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Microbial C, N and P in soils of Mediterranean oak forests: influence of season, canopy cover and soil depth

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Abstract In Mediterranean ecosystems the effect of aboveground and belowground environmental factors on soil microbial biomass and nutrient immobilization-release cycles may be conditioned by the distinctive seasonal pattern of the Mediterranean-type climates. We studied the effects of season, canopy cover and soil depth on microbial C, N and P in soils of two Mediterranean forests using the fumigation-extraction procedure. Average microbial values recorded were 820 μg C g^{-1}, 115 μg N g^{-1} and 19 μg P g^{-1}, which accounted for 2.7, 4.7 and 8.8% of the total pools in the surface soil, respectively. Microbial N and P pools were about 10 times higher than the inorganic N and P fractions available for plants. Microbial C values differed between forest sites but in each site they were similar across seasons. Both microbial and inorganic N and P showed maximum values in spring and minimum values in summer, which were positively correlated with soil moisture. Significant differences in soil microbial properties among canopy cover types were observed in the surface soil but only under favourable environmental conditions (spring) and not during summer. Soil depth affected microbial contents which decreased twofold from surface to subsurface soil. Microbial nutrient ratios (C/N, C/P and N/P) varied with seasons and soil depth. Soil moisture regime, which was intimately related to seasonality, emerged as a potential key factor for microbial biomass growth in the studied forests. Our research shows that under a Mediterranean-type climate the interaction among season, vegetation type and structure and soil properties affect microbial nutrient immobilization and thus could influence the biogeochemical cycles of C, N and P in Mediterranean forest ecosystems.

Keywords Microbial biomass · Nitrogen · Nutrient immobilization · Phosphorus · Plant–soil interactions · Seasonal dynamics · Vegetation cover

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Introduction

Soil microbes play an essential role in the main biogeochemical transformations of organic matter and in soil fertility (Jenkinson and Ladd 1981). During the mineralization process, an important fraction of the C, N and P in the decomposing residues is immobilized in the microbial biomass as part of their cellular constituents (e.g. phospholipids and proteins), and then released upon microorganism death (Anderson and Domsch 1980; Jonasson et al. 1999). The capacity of microorganisms to act both

as a sink and a source of nutrient resources is particularly relevant for plant nutrition since most of the annual N and P requirements of land plants are supplied from the decomposition of organic matter in the soil (Singh et al. 1989). Changes in biomass, physiology, composition and activity of soil microbes may affect their functional capacity and thus the ecosystem geochemical processes (Balser and Firestone 2005; Crenshaw et al. 2008).

Both aboveground and belowground factors affect microbial biomass and, therefore, nutrient availability (García et al. 2002; Schade and Hobbie 2005). Vegetation structure and composition exert a control on microbial growth through litter quality and quantity and root exudates that determine the input fluxes of labile C and nutrients (N, P) (Fisk and Fahey 2001; Kara et al. 2008). Soil chemical and physical characteristics, like soil organic matter (SOM) and soil structure and texture, may also constrain microbial developments (Hassink 1994), and the variability of these properties along soil profile is reflected in the microbial communities (Fierer et al. 2003). Climatic conditions have a direct effect on microbial communities through soil moisture and temperature (Ley et al. 2004; Nielsen et al. 2009), but they may also have an indirect effect thought interactions with other factors such as vegetation, topography and landscape (Malchair and Carnol 2009; Myers et al. 2001). Temporal patterns of microbial growth and nutrient immobilization-release cycles usually reflect seasonal changes (but see Raubuch and Joergensen 2002) although such a response vary among ecosystems depending on their particular moisture and temperature regimes (Bohlen et al. 2001; Ley et al. 2004; Nielsen et al. 2009).

In this study we investigated the effects of several abiotic and biotic factors on the soil microbial C, N and P in Mediterranean oak forests. Mediterranean ecosystems are subjected to a marked seasonality that imposes a severe summer drought after a favourable rainy autumn and spring, that is reflected in soil microbial dynamics (Quilchano and Marañón 2002). Vegetation traits that overcome Mediterranean summer drought, such as long-lived and hard leaves (sclerophylly), influence litter quality and quantity and thus decomposition processes (Gallardo and Merino 1993; Rutigliano et al. 2004). In addition Mediterranean forests have large environmental heterogeneity (species composition, canopy structure, soil properties) that might affect microbial spatial patterns (Joffre et al. 1996; Quilchano et al. 2008). The interplay of driving factors on the microbial dynamics—climate, vegetation and soil—is key to understanding the biogeochemical cycles in Mediterranean forests (Bohlen et al. 2001); however there are few studies on microbial biomass and nutrient dynamics in this type of ecosystem (Gallardo et al. 2009).

Mediterranean ecosystems, as other areas under semiarid climate conditions, are predicted to experience warmer and drier conditions due to climate change (Bates et al. 2008). Information on how microbial nutrients immobilization-release cycles are affected by environmental factors under the characteristic Mediterranean seasonal pattern could increase our understanding on how climate change may affect microbial controls over nutrient availability in this and other ecosystems and regions.

Our main objective was to investigate the main factors affecting microbial C, N and P content in Mediterranean forest soils. In particular we tested the following hypotheses: (i) under Mediterranean climatic conditions there are seasonal patterns with higher microbial growth and nutrient retention during the warm and wet season (spring), and a decline in microbial population and nutrient retention during the hot and dry season (summer); (ii) distinct vegetation cover and composition affect soil microbial properties, with higher microbial carbon under deciduous trees which have a nutrient richer litterfall; and (iii) microbial nutrient content is higher in surface than in the subsurface soil. We also tested whether these soil microbial patterns are consistent in different forest sites, and analysed the interactions between these factors.

Methods

Site description

This study was conducted in the oak forests of Aljibe Mountains, near the Strait of Gibraltar, in southern Spain (Fig. 1). Acidic, nutrient-poor soils (*Palexeralfs*; Soil Survey Staff 2006) are developed over Oligo-Miocene sandstone bedrock that is frequently interspersed with layers of marl sediments yielding soils richer in clay (*Haploxererts*; Soil Survey Staff

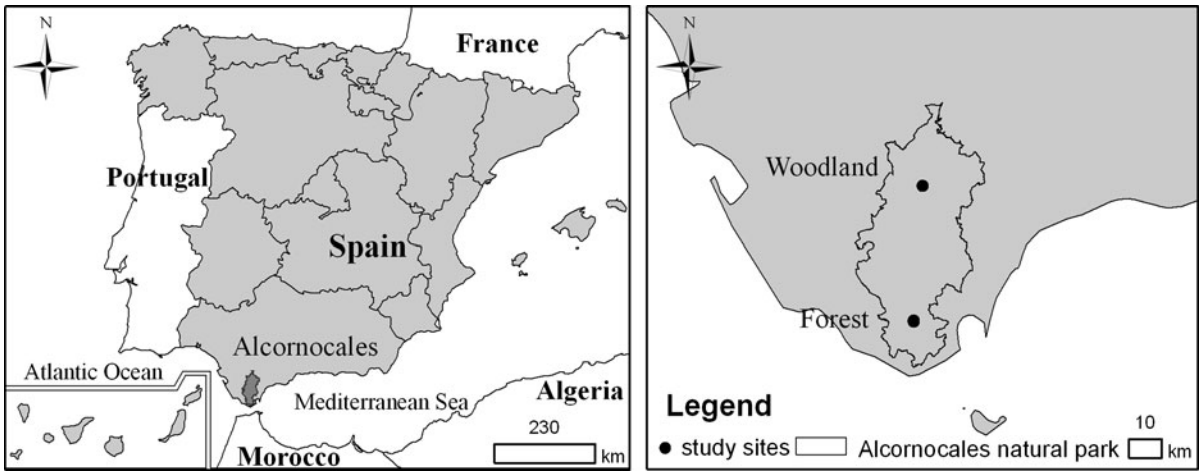

Fig. 1 Location of the study area and the two forest sites in the Iberian Peninsula

2006). Climate is subhumid Mediterranean-type with warm, dry summers, and humid, mild winters. Temperatures average 24°C in summer and 8.5°C in winter. Mean annual rainfall varies from 701 to 1331 mm, depending on the mountain topography, and most (95%) of it falls from October to May. Vegetation is dominated by sclerophyllous evergreen cork oak (*Quercus suber* L.), mixed with the winter-deciduous Algerian oak (*Q. canariensis* Willd.) which is locally abundant in the valley bottoms (Urbieta et al. 2008). Both oak species differ in their leaf fall and litter quality: *Q. canariensis* has a higher nutrient content (Ca, K, Mg, S) and a lower C/N ratio compared to *Q. suber*, what induces distinct soil conditions via litter decomposition (Aponte et al. unpublished data). There is a diverse shrubby and arborescent understorey of *Phillyrea latifolia*, *Erica* spp. and *Pistacia lentiscus*. See detailed description in Ojeda et al. (2000).

For this study two forest stands of different structure, 40 km apart, were selected within the study area. The first one, at San Carlos del Tiradero (36°9′46″N 5°35′39″W) hereafter called "Forest", was located in the south of the study area close to the coast, at 335–360 m a.s.l. on a NE slope. The second stand hereafter called "Woodland" was located at La Sauceda (36°31′54″N 5°34′29″W) and stood inland, in the north of the area, at 530–560 m a.s.l. on a NW slope. The Forest site had a higher density of trees and a close canopy cover while the Woodland site had higher canopy heterogeneity and fewer trees mixed with abundant shrubs and gaps (Table 1). Both sites presented a large heterogeneity in their chemical and physical soil characteristics; see details on the forest sites in Quilchano et al. (2008) and Pérez-Ramos et al. (2008).

Field sampling

Soil samples were taken in spring (May–June), summer (September) and autumn (December) 2007, and spring (May) 2008. Soils cores were extracted at two depths (0–8 cm and 8–16 cm) using an auger. Each sample was composed of four subsamples. At the Woodland site four microhabitats corresponding to different vegetation cover types were studied i.e. soil beneath the canopy of *Q. canariensis* (Qc), beneath *Q. suber* (Qs), under shrubby cover (S) and in gaps with grass cover (G). At the Forest site, soils beneath two types of canopy cover—*Q. canariensis* and *Q. suber*—were studied. To minimize the effect of the inherent site variability 10 replicates of each microhabitat were sampled at each season and soil depth, which made up a total of 480 samples. Two 30 × 30 cm quadrates were used to assess the thickness of the litter layer, using a folding rule, and the litter biomass, by the harvesting and drying method (expressed as kg dry mass m^{-2}) at each sampling point. This sampling design allowed us to assess the effects of three factors: season, vegetation cover type and soil depth on microbial C, N and P, and to test their consistency between the two sites.

Table 1 Climate and vegetation structure of the studied forest sites

	Forest	Woodland
Mean rainfall (mm)		
Spring	216.8	258.9
Summer	21.1	28.0
Autumn	262.3	319.2
Winter	472.2	526.4
Total	972.3	1132.4
Mean temperature (°C)		
Annual	16.6	15.5
Minimum	4.1	1.8
Maximum	23.4	23.6
Vegetation structure		
Density of trees (stems ha^{-1})	768.8	218.8
Density of arborescent shrubs (stems ha^{-1})	256.3	450.0
Basal area (m^2 ha^{-1})	47.0	22.1
Leaf area index (m^2 m^{-2})	2.26	1.84

Sources are the AEMET for climate and Pérez-Ramos et al. (2008) for vegetation

Laboratory analysis

Soil samples were brought to the laboratory in an ice-box and they were stored at 4°C for a maximum of 3 days. Stones, roots and other recognizable plant parts contained in the samples were removed and the soil was homogenised through a 2 mm sieve. A subsample of 1 g was used to determine the water content gravimetrically by weighing the fresh and dried (105°C) soil. The same subsample was then incinerated for 4 h at 550°C to determine the SOM content by calcination method (Sparks 1996).

Microbial C, N and P were estimated in the fresh soils using a chloroform fumigation-extraction procedure (Brookes et al. 1982, 1985; Vance et al. 1987). Two soil subsamples (10 and 5 g) were extracted using 50 ml of 0.5 M K_2SO_4 or 50 ml of 0.025 N HCl + 0.03 N NH_4F for subsequent determination of microbial C and N or microbial P, respectively. The other two soil subsamples (10 and 5 g) were fumigated with chloroform for 24 h in a vacuum desiccator, followed by the same extraction procedure as the unfumigated samples. The soil extracts were frozen until their C, N, P content were measured. The C and N in the fumigated and unfumigated soil extracts were determined using a Total Dissolved Organic Carbon and Nitrogen Analyzer (TOC-Vesh). Microbial C and N were estimated as the difference in K_2SO_4-extractable dissolved organic carbon or nitrogen between fumigated and unfumigated soils using as extractability correction factors: $K_C = 0.45$ for C and $K_N = 0.40$ for N (Jonasson et al. 1996; Rinnan et al. 2008). Available P in NH_4F soil extracts was measured using the Bray Kurtz method (Bray and Kurtz 1945). Microbial P was estimated as the difference in available P between the fumigated and the unfumigated soil using a correction factor $K_P = 0.40$ (Brookes et al. 1982).

Soil ammonium and nitrate and total C, N and P contents were analysed in the unfumigated soils. Inorganic nitrogen (NH_4^+ and NO_3^-) was extracted using 2 M KCl and determined by distillation–titration in a Bran–Luebre Autoanalyzer. Soil total C and N were estimated using an Autoanalyzer LECO. Soil total P was determined by acid digestion and ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometer) analysis (Sparks 1996). Concentrations of the elements are given on a dry weight basis (105°C).

Data analysis

To evaluate the effects of seasonality and canopy cover type on the soil microbial properties of the two forest sites at the two soil depths we used repeated-measures analysis of variance (ANOVA) on a split-plot design with sampling season defined as a within-factor of four levels, each one divided into two forest types (large units in the split-plot design) and each one further divided into canopy cover types (split-plots). Because of the unbalanced design, we first run the analysis including only the common cover types (*Q. canariensis* and *Q. suber*) of the two forest sites, and then analysed the differences between the microhabitats within each site. Variables were transformed (log, arcsine) to meet necessary assumptions of normality and homocedasticity. Post-hoc comparisons were done using Fisher LSD test and type I error inflation resulted from repeated tests was controlled using the False Discovery Rate (García 2003). This technique was preferred over Bonferroni-related procedures that notably increases power losses. General trends of soil microbial C, N and P values were related to other soil features (moisture, organic matter, inorganic nutrients) by correlation analysis.

Results

Soil patterns

There were significant differences in soil properties between canopy cover type and soil depth, and also between sites (Appendix 1). Soil water content varied across season attaining minimum values in summer (average of 10.9%) and maximum values in spring (average of 19.2%) (Fig. 2). Moisture decreased with soil depth across all studied soils ($F = 36.89$, $p < 0.0001$). Differences in soil moisture between the forest sites were only found in the spring ($p < 0.0001$) when soil in the Woodland site had a higher gravimetric water content than in the Forest. In each site, soil moisture values were similar for all vegetation covers in each season. Soil texture varied significantly between the forest sites with clayey soils in the Woodland ($\approx$30% clay, 49% sand) and sandy soils in the Forest ($\approx$16% clay, 65% sand). These differences affect the water holding capacity and moisture availability of their soils. Thus, under similar water content, sandy soils would proportionally have more water held at available potentials than clayey soils.

SOM estimated as loss on ignition averaged over all samples 10.5% and varied from 0.77 to 24.93% in the Woodland and from 4.11 to 24.03% in the Forest (Appendix 1). Surface soil contained higher SOM (average of 12.7%) than the deeper layer (average of 8.35%) in all studied soils ($F = 71.42$, $p < 0.0001$). In both sites higher values of SOM were found in the soils beneath *Q. canariensis* and shrub than in the soils under *Q. suber* and herbaceous cover. Maximum litter biomass values (1.69 kg m^{-2}) were recorded in the Forest. Gaps with grass cover (in the Woodland site) had a significantly lower litter mass (0.09 kg m^{-2}) and litter layer thickness (0.56 cm) than any other microsite ($p < 0.0001$).

Soil ammonium averaged 7.3 μg g^{-1} in the Woodland and 4.0 μg g^{-1} in the Forest. Similar available phosphorus values were recorded in all soils (average of 2.5 μg g^{-1}). In general the concentration of both nutrients decreased with soil depth ($p < 0.022$) except in the case of ammonium in the Forest ($p < 0.850$).

Soil total C and N ranged from 1.0 to 7.7% and from 0.1 to 0.7% respectively. Both soil total C and N decreased with soil depth ($p < 0.0001$) and total N content varied among soils. The Ct/Nt ratio averaged 14.7 and the ratio increased with soil depth ($F = 43.75$, $p < 0.0001$). The highest Ct/Nt values were recorded in *Q. suber* soils (>15.5) while soils beneath *Q. canariensis* in the Woodland had the lowest carbon to nitrogen ratio (12.9). Total P in the soil ranged from 119 to 484 μg g^{-1} and average values were higher in the Woodland (312 μg g^{-1}) than in the Forest (259 μg g^{-1}). In the Woodland total P varied significantly ($F = 24.941$, $p < 0.0001$) among canopy cover types, with maximum average values (for the two soil depths) beneath *Q. canariensis* (368 μg g^{-1}) and minimum in the grasslands (282 μg g^{-1}).

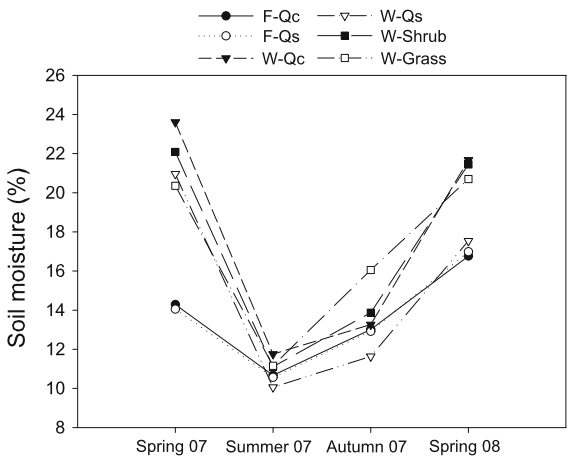

Fig. 2 Soil water content estimated gravimetrically at the two forest sites (*F* Forest, *W* Woodland) and the four studied vegetation cover types (*Qc Q. canariensis*, *Qs Q. suber*, *S* shrub, *G* grass) at each sampling season

Microbial C, N and P pools

Microbial C pool (Cm) averaged over all samples 820 μg g^{-1} and ranged from 121 to 3232 μg g^{-1} (Table 2). The proportion of microbial C to total soil C averaged 2.2%. Microbial N (Nm) averaged 115 μg g^{-1} and contributed 4.0% (range of 0.7–9.9%) to the total soil nitrogen while inorganic N (NH_4^+ and NO_3^-) contribution averaged 0.3%. Microbial P (Pm) in soils was, in average, 19 μg g^{-1} and, in the surface soil, it comprised about 9% of the total soil P. The proportion of inorganic available P to the total soil varied from 0.04 to 3.5% (mean of 0.89%). Average microbial nutrient ratios recorded were Cm/Nm: 8.6, Cm/Pm: 78.1 and Nm/Pm: 9.3. Microbial C, N and P values were positively and

Table 2 Microbial P, C and N (mean and standard error) and their ratios

	Pmic ($\mu g\ g^{-1}$)	Cmic ($\mu g\ g^{-1}$)	Nmic ($\mu g\ g^{-1}$)	Cm/Nm	Cm/Pm	Nm/Pm	Cm/Ct (%)	Nm/Nt (%)	Pm/Pt (%)
Woodland									
Q. canariensis	25.67 (2.03)	1119.62 (70.32)	147.68 (10.85)	9.60	62.29	6.88	2.93	4.13	6.8
Q. suber	22.46 (2.03)	825.87 (48.72)	108.31 (8.32)	10.17	63.48	6.58	2.29	4.18	7.9
Shrub	21.32 (1.86)	966.27 (53.81)	138.58 (10.21)	8.97	79.61	8.97	2.50	4.11	7.0
Grass	12.18 (0.88)	772.75 (38.87)	107.86 (6.11)	7.57	93.80	12.19	2.11	4.14	4.4
Forest									
Q. canariensis	16.88 (1.38)	596.81 (32.39)	95.91 (5.55)	6.98	77.43	10.37	1.71	3.70	6.2
Q. suber	16.26 (1.43)	638.29 (37.64)	89.44 (4.99)	8.03	92.26	11.06	1.67	3.94	6.3
Woodland	20.39 (0.92)	921.13 (27.98)	125.61 (4.62)	9.08	74.81	8.66	2.46	4.14	6.5
Forest	16.57 (0.99)	617.55 (24.80)	92.68 (3.73)	7.51	84.85	10.71	1.69	3.82	6.2
All average	19.14 (0.70)	819.93 (21.41)	114.63 (3.39)	8.56	78.12	9.34	2.20	4.03	6.4

Seasonal and soil depth values are averaged for forest sites (Woodland and Forest), and canopy cover type (under *Q. canariensis*, *Q. suber*, shrub and grass)

significantly correlated with soil moisture ($r > 0.46$, $p < 0.0001$) and SOM ($r > 0.62$, $p < 0.0001$).

Microbial response to seasonal conditions

Soil microbial biomass did not significantly differ among seasons (Table 3, Fig. 3). Microbial carbon pool was significantly higher in the Woodland, where it ranged from 253 to 3232 $\mu g\ g^{-1}$ and contributed 2.15% to total soil C, than in the Forest where it varied from 1201 to 1772 $\mu g\ g^{-1}$ and amounted 1.69% of total soil C ($p < 0.0001$). This difference was consistent across all seasons.

Microbial N and P pools varied seasonally with maximum values in spring and minimum values in summer (although the seasonal patterns differed between forest sites). The most important seasonal variations were observed for microbial P which values changed twofold from the spring ($\sim$24 $\mu g\ g^{-1}$) to the summer (10 $\mu g\ g^{-1}$). On average, soils in the Woodland had higher microbial N (126 $\mu g\ g^{-1}$) and microbial P (20 $\mu g\ g^{-1}$) than soils in the Forest (N: 93 $\mu g\ g^{-1}$, P: 17 $\mu g\ g^{-1}$) although this pattern was reversed in the autumn when Pm pool was larger in the Forest. Summer Nm and Pm values were similar for both forest sites while the largest differences between sites were recorded during the spring ($p < 0.003$), when the Woodland presented the highest values.

Microbial ratios (Cm/Nm; Cm/Pm; Nm/Pm) changed seasonally ($p < 0.0001$) and generally differed between the two forest sites (except for the summer), due to their distinct values of Nm and Pm (Figs. 3 and 4).

Season also affected the non microbial pools of available nutrients (Fig. 5). K_2SO_4-extractable dissolved organic carbon values increased in summer matching up with a slight but not significant decrease in Cm, and declined in spring when the microbial C values were higher. K_2SO_4-extractable dissolved organic nitrogen, inorganic N (ammonia and nitrate) and available inorganic P declined in summer and autumn and increased in spring, mirroring the pattern observed for Nm and Pm ($r \approx 0.35$, $p < 0.0001$).

Effect of vegetation cover type on soil microbial biomass

In the Forest site there were no significant differences in microbial C, N, P pools and their ratios between the soils of the two *Quercus* species at any sampling time and soil depth (Figs. 3 and 4).

In the Woodland the effect of the vegetation cover type varied across seasons: significant differences among cover types were observed in the spring while similar microbial values were recorded for all vegetation types in summer. The effect of cover type on soil microbial pools was larger in the

Table 3 Repeated measures ANOVA for microbial C, N and P of surface soil (0–8 cm) and subsurface soil (8–16 cm) from the two studied forest sites measured across four seasons

	Cm						Nm						Pm					
	F	*p*	Sp07	Su07	A07	Sp08	*F*	*p*	Sp07	Su07	A07	Sp08	*F*	*p*	Sp07	Su07	A07	Sp08
Surface soil																		
Forest site	40.45	**0.000**	**	**	***	***	15.55	**0.000**	**	**		***	18.127	**0.000**	***		*	***
Season	0.99	0.399					36.82	**0.000**					32.455	**0.000**				
Season × Forest site	6.56	**0.000**					10.74	**0.000**					24.233	**0.000**				
Vegetation cover	4.21	**0.042**	**				10.74	**0.001**	**			**	0.552	0.459				
Forest site × Vegetation cover	13.74	**0.000**	**			***	3.79	0.053	*				0.503	0.479				
Subsurface soil																		
Forest site	17.25	**0.000**	*		*	**	1.000	0.324	***		***	**	11.500	**0.002**	***		***	
Season	2.05	0.110					34.594	**0.000**					22.780	**0.000**				
Season × Forest site	1.88	0.137					28.598	**0.000**					22.646	**0.000**				
Vegetation cover	3.99	**0.048**					3.158	0.078	*				3.051	0.083				
Forest site × Vegetation cover	2.77	0.098	*				1.434	0.233					0.149	0.700				

F and *p*-values for between effects (forest site and canopy cover type), within effect (season) and two way interactions are presented

Significant univariate results for each sampling season are indicated as *($0.05 > p \geq 0.01$), **($0.01 > p \geq 0.001$), ***($p < 0.001$)

Forest site: Woodland and Forest

Vegetation cover type: *Q. canariensis* and *Q. suber*

Season: spring 07, summer 07, autumn 07, spring 08

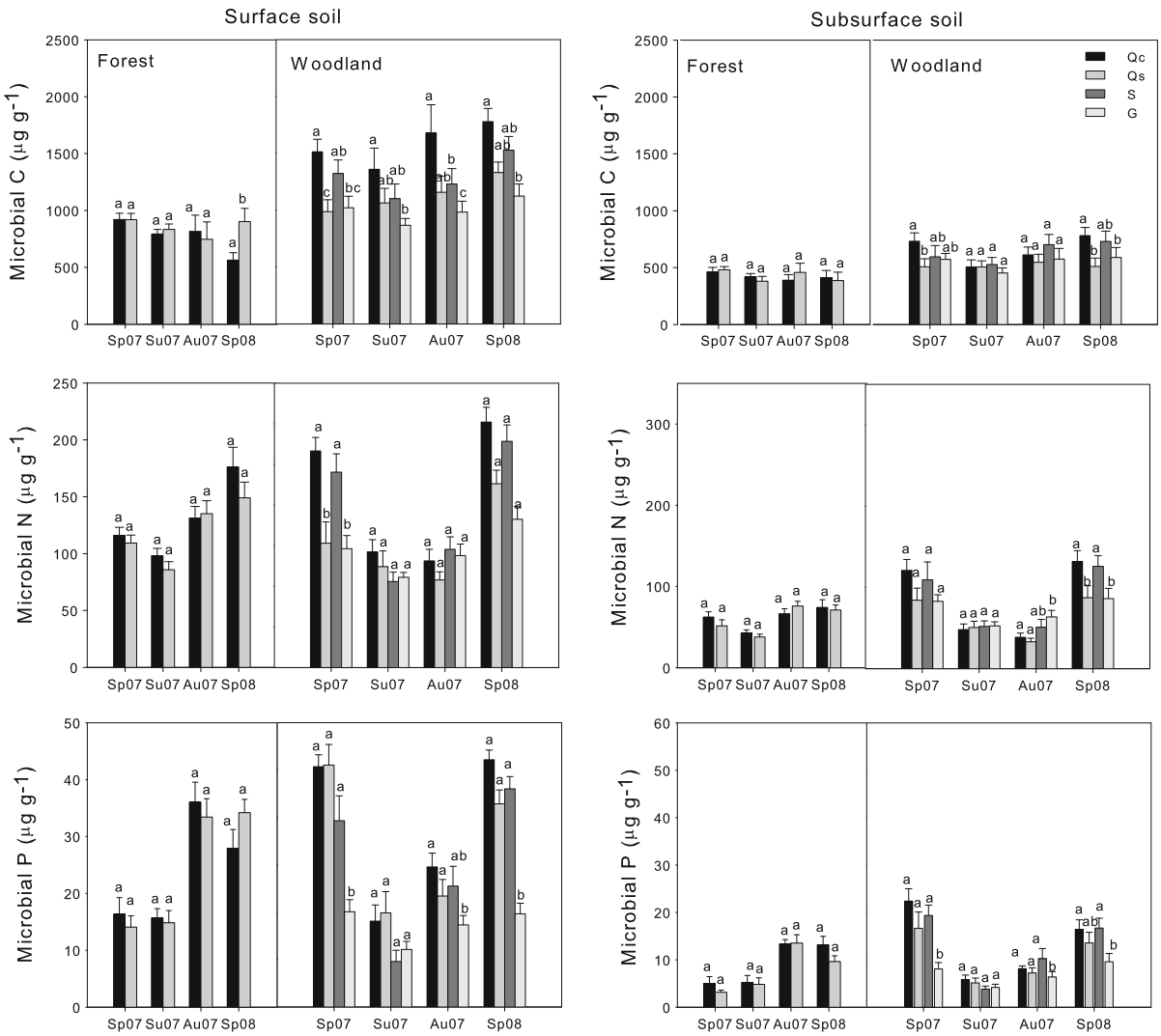

Fig. 3 Microbial C, N and P estimated in the soils under the different types of vegetation cover (*Q. canariensis* (*Qc*), *Q. suber* (*Qs*), shrub (*S*) and grass cover (*G*)) in the two studied forest sites (Forest and Woodland) across the four sampling seasons (Spring 07, Summer 07, Autumn 07, Spring 08). Data is presented for the two soil depths, surface soil (0–8 cm) and subsurface soil (8–16 cm). *Letters* indicate differences between groups for each season ($p < 0.05$ after FDR corrections). *Bars* represent standard error of the mean

upper soil layer ($p < 0.002$ for Cm, Nm and Pm) than in the subsurface soil ($p < 0.029$ for Pm). Higher values of microbial C were recorded in *Q. canariensis* and shrub soils compared to those estimated in soils beneath *Q. suber*. Soils beneath grass cover in forest gaps constantly showed the lowest Cm values and were not affected by seasonality. As occurred with Cm, soils of *Q. canariensis* and shrubs had higher microbial N than soils under *Q. suber* and herbaceous cover. In contrast microbial P was similarly high beneath the shrubs and the two oak trees while minimum values were consistently recorded in the soils under grass cover. The largest changes in Cm/Nm among vegetation cover types were observed in autumn when the ratio decreased from the two *Quercus* species to shrub soils and grass soils. Cm/Pm and Nm/Pm values tended to vary seasonally and the highest values were recorded in summer related to the limited amount of microbial P. Soils beneath grass cover, in contrast to other

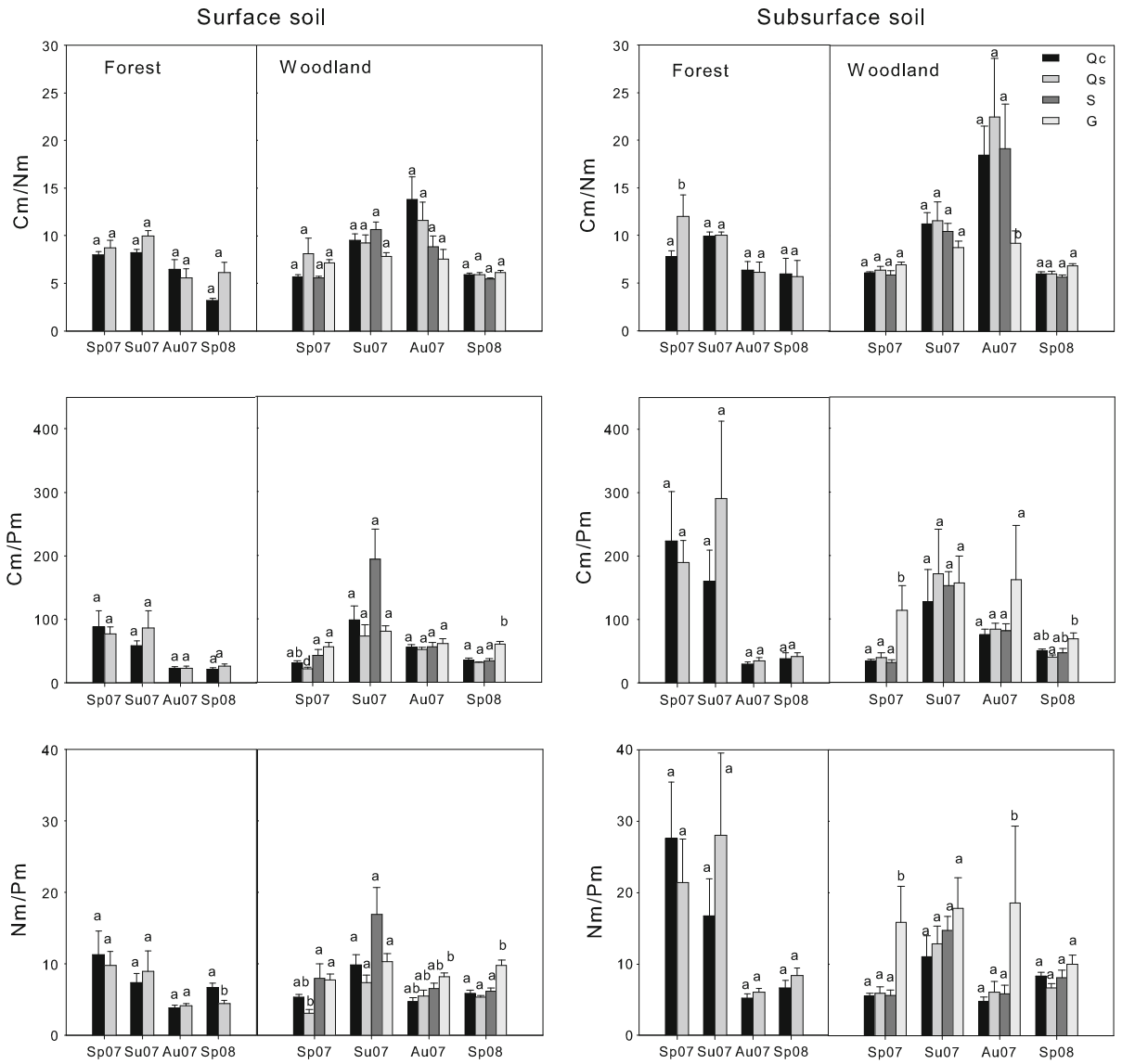

Fig. 4 Microbial ratios—Cm/Nm, Cm/Pm and Nm/Pm—for each season (Spring 07, Summer 07, Autumn 07, Spring 08), and vegetation cover type (*Q. canariensis* (*Qc*), *Q. suber* (*Qs*), shrub (*S*) and grass cover (*G*)) in the two forest sites (Forest and Woodland). Data is presented for the two soil depths (0–8 cm and 8–16 cm). *Letters* indicate differences between groups for each season ($p < 0.05$ after FDR corrections). *Bars* represent standard error of the mean

cover types, showed no similar Cm/Pm and Nm/Pm values across seasons.

Microbial properties and soil depth

All microbial C, N and P decreased twofold with soil depth ($p < 0.0001$); in particular carbon from 1106 μg g^{-1} in the upper soil to 534 μg g^{-1} in the subsurface soil, nitrogen from 158 to 71 μg g^{-1}, and phosphorus from 28 to 10 μg g^{-1}. Cm/Nm was higher in the subsurface soil, although this difference was only significant in autumn, when values increased from 8.9 to 13.6. Cm/Pm and Nm/Pm ratios were always significantly higher in the subsurface soil (104.2 and 10.3) than in the upper soil (64.0 and 5.2 respectively) ($p < 0.0001$).

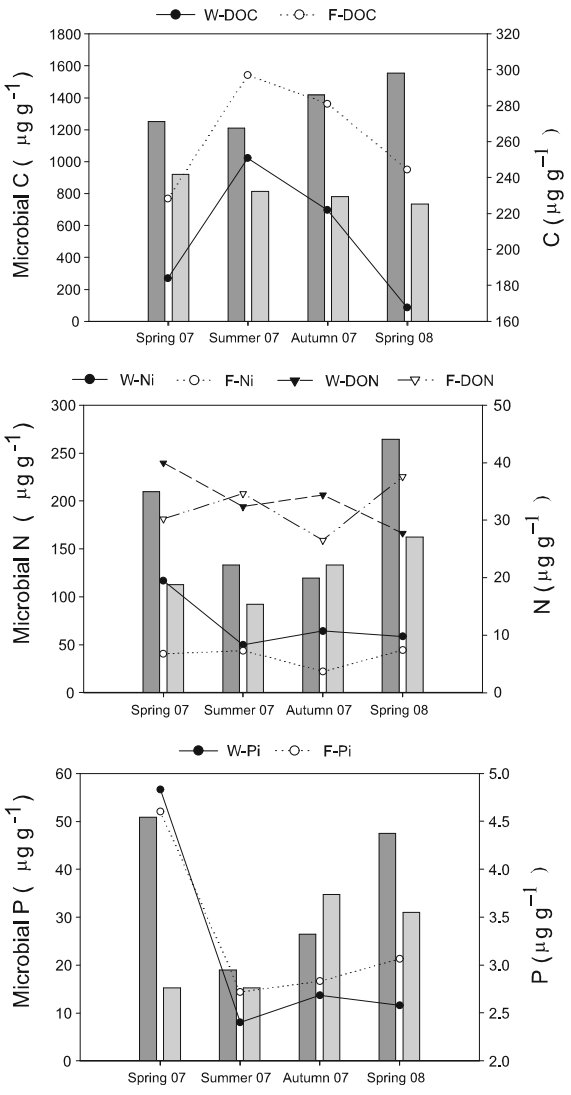

Fig. 5 Microbial C, N and P, K_2SO_4-extractable dissolved organic carbon (DOC) and nitrogen (DON), inorganic nitrogen (extractable ammonium and nitrate; Ni) and available inorganic phosphorus (Pi) estimated in the surface soil (0–8 cm) beneath *Quercus* cover in the Woodland and the Forest site across the sampling seasons

Discussion

Soil microbial carbon, nitrogen and phosphorus in Mediterranean forests

Microbial C and N averaged 820 and 115 µg g^{-1} in the studied Mediterranean forest soils. These values fell within the range of Cm and Nm estimates presented by Wardle (1992) for tropical (Cm: 653–986 µg g^{-1}; Nm: 65–100 µg g^{-1}) and temperate forests (Cm: 736–877 µg g^{-1}; Nm: 93 µg g^{-1}). They were also in accordance with the few examples of Mediterranean forests: Cm range of 975–1601 µg g^{-1} in a pine forest (Hernández et al. 1997) and Nm of 72–178 µg g^{-1} in an oak savanna (Gallardo et al. 2000).

Recently Cleveland and Liptzin (2007) have revealed the existence of a C:N:P Redfield-like ratio for the soil microbial biomass at the global scale (60:7:1) and for forest soils (74:9:1) that is very close to the average microbial ratio that we have found (78:9:1). Microbial stoichiometry relations are partly dependent on the validity of the microbial C, N and P estimates which, in fumigation procedures, are dependent on the *k*-factors used (Ross et al. 1996). Our results confirm that microbial biomass stoichiometry is well constrained and considering that the use of laboratory-determined fixed factors may not be accurate for a diverse soil community and could mask differences between soil types and depths, microbial N:P could be cautiously used, in addition to N:P ratios in plants and soils, to estimate nutrient deficiency in terrestrial ecosystems (Cleveland and Liptzin 2007).

The microbial P recorded in the studied soils (19 µg g^{-1}) was close to the lowest Pm values recorded by Joergensen et al. (1995) in 38 beech forest soils (18–174 µg g^{-1}). However the proportion of total P immobilised by microorganisms in the surface soil (8.8%) was higher that that of C (2.7%) and N (4.7%) what suggests that microbial biomass may play an important role in regulating plant phosphorous supply in the studied forests (Joergensen et al. 1995; Jonasson et al. 1996). The microbial fraction of the total N and P pool was almost 10 times higher than the estimated available inorganic N (NH_4^+ and NO_3^-; 0.38% of the total N) and available P (0.89% of total P). This could implicate that nutrient availability for plants might be strongly controlled by microbial dynamics: a flush of available N and P for plants could be released after microbial population decline while microbial population growth might be associated with a strong competition for these growth-limiting resources (Lipson et al. 1999; Schmidt et al. 2007).

Seasonal variations of microbial C, N and P

Microbial C, N and P in the studied soils showed distinct seasonal patterns that were reflected in

changes in the microbial component ratios. Seasonal variations of soil microbial carbon had been registered in many ecosystems (Bohlen et al. 2001; Díaz-Raviña et al. 1993; Miller et al. 2009) including Mediterranean and semi-arid zones (Goberna et al. 2007; Mlambo et al. 2007). Seasonality seems to affect microbial populations through changes in soil moisture and temperature, but it may also indirectly modulate substrate availability through plant phenology (Rinnan et al. 2008).

In this Mediterranean-type climate we expected a decline in microbial carbon during the summer as a result of drought stress. However, microbial C showed no significant differences across seasons, although values tended to be lower during the summer time and they were correlated with soil moisture content. Several processes can occur during summer drought that would explain these results. Under low moisture conditions, soil microorganisms may become isolated in a landscape of disconnected water pockets that impedes the diffusion of substrate, limit microbial growth and may cause death by starvation (Killham et al. 1993; Xiang et al. 2008). On the other hand this disconnection also prevents microorganism to be predated by soil fauna (protozoa, amoeba) which activity and mobility are reduced by soil drought (Kuikman et al. 1989).

Drought stress also affects microbial physiology. Low water potential induces microbial dehydration and might eventually cause death. Drought-tolerant microbes can be inherently protected against low moisture (e.g. thicker cell walls of gram-positive bacteria) or have the capacity to adapt to the external low water availability by accumulating osmolites in their cytoplasm) (Schimel et al. 2007). As a result of drought stress up to 30% of carbon resources can be bound in cytoplasmic osmotic protection molecules, what negatively affects microbial activity and population growth (Schimel et al. 1989). In this study microbial C was estimated using the fumigation-extraction technique that recovers only a fraction of the total microbial C, most of which is cytoplasmic, and relates it to the total by an empirical constant (K_C). Changes in the concentration of cytoplasmic C may therefore be a source of error in microbial biomass measurements (Ross et al. 1996; Schimel et al. 1989). We suggest that in our studied soils a fraction of the total microbial population might have died during summer drought, what together with summer root decay (Joergensen et al. 1994) could account for the K_2SO_4-extractable DOC peak recorded in this season. The estimated Cm values may instead reflect the increased cytoplasmic concentrations resulting from the physiological survival strategy of the resistant microbial fraction.

In contrast to Cm, a large decrease of microbial N and P was recorded during summer that supported our hypothesis. Cytoplasmic osmolytes used by bacteria to withstand drought stress are amino compounds (Csonka 1989), while fungi use polyols that do not contain N nor P (Witteveen and Visser 1995). We can speculate whether the observed decrease in Nm and the change in the microbial C/N ratio could indicate that during the summer drought microbial community composition shifted to a higher abundance of fungi (Schimel et al. 2007).

Increased soil microbial N and P were observed during the wet seasons (spring and autumn). In a similar seasonal study, Nielsen et al. (2009) observed that the higher water content increased the accessibility of nutrients and enhanced microbial growth, and consequently N and P immobilization in the microbial biomass. In addition root exudates, root decay and fresh litter shed by the winter-deciduous *Q. canariensis* could constitute an important input of easily decomposable organic substrate for soil microorganism growth (Gallardo and Merino 1993; Joergensen et al. 1994). Both mechanisms could account for the increased microbial nutrient immobilization.

There were also seasonal changes in the concentration of available inorganic nutrients that could be related to variations in temperature, moisture and quality of organic matter, which control microbial processes such as mineralization and immobilisation (Gallardo and Merino 1992; Schmidt et al. 1999). Despite the differences in soil water availability in the two forest sites due to distinct soil texture and water holding capacity, common patterns were found for inorganic nutrients dynamics: High levels of available N and P, probably resulting from net mineralization and liberation, were recorded during Spring 2007 when conditions were favourable for microbial growth and activity (Fig. 2). During summer Nm and Pm decreased, but there is no evidence of increasing available nutrients thus it is possible that inorganic nutrients resulted from microorganisms

decay may have been taken up by plants. In Autumn and Spring 2008, N and P were immobilized in the growing microbial biomass. From the synchrony between the available nutrients temporal patterns and microbial biomass dynamics we could infer that in these forests microbes might not be competing with plants for soil resources, but instead could be covering plants nutrient demand. However this hypothesis remains to be critically tested.

Microbial biomass is related to forest site conditions

In a previous study carried out in the same forest sites Quilchano and Marañón (2002) observed lower enzymatic (dehydrogenase) activity in the Forest site. In accordance, our results indicated that microbial C was higher in the Woodland, particularly in soils of *Q. canariensis*, than in the Forest. This difference was consistent across seasons suggesting that the driving mechanism of this variation was not affected by seasonal changes in the environmental conditions. Both sites differ in several physiographical aspects that could contribute to their distinct soil microbial properties; however we suggest that the higher clay content in the Woodland soils may be among the most important factors accounting for this difference since clay has a higher capacity to adsorb nutrients and organic matter. It also reduces decomposition rates, buffers pH changes, provides microorganisms shelter against microbivores and increases the soil water holding capacity, all of which promote microbial biomass growth (Oades 1988; Van Veen and Kuikman 1990; Wardle 1992).

Season determines canopy cover type effect on microbial biomass

Canopy cover type accounted for main differences in soil microbial parameters within the oak forests; although those patterns were only apparent under favourable environmental conditions, once the main constraining factor (water stress) disappeared. The influence of seasonal conditions on the effect of vegetation on microbial C, N and P has been also detected in other studies (Goberna et al. 2007; Malchair and Carnol 2009). In addition, the effect of canopy cover type was mostly observed in the upper soil layer, while conditions were more homogeneous in the subsurface soil suggesting a plant cover effect on soil probably through differences in litter fall quantity and quality (Augusto et al. 2002). Billore et al. (1995) found a strong positive correlation between microbial and root biomasses, thus vegetation could have also controlled soil microbial processes through differences in the root systems. Nevertheless, not all the variability observed among microhabitats should be attributed the canopy cover type but to other belowground characteristics (e.g. soil depth) that could in turn be the underlying reason for the distinct cover type.

Soils underneath *Q. canariensis* and shrub sustained a higher microbial C and microbial N than soils beneath *Q. suber*. Differences in the litter quality among the vegetation types (lower N content and higher C/N ratio in *Q. suber*; Aponte et al. unpublished data) would have induced a distinct SOM content, total soil nitrogen and C/N ratio and subsequently affected soil microbial C and N (Kara et al. 2008; Rinnan et al. 2008; Smolander and Kitunen 2002). Soils in the forest gaps had the lowest microbial values compared to the other studied microsites. These soils had a higher proportion of nitrogen (Cm/Nm: 7.6) but a lower fraction of phosphorus (Cm/Pm: 93.8), which could be interpreted as a P limitation (Cleveland and Liptzin 2007). The lack of canopy cover in the forest gaps reduced litter input (0.09 kg m^{-2}). The small inputs of P could constrain microbial growth and activity (García et al. 2002; Goberna et al. 2007) and may also increase exposure of this soil to rapid shifts in soil temperature and moisture. In contrast to the Woodland, no differences were found in the microbial components between the soils beneath the two *Quercus* species in the Forest. The vegetation structure of this site, where oak trees had a higher density (Table 1) and formed a closed canopy, promoted higher homogeneity of the litter layer that was reflected in the soil chemical and microbiological properties (Quilchano et al. 2008).

Microbial properties and soil depth

In the studied forest sites all measured microbial constituents, as well as most of the studied soil variables which may influence the microbial pool

(soil moisture, SOM, total N, C and P), where higher in the surface soil than in the subsurface soil. A similar pattern has been reported by other authors who also detected a decline in microbial activity with soil depth (Ross et al. 1996). Two main mechanisms could be driving this pattern: first a decrease in the quality and quantity of substrate; the subsurface soil contained less organic matter and probably had a higher fraction of recalcitrant compounds resulted from an advanced decomposition (Gaudinski et al. 2000). Second, the lower moisture content of the subsurface soil could impede the diffusion of the scarce substrate thought the disconnected water pockets and limit its supply to the isolated microbial populations (Xiang et al. 2008). Both mechanisms may act simultaneously inducing microbial starvation and limiting microbial population growth in the subsurface soil (Fontaine et al. 2007).

Microbial ratios increased with soil depth in our soils. Contradictory patterns of change in Cm/Nm have been found in other studies. For example Ross et al. (1996) observed that Cm/Nm declined with depth in forest soils, whereas Raubuch and Joergensen (2002) detected only a small difference in the microbial C-to-N ratios in the organic layer (6.0) and in mineral soil (7.1). Variations in the C/N ratio are commonly related to shifts in microbial community composition (bacteria versus fungi), since the fungi have higher carbon: element ratios (C:N = 5–17; N:P = 15) than the bacteria (C:N = 6.5; N:P = 7) (Cleveland and Liptzin 2007). In his work Fierer et al. (2003) observed that decreasing substrate and moisture availability along the soil depth profile induced changes in microbial community composition and lead to a community dominated by drought and starvation-tolerant organisms.

Conclusions

- In the studied Mediterranean forests soil microbial biomass was affected by season, vegetation cover type and structure, and soil depth.
- Seasonal changes in microbial nutrient content were observed for N and P, which had higher values during the wet seasons (spring and autumn), unlike microbial C.
- Differences in the soil microbial properties between forest sites or among canopy cover types were found in spring but not in summer.
- Microbial C, N and P significantly decreased from surface to subsurface soil, in every season and forest site.

The conjoint study of the effects of season, vegetation cover type and structure and soil depth on microbial biomass in two forest sites has shown the existence of relevant seasonal interactions between most of these factors.

The typical seasonal pattern of Mediterranean climate strongly determines the soil moisture regime, affects microbial growth and conditions the influence of other biotic factors on microbial biomass, playing an important role in the nutrient release-immobilization cycles and in the nutrient availability for plants in these forests.

Overall, this study provides valuable information on soil microbial seasonal dynamics in Mediterranean forests that may contribute to enhance our understanding on how climate change could affect to microbial control on nutrient availability.

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Appendix 1

See Table 4.

Table 4 Characteristics of the surface (0–8 cm) and subsurface (8–16 cm) soil beneath the studied vegetation cover types in the two forest sites

	Organic matter[1] (%)	Total C (%)	Total N (%)	Total P (ppm)	Ct/Nt	NH_4–N (ppm)	NO_3–N (ppm)	Available P (ppm)	Clay[2] (%)	Sand[2] (%)	Litter mass[3] (kg m^{-2})	Litter thickness[3] (cm)
Superficial soil												
Woodland												
Q. canariensis	14.93 (0.51) a	4.36 (0.19) a	0.43 (0.02) a	411.9 (9.5) a	10.67 (0.50) a	9.87 (1.24) a	2.56 (0.28) a	3.17 (0.31) ac	32.7 (2.1) a	46.4 (2.5) a	0.87 (0.07) ab	4.25 (0.26) ab
Q. suber	12.39 (0.37) bc	4.42 (0.18) a	0.33 (0.01) b	332.6 (8.5) b	13.84 (0.43) b	9.05 (0.76) a	2.66 (0.27) a	3.07 (0.27) ac	24.8 (2.4) ab	55.2 (4.1) ab	1 (0.15) b	2.95 (0.40) c
Shrub	14.15 (0.58) ab	4.37 (0.18) a	0.40 (0.02) a	322.1 (10.1) b	12.04 (0.66) ac	9.09 (0.85) a	2.95 (0.30) a	2.56 (0.20) ab	30.1 (3.3) a	46 (4.7) a	0.63 (0.04) a	5 (0.44) a
Grass	11.40 (0.50) c	4.12 (0.13) a	0.31 (0.01) bc	282.0 (9.3) c	14.11 (0.45) bd	5.7 (0.60) b	2.6 (0.28) a	2.31 (0.24) b	30.8 (3.5) a	47.4 (5.0) a	0.04 (0.01) c	0.5 (0.18) d
Forest												
Q. canariensis	12.51 (0.79) c	3.95 (0.20) a	0.32 (0.02) bc	297.8 (10.4) bc	13.44 (0.70) bc	4.79 (0.38) b	2.05 (0.13) a	3.68 (0.24) ac	13.1 (1.3) bc	67.8 (1.5) bc	1.69 (0.10) d	4.5 (0.27) ab
Q. suber	10.81 (0.44) c	4.28 (0.14) a	0.28 (0.01) c	299.1 (8.9) bc	16.02 (0.51) bd	4.26 (0.29) b	1.46 (0.14) b	2.92 (0.17) ac	19.2 (2.2) c	63.4 (2.5) c	1.69 (0.13) d	3.53 (0.33) bc
Subsuperficial soil												
Woodland												
Q. canariensis	9.17 (0.37) a	3.47 (0.13) a	0.24 (0.01) a	324.4 (11.4) a	15.09 (0.33) a	5.72 (0.73) a	2.02 (0.27) ab	2.03 (0.14) a	na	na	–	–
Q. suber	7.08 (0.35) b	2.87 (0.11) b	0.17 (0.01) b	228.2 (11.9) b	17.19 (0.38) a	5.8 (0.51) a	1.73 (0.21) ac	1.98 (0.14)a	na	na	–	–
Shrub	9.32 (0.44) a	3.57 (0.16) a	0.25 (0.01) a	317.3 (13.3) a	15.34 (0.52) a	6.62 (0.71) a	2.13 (0.22) a	1.46 (0.12) b	na	na	–	–
Grass	8.32 (0.29) a	3.13 (0.11) ab	0.21 (0.01) ac	281.1 (8.6) c	15.44 (0.48) a	6.01 (0.50) a	2.54 (0.28) a	1.82 (0.18) ab	na	na	–	–
Forest												
Q. canariensis	8.94 (0.66) a	3.24 (0.17) ab	0.22 (0.01) a	230.7 (8.7) b	15.63 (0.71) a	3.58 (0.40) b	1.35 (0.22) bd	2.64 (0.21) c	na	na	–	–
Q. suber	7.30 (0.29) b	3.05 (0.11) ab	0.19 (0.01) bc	209.4 (8.1) b	17.03 (0.42) a	3.41 (0.36) b	1.17 (0.14) cd	1.93 (0.13) a	na	na	–	–
All average	10.53 (0.18)	3.73 (0.05)	0.28 (0.01)	294.7 (3.7)	14.7 (0.2)	7.41 (0.36)	2.1 (0.07)	2.47 (0.06)	25.1 (1.4)	54.35 (1.8)	1.29 (0.08)	3.76 (0.18)

Data represent mean values and standard error. *Letters* indicate differences between groups $p < 0.05$ after FDR correction

[1] OM measured by loss on ignition

[2] Textural variables were determined only for 0–25 cm soil depth, na mean non-available data

[3] Litter variables were measured on the surface of each sample point

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