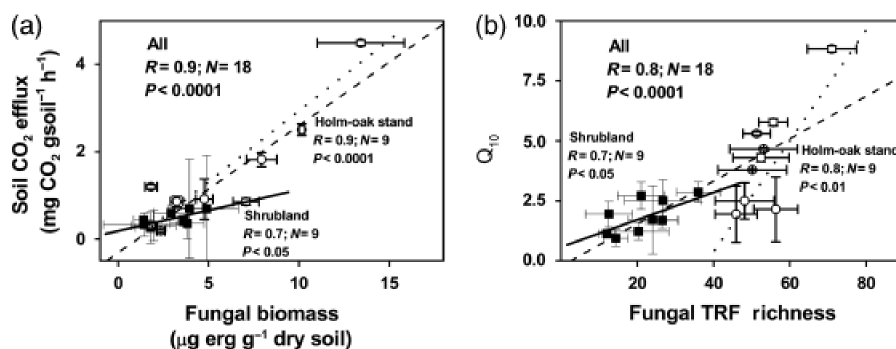

Fig. 2 Averaged values of soil CO_2 efflux (grouped by season and treatment) (Fig. 1a) and Q_{10} (temperature sensitivity coefficient) (Fig. 1b) as a function of the fungal biomass (left) and the fungal terminal restriction fragment (TRF) richness (right), respectively. Fungal biomass is given in ergosterol (erg) content units. Solid and dotted lines represent the linear fit of variables at each site (shrubland and forest, respectively) separately ($N = 9$ means $\pm$ SE of three plots shown by bars). Dashed lines represent the linear fit when both sites were pooled together ($N = 18$ means $\pm$ SE of three plots). The statistics of the linear fit (coefficient of correlation, R and P value of the fit) are provided for the entire data set (All), and for Garraf shrubland and Prades holm-oak forest separately.

variance in Q_{10} (semipartial correlation = 0.52, $P < 0.01$), particularly in the holm-oak forest (see Table 2), surpassing soil moisture, fungal biomass, bacterial TRF richness, or soil organic C (semipartial correlations −0.29, 0.11, −0.05 and −0.01 respectively) (Table 4). While the positive correlation was significant at both sites and within sites (Table 3), the effect of diversity on Q_{10} was especially strong in soils from the holm-oak forest (red line in Fig. 2, Table 3), where both fungal biomass and fungal diversity were typically higher.

Discussion

Effect of site, season and treatment on the microbial community

Site, season and treatment exerted a strong influence over biomass, diversity and the structure of bacterial and fungal communities (Tables 1 and 2, Fig. 1). Site substantially influenced biomass (microbial and fungal), diversity of fungi and the bacterial and fungal community structure (Tables 1 and 2). Microbial and

Table 3 Pearson's correlation coefficients of the individual linear correlations of SOM decomposition (SOM dec) and Q_{10} with the independent explanatory variables studied

	All data		Garraf shrubland		Prades forest	
	SOM dec	Q_{10}	SOM dec	Q_{10}	SOM dec	Q_{10}
Soil moisture						
Pearson correlation	0.414	−0.409	0.271	−0.301	0.258	−0.608
Significant (two-tailed)	0.087	0.092	0.481	0.432	0.503	0.082
N	18	18	9	9	9	9
Fungal biomass						
Pearson correlation	**0.802**	0.265	**0.697**	0.567	**0.902**	−0.22
Significant (two-tailed)	**0.001****	0.287	**0.037***	0.111	**0.001****	0.57
N	18	18	9	9	9	9
Bacteria richness						
Pearson correlation	0.193	−0.184	−0.127	−0.14	0.582	−0.22
Significant (two-tailed)	0.443	0.465	0.745	0.72	0.1	0.57
N	18	18	9	9	9	9
Bacteria Shannon–Weaver						
Pearson correlation	0.171	−0.183	−0.246	−0.197	**0.753**	0.151
Significant (two-tailed)	0.498	0.467	0.524	0.611	**0.019***	0.698
N	18	18	9	9	9	9
Fungal richness						
Pearson correlation	**0.492**	**0.798**	0.373	**0.67**	−0.071	**0.82**
Significant (two-tailed)	**0.038***	**0.0001*****	0.323	**0.048***	0.856	**0.007****
N	18	18	9	9	9	9
Fungi Shannon–Weaver						
Pearson Correlation	0.459	**0.762**	0.303	**0.67**	0.066	**0.697**
Significant (two-tailed)	0.055	**0.0001*****	0.429	**0.048***	0.865	**0.037***
N	18	18	9	9	9	9
Microbial biomass						
Pearson correlation	0.273	0.179	0.409	−0.19	−0.432	−0.069
Significant (two-tailed)	0.416	0.598	0.494	0.76	0.392	0.896
N	11	11	5	5	6	6
Total organic C						
Pearson correlation	0.165	0.11	0.185	0.024	0.297	0.022
Significant (two-tailed)	0.513	0.665	0.634	0.951	0.438	0.955
N	18	18	9	9	9	9

The significant explanatory variables for SOM decomposition and Q_{10} are highlighted in bold type.
*Significant at 0.05; **0.005; and ***0.0005 confidence interval.
SOM, soil organic matter.

fungal biomasses, SOM decomposition rates and fungal diversity were all significantly higher in the holm-oak than in the Mediterranean shrubland (Tables 1 and 2). Indeed, the holm-oak forest holds more soil C (averaging 2.2% and 3.5% C in Garraf and Prades, respectively) and biomass (Ogaya & Peñuelas, 2007; Peñuelas *et al*., 2007) compared with the Garraf shrubland. However the forest also emits more soil CO_2 (Asensio *et al*., 2007, 2008) and shows higher soil enzymatic activity (Sardans *et al*., 2008; Sardans & Peñuelas, 2010). Therefore our results coincide quite well with the differences in biological activity between sites observed in former studies. The low fungal diversity in Garraf might be interpreted as an effect of the harsh environmental conditions and low productivity of this site (Rajaniemi, 2003) with respect to Prades. On the other hand, our results indicate that bacterial diversity was not sensitive to site. It should be noted, however, that the values of bacterial diversity obtained in this study were at the very low end of those observed in soils with similar pH's (Fierer & Jackson, 2006), indicating that bacterial diversity might have been equally affected at both sites.

Fungal biomass was also strongly affected by seasonality at both sites (Tables 1 and 2) particularly because of the high fungal biomasses observed during winter (Table 1). This high fungal activity could be due to the combination of low plant-resource competition in win-

Table 4 Stepwise multiple regression coefficients (β, partial and semipartial correlation, tolerance, R^2 and P value of the t-test) for the relationships of soil organic matter (SOM) decomposition and Q_{10} to the explanatory variables, soil moisture, organic carbon, bacteria richness and fungal biomass

	SOM decomposition					Q_{10}				
	Soil moisture	Organic C	Bacteria richness	Fungi richness	Fungal biomass	Soil moisture	Organic C	Bacteria richness	Fungi richness	Fungal biomass
β	0.184	0.056	0.191	0.288	**0.614**	−0.355	0.010	−0.057	**0.680**	0.142
Pearson correlation	0.294	0.092	0.311	0.409	**0.681**	−0.495	0.015	−0.094	**0.713**	0.202
Significant correlation	0.152	0.046	0.162	0.221	**0.459**	−0.293	0.008	−0.048	**0.523**	0.106
Tolerance	0.681	0.663	0.716	0.593	**0.558**	0.681	0.663	0.716	**0.593**	0.558
R^2	0.319	0.337	0.284	0.407	**0.442**	0.319	0.337	0.284	**0.407**	0.442
P level	0.308	0.753	0.279	0.146	**0.007****	0.072	0.959	0.750	**0.004****	0.488

Only the variables measured during the three seasons were used for this analysis. The significant explanatory variable for SOM decomposition and Q_{10} are highlighted in bold type. **Significant at 0.05.

ter and good abiotic conditions (high moisture and mild temperatures) typical of the Mediterranean winters. Indeed soils of these Mediterranean ecosystems maintain a high potential enzymatic activity during winter time (Sardans *et al.*, 2008; Sardans & Peñuelas, 2010) and the high SOM decomposition rates observed in winter in this study (Table 1) support this idea.

Season also affected the diversity of bacteria as well as bacterial and fungal community structure (Tables 1 and 2). Nevertheless, the observed seasonality of bacterial diversity was not necessarily positively correlated with moisture (Tables 1 and 2), which in Mediterranean ecosystems is generally the limiting factor on the seasonal scale (J. C. Yuste, S. Vicca, I. A. Janssens, M. Bahn, S. Luyssaert, B. Longdoz, D. D. Baldocchi, E. A. Davidson, J. Peñuelas, M. Reichstein, N. Arriga, J. M. Aubinet, N. Buchmann, A. Carrara, C. Gimeno, T. Gruenwald, B. F. Lobster, S. Ma, M. Mencuccini, L. Misson, L. Montagnani, F. Moyano, M. Pavelka, J. Pumpanen, Richardson, N. A. Rurh, M. Suleau, J. W. Tang, M. S. Torn, R. Vargas, G. Wohlfahrt & W. Kutsch, unpublished results). Seasonality does, however, not only implies changes in climate but also in plant productivity and hence supply of resources to soils. The relationship between microbial diversity and productivity is rather complex (Waldrop & Firestone, 2006a, b) because few species can withstand low resource levels. But as the resource level increases and more species meet their minimum resource requirements, other processes such as competitive exclusion (Rajaniemi, 2003) and/or top-down predatory interactions (Worm *et al.*, 2002) may also lead to a reduction in diversity. Therefore the relationships between bacterial diversity and seasonality are quite complex and in our study no single environmental factor appeared to control the observed seasonal variations.

Results from the PCA analyses further demonstrated that season exerted a stronger effect over the structure of the microbial community than treatment. This can be seen by the position in the ordination plot in dimensions 1 and 2 of the PCA analyses (Fig. 1, Table 2). Unlike fungal diversity (Table 2), fungal community structure showed strong sensitivity to season at both sites (Fig. 1), indicating that the phylotypes associated to the TRF peaks changed seasonally without a general loss in diversity. Indeed, the microbial community can possess the metabolic and genetic capability of adapting to changing environmental conditions on short-time scales, e.g. seasonally (Lipson *et al.*, 2002, 2009; Waldrop & Firestone, 2006a, b; Schmidt *et al.*, 2007; Williams, 2007). Our results therefore indicate that as the seasonal environmental conditions changed, the phylotype composition of the microbial communities could adapt accordingly without a general loss in diversity.

Treatment, on the other hand, affected preferentially the bacterial more than the fungal community (Table 2). At both sites we observed a greater, though not significant, decline in microbial biomass, covering both fungal and bacterial biomasses, than in fungal biomass alone (Table 1). This suggests that drought probably affected the bacterial biomass more than the fungal. Furthermore, the 10 years drought resulted in a significant decline in bacterial diversity at both sites especially in holm-oak forest soils, while no effect on fungal diversity was observed (Table 1 and 2). Our results suggest that increasing drought conditions appear to favor a fungal-rich microbial community, as fungi were able to overcome better the disadvantages of drier conditions than bacteria. These results, therefore, raise the question as to whether future 'drier conditions,' as projected by models for the Mediterranean regions (Gibelin & Deque, 2003) might eventually favor fungal dominated microbial communities. A shift to fungal-dominated communities may have important consequences for soil nutrient cycling because it is believed that fungal-dominated communities sequester more C than bacterial-dominated communities (Martin & Haider, 1979; Holland & Coleman, 1987; Beare *et al*., 1997; Bailey *et al*., 2002; Six *et al*., 2006). In our study, long-term drought affected significantly (0.05% conf. *T*-test) soil organic C concentration in Garraf soils ($1.9 \pm 0.2\%$ C content in treatment with respect to $2.5 \pm 0.3\%$ C in drought soils, at 10 cm depth), but did not affect soil organic C concentration in Prades soils (3.4% C content at both soils, ctrl and treatment). Nonetheless soil organic C depends not only on the microbial community responsible for OM decomposition but also on the productivity (inputs) of the system, that were not measured in this study. Although no conclusive results could be drawn, the relation between the fungal community and the stabilization of SOM in Mediterranean ecosystems could be a key issue that requires further study.

Drivers of SOM decomposition and Q_{10} variability

We also report strong correlations between fungal biomass and SOM decomposition (Fig. 2a, Tables 3 and 4) and between fungal diversity and response to temperature (Q_{10}) (Fig. 2b, Tables 3 and 4). This indicates that besides the observed capacity of the fungal community to resist both long-term- and seasonally induced environmental changes better than bacteria, the biomass and diversity of fungi played a central role in the decomposition of SOM. These results add to a growing number of studies that confirm the important and active role that the microbial community can play in soil C balance (Balser & Firestone, 2005; Aerts, 2006; Waldrop & Firestone, 2006a, b; Balser & Wixon, 2009; Strickland *et al*., 2009). This contrasts with the more classical vision of the microbial community having a passive role in the oxidation of SOM, as a result of a high degree of functional redundancy (Gitay *et al*., 1996).

Fungal biomass explained variability in SOM decomposition better than other variables such as TOC, microbial biomass or moisture. This result indicates that the decomposition of SOM in the two ecosystems studied was strongly bound to the presence of fungi. In contrast, recent studies on a subalpine conifer forest highlighted the role of bacteria over that of fungi in the seasonality of heterotrophic respiration (Lipson *et al*., 2009). However fungi are a key functional group within the soil microbial community that overall contributes substantially to the decomposition of SOM. Fungi dominate the production of a wide range of extracellular enzymes that break down complex high molecular organic matter, particularly lignocelluloses and humus (Peay *et al*., 2008). The microbial community therefore benefits greatly from the presence of fungi as important providers of assimilable (low molecular weight) substrate and nutrients to the whole microbial community (Beare *et al*., 1992). This together with the observed resistance of fungi to the relatively harsh environments of these Mediterranean ecosystems (Tables 1 and 2) suggests that the fungal community is playing a central role in the soil C balance of those ecosystems. It follows that our results indicate that the relative role of bacteria and fungi may vary according to the abiotic/biotic conditions of different ecosystems. While this is only speculative, future studies should be designed to understand the different roles of fungal and bacterial communities in SOM decomposition in different ecosystems.

In addition our results show that the response of SOM decomposition to temperature was highly correlated to the diversity of the fungal community, within and among sites (Fig. 2b). It is difficult to interpret the possible cause-effect relation between diversity and Q_{10} because this is, to the best of our knowledge, the first time it has been shown. It is possible that the strong/sudden changes in temperature (40 °C in 48 h) favored more diverse fungal communities, because they are more likely to contain species able to respond to the fast warming of soils. Microbes can sense changing local conditions in seconds to minutes, and they come out of dormancy within minutes in response to a change (e.g. Fenchel, 2002). Therefore high Q_{10}'s may represent the response of communities which are better adapted to temperature perturbations. This is in accordance with the 'insurance hypotheses,' which states that the more diverse a community is, the more likely it contains species able to tolerate and take advantage of a disturbance such as a temperature change (Chapin III *et al*.,

1992; Naeem & Li, 1997). At least in the relatively harsh environments of Mediterranean ecosystems the redundancy of organisms within a functional group would be paramount for microbial communities to maintain their activity when faced with climatic disturbances.

Nonetheless we cannot conclude that the observed correlation between fungal diversity and Q_{10} was necessarily causal. During the temperature cycles microbial communities may have undergone physiological and/or community changes beyond simple kinetics, e.g. temperature-induced stress, reflected in increases in the metabolic quotient at higher temperature (Pietikainen *et al*., 2005). However it is difficult to explain the mechanisms that make diverse communities more stressed than less diverse communities. Furthermore if high Q_{10}&apso;s were related to stress, the Q_{10} of drought stressed soils should have been higher than Q_{10} for control soils, which was not the case (Table 1). Because ambient temperatures also change the optimal temperatures of soil microbial communities (Barcenas-Moreno *et al*., 2009), the observed trend might be an acclimatization response by these microbial communities to the ambient temperatures at sampling date. This would explain the high responsiveness to temperature (high Q_{10}'s) of the holm-oak soils collected during summer (Table 1). While such conclusions are not definitive as yet, our results suggest that the structure and diversity of the microbial community might not only affect its functionality, which has been previously observed in a number of studies, but it may also affect the responsiveness of soil ecosystems to climate.

Concluding remarks

Our results, therefore, support the three hypotheses raised in the study. Firstly, environmental changes associated to site and seasonality exerted a strong influence over the microbial structure, the diversity and the biomass of the microbial community. Fungal diversity was less sensitive to seasonal changes in moisture, temperature and plant activity than bacterial diversity. On the other hand, fungal communities showed the ability to dynamically adapt to changing environments without a loss of diversity. Secondly, fungi resisted the 10-year precipitation exclusion performed in water-limited Mediterranean ecosystems better than bacteria. The high resistance of the fungal community to changes raises the question as to whether future 'drier conditions' for the Mediterranean regions might favor fungal dominated microbial communities. Finally, our results further support our third hypothesis: The capacity of fungi to overcome disturbances played a pivotal role in shaping the microbial community and exerted a strong influence over the temporal and spatial variability of SOM decomposition, and hence over soil C dynamics. Fungal biomass and diversity, therefore, should be extensively and intensively explored in order to understand and correctly predict soil CO_2 emissions from different types of ecosystems, especially in the frame of current human-driven alterations in the C cycle and the resulting climate change.

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