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Abstract

Background and aims

Intensive land use has led to degradation and abandonment of Portuguese oak woodlands, and subsequent shrub encroachment may have altered the spatial heterogeneity of soil C and N pools. The aim of this study was to evaluate the effects of shrub invasion on soil C and N dynamics in an oak woodland in Southern Portugal.

Methods

Soil was sampled beneath and outside scattered *Quercus suber* L. canopies, considering non encroached areas and areas encroached by shrubs (*Cistus ladanifer* L. or *Cistus salviifolius* L.).

Results

The spatial heterogeneity of soil C and N contents was mainly associated with tree presence. Outside tree canopies, the labile C pools were larger (mainly beneath *C. ladanifer*) and C cycling was faster in encroached areas than in non encroached areas. Net and gross N mineralization and urease and protease activities were also higher in encroached than in non encroached areas; however, the metabolic quotient and the Cmicrobial/Corganic ratio were not significantly affected. Beneath the tree canopy, significant effects of encroachment included a small increase in soil labile C and the enzymatic activity beneath *C. ladanifer*.

Conclusions

The results indicate the potential capacity of shrub encroachment to accumulate soil organic C in the long term. The rate of soil C and N turnover promoted by shrub encroachment may depend on the *Cistus* species present.

Keywords: ^{15}N isotope dilution technique; gross N mineralization; hot water soluble C; particulate organic matter; soil respiration

Introduction

In the Iberian Peninsula, evergreen oak woodlands occur in an agroforestry system (known as "montado" in Portugal and "dehesa" in Spain) characterised by the combination of open tree woodland and an understorey of grassland, cereal crops or Mediterranean shrubs (Eichhorn et al. 2006). In Portugal, oak woodlands cover about 1.3 million ha (IFN, 2006), most of which are located in dry sub-humid to semi-arid areas of the southern part of the country. These agroforestry systems have been managed for centuries (Eichhorn et al. 2006) with a combination of extensive livestock (sheep, black pig and cattle) and shifting cultivation of cereals. However, in some areas, intensification of cultivation and overgrazing during the last few decades has gradually reduced soil fertility and enhanced soil compaction and erosion, leading to soil degradation and land abandonment (Pinto Correia and Mascarenhas 1999). Intensive land use has also been associated with increased tree mortality and poor natural tree regeneration (Pulido et al. 2001). In view of this, the sustainability of this system has been questioned (Plieninger et al. 2003).

In abandoned areas, herbaceous species are often replaced by native shrubs dominated by *Cistus* species (Cistaceae), mainly *C. ladanifer* L. and *C. salviifolius* L. Although both species are abundant in areas subjected to repeated and intense disturbance,

particularly after fire, *C. ladanifer* is more xeric than *C. salviifolius* and usually occupies the most degraded habitats (Bastida and Talavera 2002). Shrub clearing and rotational ploughing have been carried out in recent years to control shrub invasion, reduce risk of fires and increase pasture yield (Cubera and Moreno 2007). However, shrub encroachment may have some advantages, as it reduces soil surface temperature and increases soil water content (Moro et al. 1997), and improves soil structure (Pinto Correia and Mascarenhas 1999) and nutrient content (Moreno and Obrador, 2007; Simões et al. 2009; Tárrega et al. 2009) beneath shrub canopies. Despite the negative effects of competition for water and light observed in encroached woodlands (Pérez-Devesa et al. 2008), such favourable conditions may facilitate the establishment of oak seedlings and promote natural tree regeneration (Pulido and Diaz 2005). Shrub development may also promote the invasion of more nutrient-demanding species, since local areas of high fertility are likely to be favoured for vegetation regeneration (Simões et al. 2009). Therefore, shrub encroachment may play a valuable ecological role in soil functions and the long-term ecosystem sustainability of Mediterranean oak woodlands.

Evaluation of spatial heterogeneity of soil C and N in oak woodlands has mainly been carried out at tree level, showing that scattered trees enhance soil fertility and improve soil physical conditions and biological activity (Gallardo et al. 2000; Howlett et al. 2011; Gómez-Rey et al. 2012b) beneath the canopies. However, soil spatial variability induced by a shrub layer is not well known and, to our knowledge, the effect of encroachment beneath oak canopies has not yet been evaluated. Although previous studies have shown that invasion by shrubs, including *Cistus* species, potentially contributes to soil organic C accumulation in these systems, the results do not clearly show changes in total organic C contents (Moreno and Obrador 2007; Simões et al. 2009; Tárrega et al. 2009), which may be associated with suppression of grasses and herbs under *Cistus* shrubs (Rivest et al. 2011). Therefore, short-term changes could be detected in labile fractions of soil organic matter (SOM), since these have been suggested to be sensitive indicators of soil management effects (Haynes 2005). In addition, examination of active labile fractions of SOM may provide a better understanding of nutrient cycling dynamics because these fractions are easily decomposed by microorganisms and are closely associated with microbial growth and activity (Rovira et al. 2010). Improved soil and water conditions and microclimate beneath the shrubs may create a favourable environment for biological activity and presumably faster nutrient cycling; however, few studies have evaluated the effect of shrub encroachment on biological soil properties (Castells and Peñuelas 2003; Maestre et al. 2011) and there is no information about *Cistus* growing in Mediterranean oak woodlands.

In this context, a study was carried out in an oak woodland (previously studied by Simões et al., 2008, 2009), where grazing has been excluded since 1991. It was hypothesized that shrub encroachment changes the spatial heterogeneity of soil C and N dynamics, and that the effect may depend on shrub species and oak tree presence. To test this hypothesis, soil C and N pools (total and labile) and C and N mineralization rates were evaluated in an unmanaged cork oak woodland invaded by two shrub species (*C. salviifolius* and *C. ladanifer*) both in the presence (below the canopy influence) and absence of trees, by determining the following : (i) changes in labile SOM and soil C and N dynamics promoted by shrub encroachment, and (ii) differences between the studied shrub species.

Material and Methods

Site description

The study was carried out at the "Centro de Estudos e Experimentação da Mitra", located close to Évora, Southern Portugal (38º32' N, 8º01' W, 243 m a.s.l.). The site has the typical wet winter and dry summer pattern of the Mediterranean-type climate. The mean annual air temperature is 15.4 ºC, ranging from 8.6 ºC in winter (January) to 23.1 ºC in summer (August). The mean annual rainfall is 665 mm, 90 % of which occurs from autumn to early spring (data collected between 1951–1980: Reis and Gonçalves 1987).

The site is characterised by gently undulating topography, and the predominant slope gradient is 3-8%. Soils are mostly Dystric Leptosols and Dystric Cambisols (IUSS Working Group 2006) developed over granites and gneisses. The soils have a sandy loamy texture, low organic-matter content (6.0-23.9 g kg^{-1} of organic C) and P (0.5-2.0 mg kg^{-1}) status, and they are acidic (pH about 5.7). The vegetation in the study site is a mature open woodland (10-45 trees ha^{-1}, cover canopy of 11-51%) of *Quercus suber* L. (cork oak) and *Q. rotundifolia* Lam. (holm oak) with an understorey of shrubs, dominated by *Cistus salviifolius* and *C. ladanifer*, which accounted for >70% of the community cover. The herbaceous understory included *Geranium molle* L., *Ornithopus*

compressus L., *Ornithopus pinnatus* (Miller) Druce, *Rumex bucephalophorus* L. and some species of the *Poaceae* family.

The site was fenced off in 1991 to prevent grazing (it had been used to graze goats) and protect the vegetation, and it remained undisturbed thereafter (Simões et al. 2009).

Soil sampling

The six most frequent types of soil cover in the study site were identified, and six samples of each type were obtained, in April 2009. Previous studies in neighbouring oak woodlands showed that differences in N dynamics between natural and improved pastures (beneath and beyond tree crown) were similar throughout the year, but that differences were greater during spring (Nunes 2004; Nunes et al. 2012). The study area (200 m x 160 m) was divided into a grid of 20 m x 20 m cells (totalling 80 cells) and six cells were selected at random. In each cell, two tall *C. ladanifer* shrubs (taller than one metre) were selected for study: one located beneath the canopy of a mature *Q. suber* tree (CL-T) and the other was located at least five meters from the crown limit of the mature *Q. suber* tree (CL-NT). Similarly, two tall *C. salviifolius* shrubs (taller than one metre) were selected (CS-T and CS-NT, respectively). A mature *Q. suber* tree without *Cistus* shrubs below the canopy (NE-T) and a location without shrub or tree influence were also selected for study (NE-NT). For the NE-NT selection, only locations at least five metres from the tree crown limit and two metres from the shrub crown limit were considered. If a particular type of soil cover was not found in the selected cells, successive cells were chosen at random until it appeared. Shrubs were chosen by comparison with those marked during a study carried out at the site in 2004/2005 (Simões 2002); selected shrubs were at least 15 years old. Four soil cores (65 mm diameter, 0-10 cm) -one for each main wind direction- were collected from beneath the canopy of each selected shrub or tree (50 % of radius crowns). These were combined to make a composite sample. For the NE-NT type, four soil cores were collected in each location and were bulked into a composite sample. Sampling was restricted to the upper 10 cm of the soil layer because previous studies in the same (and similar) sites showed that changes in the effects of plants on soil C and N concentrations mainly occur in this layer (Nunes 2004; Simões et al. 2009). The primary rooting depth of shrubs and grasses is also within this layer (Silva de Sá 2001; Simões et al. 2012).

Fresh sub-samples of soil were sieved (< 4.75 mm) and stored at 4 ^{o}C for less than three days until incubation procedures to determine C and N mineralization. A set of sub-samples was sieved (< 2 mm) for analysis of enzymatic activities and the other set was air-dried and sieved (< 2 mm) for determination of physical and chemical properties and for labile SOM fractionation.

Laboratory procedures

Chemical properties

Soil pH was determined potentiometrically in distilled water (soil:solution ratio 1:2.5). Organic C was determined by wet oxidation, and total N was determined by Kjeldhal digestion (Digestion System 40, Kjeltec Auto 1030 Analyzer). Extractable P was obtained by shaking 1 g of soil with 20 mL of $K(SbO)C_4H_4O_6.½H_2O$ solution at pH 3.7 for 2 h (Egnér et al., 1960). Exchangeable Ca, Mg, Na and K (extracted with 1 *M* NH_4OAc, adjusted at pH 7.0) were measured by atomic absorption spectroscopy (Aanalyst 300, Perkin Elmer); extractable acidity (Al and H extracted with 1 *M* KCl) was measured by the method described by Thomas (1982) and extractable P by colorimetry. The sum of bases (SB) was calculated as the sum of exchangeable Ca, Mg, Na and K, and the effective cation exchange capacity (eCEC) was calculated as the sum of extractable acidity (Al and H) and SB.

Labile soil organic matter fractions

The air-dried soil was dispersed by shaking with distilled water (10 g / 100 mL) and was then sieved (< 50 mm). Particulate organic C (C_{POM}) and N (N_{POM}) were determined in the > 50 mm fraction by wet oxidation and Kjeldahl digestion, respectively. Hot water soluble C (Chw) and N (Nhw) were extracted with distilled water (10 g air-dried soil: 50 mL) at 80 ^{o}C for 60 min and, after centrifugation, organic C and total N in extracts were determined in an autoanalyzer (Skalar FORMAC Combustion TOC/N), with a near infrared spectroscope and a chemiluminescence detector, respectively. Microbial C and N (Cmic and Nmic) were measured by the chloroform fumigation-extraction method (Vance et al., 1987). Organic C and total N were determined in an autoanalyzer, as previously described. Cmic and Nmic were calculated as the difference between fumigated and non-fumigated samples, and K_{EC} and K_{EN} factors of 2.64 and 1.85, respectively were applied.

Soil C and N mineralization

Carbon mineralization was measured during 20 weeks by incubating fresh soil equivalent to 50 g of dw at 25 ^{o}C in a 1.5 L sealed glass jar containing a

30 mL trap of 0.5 *M* NaOH. A container of water was also placed in the jars to minimize soil drying. The NaOH solution was replaced periodically and titrated with 0.5 *M* HCl to phenolphthalein endpoint, after addition of 1.5 *M* $BaCl_2$. The metabolic quotient (qCO_2) was calculated as the ratio of hourly basal respiration, after 3 days, to the corresponding initial Cmic [mg CO_2-C (g Cmic)$^{-1}$ h^{-1}].

Anaerobic net N mineralization was measured in sub-samples of 5 g soil to which 12.5 mL of distilled water were added. The samples were incubated for 14 days at 40 ºC in 50 mL bottles and 12.5 mL of 4 *M* KCl were added. The samples were then shaken for 1h and filtered, and the NH_4^+-N concentrations were determined, as below. Mineralizable N was calculated as the difference between the amount of NH_4^+-N before and after incubation.

Aerobic net N mineralization was evaluated fortnightly during 20 weeks. Approximately 0.5 kg (fresh weigh, < 4.75 mm) of each composite soil sample was incubated in polyethylene bags, at 25 ºC, 60% of the water holding capacity (WHC) in darkness. The NO_3^--N and NH_4^+-N before and after incubation periods were extracted by shaking 10 g of soil for 1 h with 2 *M* KCl (1:5 soil:solution ratio). Extracts were filtered, and the NO_3^--N and NH_4^+-N contents were determined, in a segmented flow autoanalyzer, by the a-naphthylamine/ sulphanilamide method (after reduction with Cd) and the modified Berthelot method, respectively. Net N mineralization was calculated as the difference between the levels of NO_3^--N and NH_4^+-N at each incubation time and their respective initial levels.

Gross N transformation rates were determined by the ^{15}N isotope dilution technique. Analyses were carried out for four of the soil cover types (NE-NT, CL-NT, CS-NT, NE-T), on four of the six soil composite samples. Two replicates of each sample (fresh soil equivalent to 10 g dw) were placed in 250-mL flasks (128 flasks: 4 cover types x 4 plots x 2 labels x 2 times x 2 replicates). Each flask was labelled with 0.8 mL of either $(^{15}NH_4)_2SO_4$ or $^{15}NO_3K$ for gross mineralization and nitrification measurement, respectively. The ^{15}N-labelled solution (98 atom % excess of ^{15}N), equivalent to an addition of 5 mg N kg^{-1} dw, was added uniformly to the soil surface with an automatic pipette. One hour after addition of the ^{15}N, half of the soil samples were extracted with 50 mL of 2 *M* KCl and filtered. The other samples were incubated for 48 h (in darkness at 25 ˚C and 60 % of WHC) and extracted as above. The inorganic N content was analyzed by a modified diffusion method (Gómez-Rey et al. 2012a). Before the diffusion, 10 mg of unlabelled NH_4NO_3 was added to each glass jar to ensure that ^{15}N labelling was within the range of certified isotopic standards of the IAEA. After titration, the resulting $(NH_4)_2SO_4$ solutions were acidified with 1 mL 0.004 *M* H_2SO_4 and dried at 60 ºC in a vacuum oven (Memmert VO400, PM400). The $(NH_4)_2SO_4$ salts were analyzed for ^{15}N in an elemental analyser (Carlo Erba CNS 1508, Milano, Italy) coupled online to an isotopic ratio mass spectrometer (Finnigan Mat, delta C). The mean values of two replicates were used to calculate gross mineralization, nitrification and immobilization rates with the equations of Kirkham and Bartholomew (1954). To enable comparison of soils with different C and N contents, the C and N mineralization rates were expressed per unit of initial organic C (mg C–CO_2 g^{-1} C) and total N (mg N g^{-1} N).

Enzymatic activities

Enzymatic activities were determined in duplicate field-moist soil samples. Urease activity (EC 3.5.1.5) was determined according to the method described by Kandeler et al. (1999). To determine protease (EC 3.4.4) activity, soil was incubated in 5.0 ml of TRIS buffer (pH 8.1, 0.05 *M*) and 5.0 mL of casein (2 % w/v) at 50 ˚C for 2.h. The released amino acids were determined by the Folin-Ciocalteu colorimetric method (Ladd and Butler, 1972). ß-glucosidase activity (EC 3.2.1.21) was measured according to the method of Tabatabai (1982). Acid phosphomonoesterase activity (EC 3.1.3.2) was determined by using 0.025 *M* *p*-nitrophenyl phosphate as substrate and incubating at pH 6.5 (MUB) and 37 ˚C. After 1h, 0.5 M $CaCl_2$ was added and the released *p*-nitrophenol was extracted with 0.5 *M* NaOH and determined as above.

Statistical analysis

Soil data were analysed by two-way ANOVA (with shrub encroachment and tree presence as factors), except for gross N mineralization, which was evaluated by one-way ANOVA. Levene's test was used to verify the equality of variances among groups. When homocedasticity was detected, significant differences between means were established (at $P < 0.05$) using the Bonferroni's test for multiple comparisons (ANOVA-2) or the Tukey's test (ANOVA-1). In the case of unequal variances, the original data were subjected to Cox-Box transformations to obtain equality of variances, and significant differences between means were

then established (at $P < 0.05$) using the Bonferroni's test (ANOVA-2) or the Tukey's test (ANOVA-1). The relative importance of each factor or interaction in two-way ANOVA was determined by the partial eta-squared (η_p^2) statistic, which measures the proportion of the variation accounted for each factor or interaction. Relationships between soil chemical properties were explored by Pearson correlations. Statistical procedures were performed using SPSS 15.0 for Windows.

Results

Soil chemical properties

Statistical analysis revealed that 25 % of the variation in soil organic C contents was explained by shrub encroachment (η_p^2= 0.249), while no significant encroachment effect was observed for soil total N contents (Table 1). Organic C and total N contents were greatly affected by tree presence (80 and 73 % of variance explained, respectively), with higher contents in soils beneath the tree canopy, either in the absence or presence of encroachment (no significant E x T interaction). Both shrub encroachment and tree presence affected the soil C/N ratio, but the significant E x T interaction showed that the encroachment effect was only observed in soils outside the tree canopies. Although the NO_3^--N contents were affected by both shrub encroachment and trees (Table 1), the E x T interaction showed that the increased contents associated with tree presence were only observed in soils not encroached by shrubs.

No significant differences due to the studied factors were found for NH_4^+-N contents or soil pH_{H2O} (Table 1). The values of SB and eCEC were strongly affected by tree presence (51 and 56 % of variance explained, respectively), but not by shrub encroachment, and the values were higher under tree canopy, irrespective of shrub presence (no significant E x T interaction). Encroachment by *C. ladanifer* led to a significant increase in extractable P in soils samples from beneath tree canopies.

Labile soil C and N pools

In pre-incubated samples, particulate organic matter accounted for a large proportion of soil organic C (28-45 %) and total N (23-34 %), while the percentages of organic C and total N in the hot water soluble fraction (2.0-9.5 %) were much lower (Table 2). Despite large differences, the POM and hot water soluble fractions were positively correlated (r= 0.93, r= 0.61 for C and N fractions, respectively, $P < 0.01$, n= 36). The organic C was strongly correlated with C_{POM} and Chw (r= 0.96 and r= 0.92, respectively, $P < 0.01$, n= 36) and total N with N_{POM} and Nhw (r= 0.65 and r= 0.94, respectively, $P < 0.01$, n= 36).

Shrub encroachment induced a significant increase in soil C_{POM} and Chw contents, but the effect (33 and 39 % of variance explained, respectively) was lower than that produced by tree presence (79 and 77 % of variance explained) (Table 2). A similar trend was observed for the proportions of C_{POM} and Chw relative to organic C. For C_{POM} contents, the significant E x T interaction indicated that the encroachment effect was only significant for *C. ladanifer* under tree canopy. For Chw contents, the effect of *C. ladanifer* encroachment was independent of tree presence, while the increase associated with *C. salviifolius* encroachment was only observed in areas not influenced by tree canopies. Tree presence, but not shrub encroachment, influenced N_{POM} and Nhw contents, with higher values beneath trees than in the open areas. The Nhw/Nt ratio was affected by encroachment and tree presence, but the significant E x T interaction indicated that the increment due to shrub encroachment was only observed in the absence of trees.

After soil incubation, C_{POM} and Chw contents decreased by 20-29% and 19-60%, respectively. The difference in Chw was positively correlated with the cumulative amount of CO_2-C respired during incubation (r= 0.71, $P < 0.01$; n= 36, Fig. 1), while the correlation with C_{POM} was much weaker (r= 0.35, $P < 0.05$; n= 36).

Soil C mineralization

At the end of the incubation period, the cumulative C mineralized was similarly affected by shrub encroachment and tree presence (48 and 53 % of the variance explained, respectively, $P < 0.001$); the increase associated with shrub encroachment was only observed in open areas (Fig. 2). A similar trend was observed for C mineralization rates expressed per unit of initial total C (Table 3). The metabolic quotient (qCO_2) was not significantly affected by either shrub or tree presence (Table 3).

Microbial C and N

Shrub encroachment and tree presence led to increases in soil microbial C contents; the significant E x T interaction indicated that the effect only applied to soil encroached with *C. salviifolius* outside tree canopies (Table 3). Microbial N contents were not affected by encroachment, but were significantly increased by the presence of

Table 1 Mean values ± standard error (SE, n=6) of the main chemical characteristics of the studied soils (0-10 cm) and results of the two-way ANOVA

	Org C	Total N	C/N	pH	NH_4^+-N	NO_3^--N	SB	eCEC	Extr.P
	g kg^{-1}	g kg^{-1}	mass ratio	H_2O	mg kg^{-1}	mg kg^{-1}	$cmol_c$ kg^{-1}	$cmol_c$ kg^{-1}	mg kg^{-1}
Encroachment									
NE	12.1±1.4^a	0.95±0.08^a	12.2±0.5^a	5.73±0.17^a	2.12±2.10^a	3.34±0.56^b	3.15±0.36^a	3.22±0.34^a	0.90±0.11^a
CL	14.9±1.5^b	1.05±0.11^a	14.2±0.5^b	5.63±0.26^a	1.79±1.29^a	1.87±0.39^a	3.36±0.33^a	3.44±0.31^a	1.34±0.16^b
CS	13.7±1.0^{ab}	1.00±0.08^a	13.7±0.4^b	5.74±0.20^a	2.49±1.22^a	2.04±0.23^a	3.30±0.22^a	3.33±0.21^a	1.05±0.10^{ab}
Tree									
NT	9.7±0.9^a	0.75±0.03^a	12.8±0.5^a	5.76±0.14^a	1.60±0.96^a	1.83±0.21^a	2.56±0.17^a	2.63±0.16^a	0.83±0.09^a
T	17.5±0.6^b	1.26±0.04^b	13.9±0.3^b	5.64±0.26^a	2.66±1.89^a	3.01±0.44^b	3.98±0.19^b	4.03±0.17^b	1.36±0.09^b
Encroachment x tree									
NE x NT	7.7±0.8^a	0.69±0.05^a	10.7±0.4^a	5.73±0.06a	1.64±0.55^a	1.96±0.40^a	2.10±0.27^a	2.21±0.24^a	0.73±1.30^a
CL x NT	10.3±1.0^a	0.75±0.08^a	14.0±0.9^b	5.69±0.06^a	1.05±0.22^a	1.75±0.54^a	2.67±0.37^{ab}	2.75±0.35^{ab}	0.97±0.20^a
CS x NT	10.9±0.6^a	0.80±0.04^a	13.6±0.2^b	5.84±0.04^a	2.12±0.23^a	1.79±0.09^a	2.90±0.16^{abc}	2.93±0.14^{ab}	0.78±0.09^a
NE x T	16.5±0.6^b	1.22±0.03^b	13.6±0.3^b	5.72±0.09^a	2.59±1.11^a	4.73±0.69^b	4.20±0.26^c	4.23±0.23^c	1.06±0.16^a
CL x T	19.4±1.0^b	1.35±0.09^b	14.5±0.6^b	5.56±0.14^a	2.52±0.58^a	1.99±0.61^a	4.05±0.40^c	4.13±0.32^c	1.71±0.09^b
CS x T	16.5±1.1^b	1.20±0.09^b	13.9±0.7^b	5.63±0.09^a	2.86±0.66^a	2.29±0.45^a	3.68±0.35^{bc}	3.73±0.32^{bc}	1.31±0.08^{ab}
η_p^2 Encroachment	0.249 *	0.066 ns	0.360 ***	0.060 ns	0.040 ns	0.255 *	0.015 ns	0.020 ns	0.268 **
η_p^2 Tree	0.799 ***	0.733 ***	0.219 **	0.084 ns	0.122 ns	0.213 *	0.505 ***	0.559 ***	0.433 ***
η_p^2 Encroach. x tree	0.147 ns	0.068 ns	0.228 *	0.043 ns	0.012 ns	0.202 *	0.127 ns	0.138 ns	0.068 ns

Different letters in the same column indicate significant differences ($P < 0.05$) within the same factor or interaction estimated by a two-way ANOVA and Bonferrony test, with shrub encroachment (NE: no encroachment, CL: encroachment of *C. ladanifer*, CS: encroachment of *C. salviifolius*) and tree (NT: no tree influence, T: under *Q. suber* canopy influence) as independent variables and soil parameters as dependent variables.

* significant at $P < 0.05$; ** significant at $P < 0.01$; *** significant at $P < 0.001$; ns, not significant.

SB: sum of bases; eCEC: effective cationic exchange capacity, η_p^2: partial eta-squared

Table 2 Pre-incubation contents of C and N (g kg^{-1}) in particulate organic matter (C_{POM} and N_{POM}) and hot water extracts (Chw and Nhw) and their percentage in relation to organic C and total N. Values are mean ± SE (n=6)

	C_{POM}	%C_{POM}/Corg	Chw	%Chw/Corg	N_{POM}	%N_{POM}/Nt	Nhw	%Nhw/Nt
Encroachment								
NE	4.38±0.71^{a}	33.6±0.2^{a}	0.40±0.08^{a}	2.96±0.34^{a}	0.30±0.03^{a}	31.3±0.5^{a}	0.07±0.01^{a}	6.57±0.90^{a}
CL	6.14±0.83^{b}	39.8±2.0^{b}	0.64±0.08^{b}	4.20±0.21^{b}	0.27±0.02^{a}	26.1±1.5^{a}	0.09±0.01^{a}	8.42±0.45^{b}
CS	5.07±0.54^{ab}	36.2±1.7^{ab}	0.53±0.06^{ab}	3.80±0.17^{b}	0.29±0.04^{a}	28.0±3.2^{a}	0.08±0.01^{a}	7.50±0.39^{ab}
Tree								
NT	3.17±0.27^{a}	32.0±1.4^{a}	0.31±0.23^{a}	3.07±0.23^{a}	0.22±0.02^{a}	29.0±1.9^{a}	0.05±0.01^{a}	6.04±0.43^{a}
T	7.23±0.38^{b}	41.1±1.2b	0.74±0.04^{b}	4.24±0.14^{b}	0.35±0.03^{b}	27.9±2.3^{a}	0.11±0.01^{b}	8.96±0.36^{b}
Encroachment x tree								
NE x NT	2.21±0.41^{a}	27.8±2.2^{a}	0.15±0.03^{a}	1.96±0.21^{a}	0.25±0.05^{ab}	34.3±0.4^{a}	0.03±0.01^{a}	4.23±0.69^{a}
CL x NT	3.57±0.37^{a}	34.7±1.7^{ab}	0.39±0.05^{b}	3.84±0.33^{bc}	0.23±0.03^{ab}	29.8±1.9^{a}	0.06±0.01^{a}	7.38±0.60^{bc}
CS x NT	3.71±0.40^{a}	33.7±2.6^{ab}	0.37±0.02^{b}	3.39±0.10^{b}	0.19±0.02^{a}	23.0±1.9^{a}	0.06±0.01^{a}	6.51±0.13^{bc}
NE x T	6.54±0.44^{b}	39.5±1.8^{bc}	0.65±0.07^{c}	3.96±0.26^{bc}	0.35±0.04^{ab}	28.3±2.8^{a}	0.11±0.01^{b}	8.91±0.93^{bc}
CL x T	8.71±0.49^{c}	45.0±2.1^{c}	0.88±0.06^{d}	4.55±0.22^{c}	0.30±0.02^{ab}	22.5±1.1^{a}	0.13±0.01^{b}	9.47±0.31^{c}
CS x T	6.43±0.64^{b}	38.8±1.8^{bc}	0.70±0.07^{cd}	4.19±0.25^{bc}	0.39±0.06^{b}	33.0±5.6^{a}	0.10±0.01^{b}	8.48±0.52^{bc}
η_p^2 Encroachment	0.326 **	0.235 *	0.393 ***	0.525 ***	0.012 ns	0.080 ns	0.166 ns	0.247 *
η_p^2 Tree	0.791 ***	0.490 ***	0.770 ***	0.567 ***	0.352 ***	0.012 ns	0.701 ***	0.549 ***
η_p^2 Encroach. x tree	0.187 *	0.086 ns	0.098 ns	0.312 **	0.058 ns	0.213 *	0.072 ns	0.184 *

Different letters in the same column indicate significant differences ($P < 0.05$) within the same factor or interaction estimated by a two-way ANOVA and Bonferroni test, with shrub encroachment (NE: no encroachment, CL: encroachment of *C. ladanifer*, CS: encroachment of *C. salviifolius*) and tree (NT: no tree influence, T: under *Q. suber* canopy influence) as independent variables and soil parameters as dependent variables.

* significant at $P < 0.05$; ** significant at $P < 0.01$; *** significant at $P < 0.001$; ns, not significant; η_p^2: partial eta-squared

Table 3 Cumulative C mineralization (C min.; mg C-CO_2 kg^{-1} soil) and C mineralization rate (mg C-CO_2 g^{-1} of initial organic C), initial metabolic quotient [qCO_2, mg C-CO_2 (g Cmic)$^{-1}$ h^{-1}]) and initial content (mg kg^{-1}) of microbial C and N (Cmic and Nmic) and their percentage in relation to initial organic C and total N. Values are mean ± SE (n=6)

	C min.	C min. rate	qCO_2	Cmic	%Cmic/Corg	Nmic	%Nmic/Nt
Encroachment							
NE	1312±205^{a}	103±6^{a}	0.49±0.06^{a}	512±72^{a}	4.3±0.3^{a}	53.3±7.9^{a}	5.3±0.4^{a}
CL	2123±150^{b}	156±14^{b}	0.47±0.05^{a}	661±63^{b}	4.5±0.2^{a}	61.6±8.1^{a}	5.8±0.4^{a}
CS	2067±159^{b}	152±8^{b}	0.43±0.05^{a}	637±35^{ab}	4.8±0.4^{a}	62.0±4.4^{a}	6.2±0.3^{a}
Tree							
NT	1429±159^{a}	146±13^{a}	0.47±0.05^{a}	490±42^{a}	5.0±0.2^{a}	42.8±3.6^{a}	5.7±0.3^{a}
T	2239±102^{b}	128±4^{a}	0.46±0.03^{a}	729±36^{b}	4.1±0.1^{b}	75.1±4.7^{b}	5.9±0.2^{a}
Encroachment x tree							
NE x NT	703±110^{a}	90±6^{a}	0.58±0.11^{a}	328±54^{a}	4.3±0.6^{a}	33.2±6.1^{a}	4.7±0.5^{a}
CL x NT	1796±222^{b}	186±34^{c}	0.46±0.06^{a}	505±57^{ab}	4.9±0.4^{a}	41.4±4.7^{ab}	5.7±0.7^{a}
CS x NT	1790±193^{b}	163±13^{bc}	0.38±0.08^{a}	637±51^{bc}	5.8±0.4^{a}	53.7±5.2^{ab}	6.7±0.5^{a}
NE x T	1921±154^{b}	116±7^{b}	0.40±0.03^{a}	733±44^{bc}	4.3±0.2^{a}	73.3±8.6^{c}	6.0±0.6^{a}
CL x T	2452±83^{b}	127±6^{bc}	0.47±0.07^{a}	816±67^{c}	4.2±0.1^{a}	81.7±10.2^{c}	5.9±0.4^{a}
CS x T	2345±210^{b}	142±9^{bc}	0.49±0.07^{a}	637±53^{bc}	3.9±0.3^{a}	70.2±5.7^{bc}	5.9±0.2^{a}
η_p^2 Encroachment	0.485 ***	0.510 ***	0.019 ns	0.172 *	0.053 ns	0.060 ns	0.095 ns
η_p^2 Tree	0.531 ***	0.038 ns	0.003 ns	0.489 ***	0.078 ns	0.510 ***	0.012 ns
η_p^2 Encroach. x tree	0.128 ns	0.317 **	0.111 ns	0.336 **	0.161 ns	0.111 ns	0.123 ns

Different letters in the same column indicate significant differences ($P < 0.05$) within the same factor or interaction estimated by a two-way ANOVA and Bonferroni test, with shrub encroachment (NE: no encroachment, CL: encroachment of *C. ladanifer*, CS: encroachment of *C. salviifolius*) and tree (NT: no tree influence, T: under *Q. suber* canopy influence) as independent variables and soil parameters as dependent variables.
* significant at $P < 0.05$; ** significant at $P < 0.01$; *** significant at $P < 0.001$; ns, not significant; η_p^2: partial eta-squared.

trees, irrespective of shrub encroachment (no significant E x T interaction). The proportion of C and N in microbial biomass did not differ significantly between soils.

Net N mineralization

Shrub encroachment and tree presence led to significant increases in the amount of nitrogen mineralized under anaerobic conditions; the E x T interaction indicated that the encroachment effect was only significant in areas not influenced by trees (Table 4). A similar trend was observed for rates of anaerobically mineralized N (per unit of initial total N).

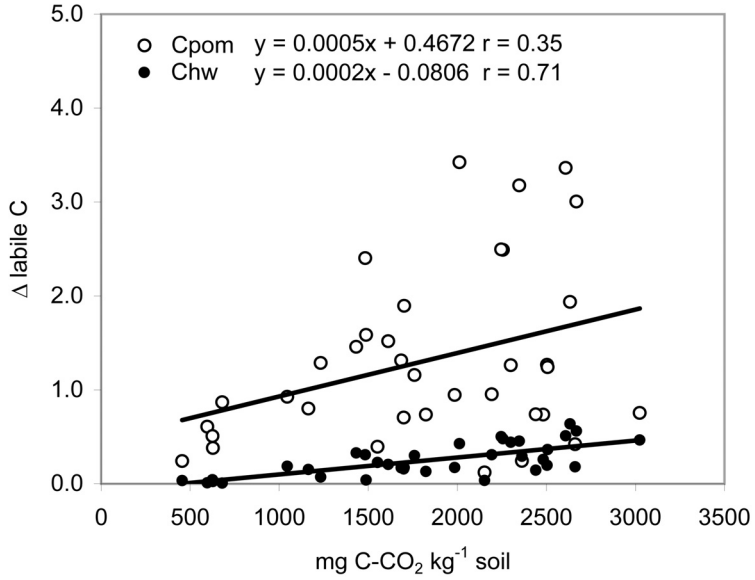

Fig. 1. Correlation between the cumulative C mineralized (mg $C\text{-}CO_2$ kg^{-1} soil) during soil incubation and differences in labile C contents (C_{POM} and Chw, g kg^{-1} soil) measured after and before 20 weeks of incubation.

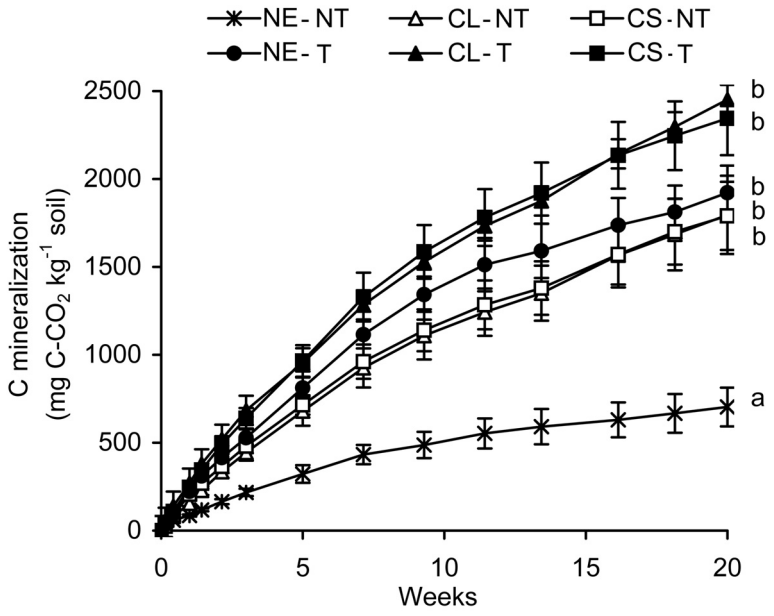

Fig. 2. Cumulative C mineralized (mg $C\text{-}CO_2$ kg^{-1} soil) during 20 weeks of incubation. Different letters for data at the end of the incubation indicate significant differences between means at the *P*= 0.05 level. Vertical lines represent ± 1 SE (n=6).

Table 4 Cumulative net N mineralization (mg N kg^{-1} soil) and net mineralization rates (mg N g^{-1} total N) after 2 weeks of anaerobic incubation and 20 weeks of aerobic incubation. Values are mean ± SE (n=6)

	Anaerobic incubation		Aerobic incubation	
	mg N kg^{-1} soil	mg N g^{-1} total N	mg N kg^{-1} soil	mg N g^{-1} total N
Encroachment				
NE	38.1±10.2^{a}	32.9±8.0^{a}	87.8±14.0^{a}	88.8±6.9^{a}
CL	67.1±8.4^{b}	65.4±6.2^{b}	129.2±15.8^{b}	117.8±7.3^{b}
CS	57.0±5.9^{b}	56.3±3.7^{b}	105.0±9.3^{b}	103.4±3.5^{ab}
Tree				
NT	31.7±5.0^{a}	41.8±6.7^{a}	70.3±5.4^{a}	95.9±6.7^{a}
T	76.5±4.8^{b}	61.3±4.0^{b}	144.4±8.6^{b}	110.8±3.7^{b}
Encroachment x tree				
NE x NT	6.2±3.2^{a}	8.3±4.1^{a}	43.9±0.8^{a}	69.5±1.8^{a}
CL x NT	45.7±4.0^{b}	63.5±7.3^{b}	84.1±7.7^{b}	116.7±13.9^{b}
CS x NT	43.3±5.6^{b}	53.6±6.1^{b}	83.0±5.8^{b}	101.7±5.0^{b}
NE x T	70.1±6.1^{bc}	57.6±4.9^{b}	131.7±9.9^{c}	108.2±7.4^{b}
CL x T	88.7±10.5^{c}	67.3±10.6^{b}	174.5±15.0^{c}	119.0±6.2^{b}
CS x T	70.7±6.9^{bc}	59.0±4.4^{b}	127.0±12.5^{c}	105.2±5.2^{b}
η_p^2 Encroachment	0.405 ***	0.459 ***	0.541 ***	0.409 ***
η_p^2 Tree	0.703 ***	0.301 **	0.823 ***	0.258 **
η_p^2 Encroach. x tree	0.208 *	0.336 **	0.388 ***	0.290 **

Different letters in the same column indicate significant differences ($P < 0.05$) within the same factor or interaction estimated by a two-way ANOVA and Bonferroni test, with shrub encroachment (NE: no encroachment, CL: encroachment of *C. ladanifer*, CS: encroachment of *C. salviifolius*) and tree (NT: no tree influence, T: under *Q. suber* canopy influence) as independent variables and soil parameters as dependent variables.

* significant at $P < 0.05$; ** significant at $P < 0.01$; *** significant at $P < 0.001$; ns, not significant; η_p^2: partial eta-squared.

Net N mineralization

Shrub encroachment and tree presence led to significant increases in the amount of nitrogen mineralized under anaerobic conditions; the E x T interaction indicated that the encroachment effect was only significant in areas not influenced by trees (Table 4). A similar trend was observed for rates of anaerobically mineralized N (per unit of initial total N).

Nitrification was the predominant N process during the aerobic incubation period (Fig. 3). After 20 weeks, cumulative net ammonification was not affected by either shrub encroachment or by the E x T interaction, but it was affected by tree presence (partial η^2= 0.595; P < 0.001), with the highest amounts in soils taken beneath the tree canopy. The highest values of cumulative net nitrification were associated with shrub encroachment (partial η^2= 0.484; P < 0.01) and tree presence (partial η^2= 0.789; P < 0.001); the E x T interaction (partial η^2= 0.396; P < 0.001) showed that the increase due to shrub encroachment was only significant in the open areas. A similar trend was observed for net nitrification rates (per unit of total N, data not shown) and for cumulative net N mineralization and net mineralization rates (Table 4).

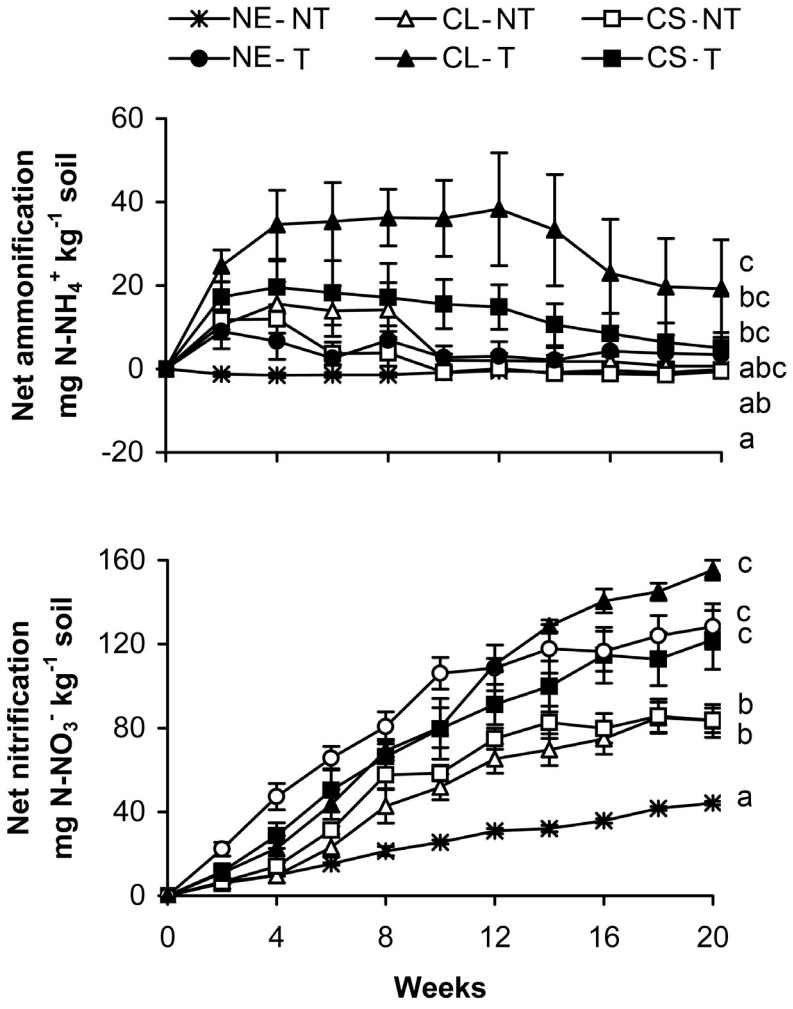

Fig. 3. Cumulative net ammonification and net nitrification during 20 weeks of aerobic incubation. Different letters for data at the end of the incubation indicate significant differences between means at the P= 0.05 level. Vertical lines represent ±1 SE (n=6).

Gross N mineralization

Shrub encroachment (in the open areas) and tree presence caused a significant increase in gross N mineralization (Fig. 4). Gross mineralization rates (N mineralized per unit of total N) were significantly higher beneath shrubs (1.21 and 1.36 mg N g^{-1} N day^{-1} for CL-NT and CS-NT, respectively) than in the non encroached area (NE-

NT, 0.08 mg N g^{-1} N day^{-1}), with intermediate values beneath trees (NE-T, 0.78 mg N g^{-1} N day^{-1}). The highest gross NH_4^+-N immobilization was observed in soils encroached with C. *salviifolius*, for which the values were significantly higher than in soils not affected by encroachment or tree presence (NE-NT).

For gross nitrification, lower values were associated with shrub encroachment (CL-NT and CS-NT, Fig. 4). Gross nitrification rates (per unit of total N) were lower in the soil influenced by shrubs and trees (0.21-0.32 mg N g^{-1} N day^{-1}) than in the open areas (0.98 mg N g^{-1} N day^{-1}). No significant differences were observed in gross NO_3^--N immobilization, the values of which significantly exceeded those of gross nitrification only in encroached soils ($P < 0.05$).

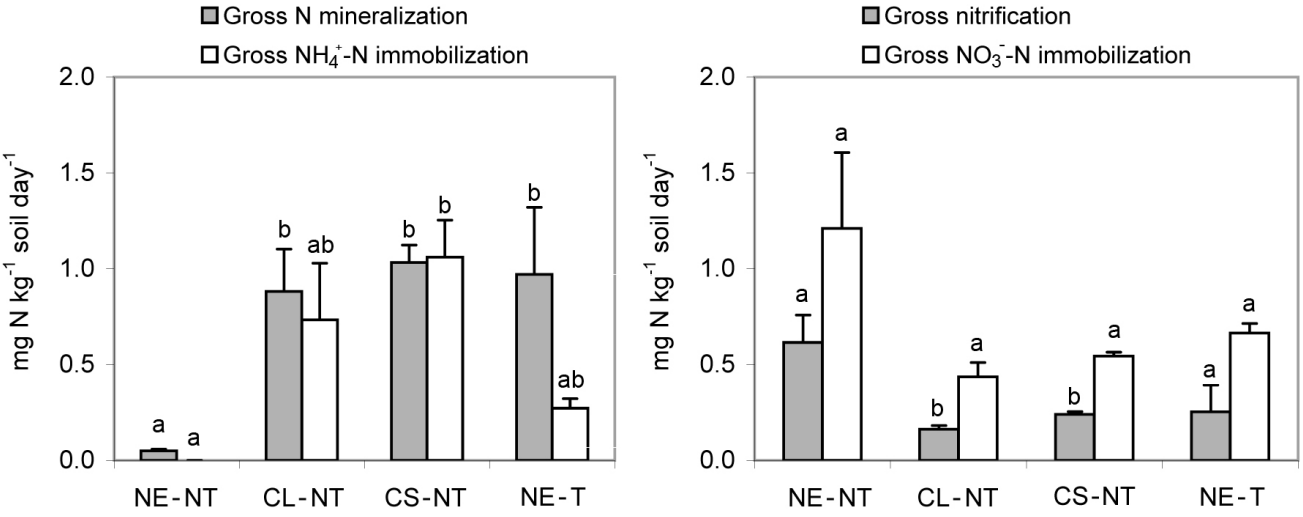

Fig. 4. Gross N mineralization, NH_4^+–N immobilization, nitrification and NO_3^-–N immobilization. Error bars refer to SE and different letters indicate significant differences between means (n= 4) of the same variable at the P= 0.05 level.

Enzymatic activities

Shrub encroachment and tree presence greatly affected urease and protease activities; the significant E x T interactions indicated that beneath the tree canopy, the increases associated with encroachment were only significant for *C. ladanifer* (Table 5). Encroachment with *C. salviifolius* led to a significant increase in phosphatase activity, both in the absence and presence of trees (no significant E x T interaction). Irrespective of shrub encroachment (no significant interaction E x T), b-glucosidase activity was significantly higher under trees and explained most of the observed variance (64 %).

Discussion

Soil C accumulation

In the woodland under study, the spatial variability in SOC contents was mainly associated with tree presence, confirming the positive influence of scattered oaks reported for similar systems (Howlett et al. 2011; Gómez-Rey et al. 2012b). Although not significant, the development of shrub understorey also tended to increase the SOC content beyond tree canopies, which is consistent with findings in dehesas encroached by shrubs after 10-20 years (Simões et al. 2009; Moreno and Obrador 2007; Tárrega et al. 2009). The low SOC accumulation associated with shrub encroachment may be mainly related to its short development period compared with that of trees (Belsky et al. 1989) rather than to differences in the aboveground input, which is similar in oak trees (Escudero et al. 1985) and some *Cistus* species (Simões et al. 2009). Changes in belowground (mostly located in the 0-10 cm soil layer; Silva de Sá 2001) and aboveground C inputs from herbaceous understory should also be taken into account. A large reduction in pasture yield beneath *C. ladanifer*, relative to oak trees, has been reported for Spanish dehesas encroached after 10-15 years (Rivest et al. 2011), which may be associated with the shallow dense root system developed by *Cistus*

Table 5 Enzyme activities in the 0-10 cm soil layer. Values are mean ± SE (n=6)

	Urease activity ($\mu mol\ NH_3\ g^{-1}h^{-1}$)	Protease activity ($\mu mol\ tyr\ g^{-1}h^{-1}$)	Phosphatase activity ($\mu mol\ PNP\ g^{-1}h^{-1}$)	β-glucosidase activity ($\mu mol\ PNP\ g^{-1}h^{-1}$)
Encroachment				
NE	1.405±0.191^{a}	0.249±0.051y	1.251±0.020^{a}	0.551±0.095^{a}
CL	2.111±0.097^{c}	0.362±0.061^{b}	1.261±0.013^{ab}	0.752±0.089^{b}
CS	1.662±0.083^{b}	0.276±0.033^{b}	1.312±0.008^{b}	0.596±0.064^{ab}
Tree				
NT	1.363±0.106^{a}	0.148±0.012^{a}	1.265±0.018^{a}	0.412±0.039^{a}
T	2.089±0.079^{b}	0.444±0.027^{b}	1.284±0.007^{a}	0.855±0.051^{b}
Encroachment x tree				
NE x NT	0.805±0.028^{a}	0.090±0.005^{a}	1.213±0.036^{a}	0.292±0.024^{a}
CL x NT	1.812±0.044^{bc}	0.169±0.014^{b}	1.270±0.026^{ab}	0.493±0.086^{ab}
CS x NT	1.472±0.084^{b}	0.185±0.017^{b}	1.311±0.137^{b}	0.450±0.060^{abc}
NE x T	2.004±0.129^{c}	0.409±0.035^{c}	1.289±0.005^{ab}	0.811±0.110^{cd}
CL x T	2.410±0.067^{d}	0.555±0.033^{d}	1.252±0.008^{ab}	1.010±0.031^{d}
CS x T	1.852±0.095^{c}	0.367±0.038^{c}	1.313±0.008^{b}	0.743±0.077^{bcd}
η_p^2 Encroachment	0.717 ***	0.513 ***	0.242 **	0.204 *
η_p^2 Tree	0.796 ***	0.900 ***	0.063 ns	0.643 ***
η_p^2 Encroach. x tree	0.471 ***	0.448 ***	0.175 ns	0.098 ns

Different letters in the same column indicate significant differences ($P < 0.05$) within the same factor or interaction estimated by a two-way ANOVA and Bonferroni test, with shrub encroachment (NE: no encroachment, CL: encroachment of *C. ladanifer*, CS: encroachment of *C. salviifolius*) and tree (NT: no tree influence, T: under *Q. suber* canopy influence) as independent variables and soil parameters as dependent variables.
* significant at $P < 0.05$; ** significant at $P < 0.01$; *** significant at $P < 0.001$; ns, not significant; η_p^2: partial eta-squared.

(Silva et al. 2003). The present results suggest that shrub encroachment may lead to significant long-term increases in SOC, following the trend reported by Springsteen et al. (2010) for a 42-year chronosequence of woody shrub expansion in temperate semi-arid grassland of North America.

Despite the potential capacity of shrub encroachment to accumulate SOC, long encroachment periods are needed to detect significant differences. Nevertheless, the hot water extractable C fraction (considering both absolute and relative contents) was useful for detecting a significant impact of shrubs in areas not influenced by trees. Similarly, hot water soluble C and C_{POM} contents were more sensitive than SOC to changes associated with *C. ladanifer* encroachment in areas beneath trees, following the findings of Ghani et al. (2003) on the impact of pastoral management practices. The positive correlation with SOC content suggests that the measured labile fractions (mainly the hot water extractable fraction) may be an early indicator of the effect of encroachment on SOM, which supports the hypothesis that shrub invasion may lead to long-term accumulation of SOC.

The effect of shrub encroachment beneath tree canopies appears to be dependent on shrub species, because accumulation of SOC was only observed for *C. ladanifer*. However, no such differences were observed in soils not influenced by trees, suggesting that competitive interactions between *C. salviifolius* shrubs and oak trees may limit C accumulation beneath shrub canopies. This may be related to the higher sensitivity to drought length period and intensity of *C. salviifolius* than *C. ladanifer* (Simões et al. 2008), which may lead to smaller inputs of residues.

In the woodland under study, the hot water extractable fraction was a better indicator of changes in SOM than the POM fraction, and it was also a better indicator of the effects of shrubs and trees than the bulk SOC. Thus, the present results suggest that the hot water extractable fraction may be useful for early detection of long-term impacts of shrub encroachment on SOC.

Soil C dynamics

The higher amount of potentially mineralizable C beneath tree canopies is consistent with the trend reported for other shrubs (McCulley et al. 2004; Moro et al. 1997) and for Mediterranean oaks in woodlands (Waldrop and Firestone 2006; Gómez-Rey et al. 2012b) and forests (Mariscal-Sancho et al. 2010; Llorente and Turion 2010). Such increases may be related to SOM accumulation beneath canopies, considering the positive correlations between cumulative C mineralized and C contents (Corg r= 0.84, C_{POM} r= 0.76, Chw r= 0.78; $P < 0.01$, n= 36). These results are consistent with the large reduction in Chw and C_{POM} contents during incubation, which may reflect the consumption of easily available labile fractions by microorganisms. The correlation between Chw reduction and cumulative C mineralized indicates that this small fraction may represent a main substrate for microorganisms (Ghani et al. 2003; Zhao et al. 2008). The lower correlation obtained for C_{POM} reduction suggests difficulty in the physical isolation of an active SOM fraction, as also reported by Rovira et al. (2010) for Mediterranean forest soils.

Despite differences in SOC content, the effect of shrub encroachment on C mineralization was of similar magnitude to that observed for the trees, in contrast with the higher amounts of potentially mineralizable C under trees than under shrubs, in the Mediterranean environment (Pérez-Bejarano et al. 2010; Rutigliano et al. 2004). In the present study, such a pattern may be explained by the proportion of hot water extractable C, which was also similarly affected by shrub encroachment and trees, thus confirming the role of the labile fraction on SOC dynamics. The high C mineralization rate associated with shrub encroachment, especially beneath *C. ladanifer*, may also limit SOC accumulation when compared with that for trees.

The enhanced SOC pool beneath canopies sustains a large microbial biomass (although not significant for CS), following the pattern observed beneath oak trees (Herman et al. 2003; Rutigliano et al. 2004) and woody shrub canopies (McCulley et al. 2004) in Mediterranean areas. The small variation in the Cmic/Corg ratios and qCO_2 values indicates that the microbial biomass supported per unit of SOC is similar in all studied soils, and that the substrate C use efficiency by the soil microbial community was not altered by shrub encroachment (Pérez-Bejarano et al., 2010). Therefore, the increased microbial activity beneath tree and shrub canopies appears to be the consequence of a higher growth rate of soil microorganisms rather than a more efficient microbial community.

The β-glucosidase activity, similar to that reported for other Mediterranean soils (Rutigliano et al. 2004; Waldrop and Firestone 2006), was enhanced only beneath oak canopies, as also found by Mariscal-Sancho et al. (2010) in Mediterranean soils. Such behaviour may be attributed to SOC

accumulation, as b-glucosidase activity was strongly correlated with SOC levels (Corg r= 0.89, C_{POM} r= 0.86, Chw r= 0.78; $P < 0.01$, n= 36).

The present results show that shrubs and trees enhanced soil C cycling, especially in soils encroached by *C. ladanifer*, in which the high C mineralization rate may limit SOC accumulation. However, the effect of encroachment was minimized beneath oak canopies and in the respective soils in which the faster C cycling was due to the presence of trees. The actual C mineralization rates may be much lower than the values obtained in this laboratory-based study, as C mineralization under field conditions will be restricted by environmental factors (temperature and humidity) and will probably be limited to winter and autumn periods. The seasonal variability in soil microbial biomass was not taken into account, and therefore different conclusions may be reached in another sampling season.

Soil N pools and dynamics

The negligible effect of shrub encroachment on soil total N, relative to that observed for organic C, led to a higher soil C/N ratio beneath shrubs, as also found in the same site by Simões et al. (2009). However, this pattern is not consistent with the significant increase in organic N reported for Spanish oak woodlands encroached by leguminous shrubs (Moreno and Obrador 1997, Moro et al. 1997), suggesting that soil N accumulation may depend on shrub species.

In the woodland under study, variables related to soil N dynamics (total and labile N, microbial N, net N mineralization and nitrification, urease and protease activities) were greatly affected by oak presence, thus confirming the higher availability of soil N reported for soils below other scattered trees in Mediterranean systems (Gallardo et al. 2000). However, shrub encroachment had a negligible effect on soil N accumulation; such differences are probably the result of a higher tree root uptake from deeper depths and increased atmospheric interception by tree canopies. Despite a small effect**,** the presence of shrubs (beyond tree canopies) also enhanced the amount of N mineralized and nitrified, as reported for shrub encroachment in Mediterranean (Maestre et al. 2011) and subtropical (McCulley et al. 2004) grasslands. As shrub encroachment did not affect the soil N content, the increase in N mineralization may be attributed to faster turnover of soil N (greater net N mineralization and nitrification rates and urease and protease activities), which was of similar magnitude to that beneath trees, thus showing the driving role of shrub encroachment for improving N availability in oak woodland systems.

Spatial variability in net mineralization and nitrification is probably mainly related to C accumulation beneath canopies, considering the positive correlations between both cumulative net mineralization and nitrification and the initial organic and labile C contents ($r > 0.89$, $P < 0.01$, n= 36). These results indicate that C availability is an important factor controlling N dynamics in the study site and suggest that microbial N cycling is limited by C. In fact, temporal and spatial differences in SOC have previously been related to changes in N dynamics in semi-arid grasslands (Chen and Stark 2000) and Mediterranean oak woodlands (Gallardo et al. 2000).

The results indicate potentially greater NO_3^--N leaching from surface soils beneath shrubs and tree canopies; however, under field conditions, the NO_3^--N may be taken up by the shallow root system of *Cistus* and herbaceous plants, especially during late winter - late spring (Simões et al. 2008), as well as by oak tree roots. Immobilization of N may also be enhanced beneath shrubs by the inputs of residues with C/N ratios (70-116 in litterfall, and 115 and 145 for fine and coarse roots, respectively; Simões et al. 2009, 2012) that are higher than in tree residues (C/N= 59 in litterfall; Sá et al. 2001).

The observed gross N mineralization and nitrification rates were relatively low compared with those reported for other Mediterranean woodlands (Herman et al. 2003) and semi-arid shrublands (Chen and Stark 2000), probably because of the low organic C content of the soils under study. Results of the isotope dilution technique supported the conclusions from net N mineralization measurements, with enhancement of gross N mineralization beneath both tree and shrub canopies, as also reported for *Cistus* (Castells et al. 2003) and oak trees in Mediterranean woodlands (Davidson et al. 1990; Herman et al. 2003). This is consistent with the significant influence of C availability on gross soil N mineralization (Booth et al. 2005). However, the results obtained for nitrification rates were contradictory, as the highest gross rates and the lowest net nitrification rates were observed in soils outside canopies. Since C provides the energy that drives heterotrophic N transformations, the lower C availability in these soils may restrict heterotrophic growth and favour autotrophic nitrification (Cookson et al. 2006).

In all of the soils under study, the NO_3^--N

produced appeared to be rapidly assimilated by microorganisms, as gross NO_3^--N immobilization tended to be higher than gross nitrification. As suggested by Herman et al. (2003) for oak woodlands, microbial assimilation of NO_3^--N, together with the above-mentioned root uptake, may lead to low NO_3^--N leaching rates.

Beneath the tree canopy, the effects of shrub encroachment on soil C and N dynamics were much less than outside the tree canopy and were restricted to *C. ladanifer*. Beneath tree canopies, the high urease and protease activities in soils encroached by *C. ladanifer* may suggest faster N cycling, but differences in N cycling rates were not significant. In these soils, the higher C availability might have increased microbial N demand, resulting in less available N for mineralization and nitrification during the incubation. This is supported by the fact that adding organic C (i.e. fine roots from trees and/or herbaceous plants) to soils of an unmanaged oak woodland reduces the net N mineralization (Gómez-Rey et al. 2011). Further measurements, including gross N fluxes, are necessary to reveal the relative importance of N immobilization process in these soils.

Although the results of a field study suggested that soil C and N cycling is expected to be slower in sites dominated by *C. ladanifer* than by *C. salviifolius* (Simões et al., 2009), the present results did not confirm this hypothesis. This discrepancy may be related to the presence of phenolic compounds released by *C. ladanifer* under field conditions, which may have reduced residue decomposition and N mineralization, as reported for soils densely covered with *C. ladanifer* (Mariscal-Sancho et al. 2010) or incubated with leachates from *C. albidus* leaves (Castells and Peñuelas 2003).

Although Simões et al. (2009) hypothesized that P cycling should be faster in sites dominated by *C. ladanifer* than by *C. salvifolius*, we found slightly higher phosphatase activity and the lowest soil extractable P contents in the latter type of sites. However, these results were not sufficient to explain the differences in residue decomposition observed by Simões et al. (2009), suggesting similar soil P cycling in both species.

In conclusion, shrub encroachment enhanced N cycling in the soil under study, and had a similar effect to tree presence. However, the effect of shrub encroachment was minimized beneath oak canopies, and the faster N cycling in the soils was due to the tree presence. Measured rates of N cycling correspond with the potential capacity of N mineralization, but different results may be obtained in situ or in a different season. Moreover, only the upper soil layer was analysed in the present study, which limits any interpretation regarding the entire soil profile.

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