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Variation in soil C and microbial functions across tree canopy projection and open grassland microenvironments

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Abstract: Mixtures of grasslands and perennial woody crops or vineyards represent a major source of potential carbon storage or release. Understanding the spatial variability of soil properties in these ecosystems is important in determining soil constraints related to the management of soil resources. The aims of the study were 1) to explore the spatial variability associated to the trees for soil C storage and its components and reactivity; and 2) to assess the similarities between microenvironments in terms of microbial functional diversity. Eight microenvironments characterized by different long-term soil management practices and different positions with respect to woody plant canopy soil vertical projections were selected in a Mediterranean agropastoral system. Four management types were considered: pasture, hay crop, grass-covered vineyard, and tilled vineyard. Soil organic C, microbial biomass, and respiration were measured to assess C storage and dynamics, while functional diversity was determined by means of soil enzyme activities. The results showed that the microenvironmental variation of soil organic C and functional microbial diversity generated by the tree canopies in the wooded grassland can be very relevant for an accurate assessment of soil organic C content and its dynamics. The same was not applicable to vineyards, where the spatial variation of both soil organic C and functional diversity was negligible, independently of the soil management practices. These results suggest that in such systems the microscale spatial variability generated by the trees is worth of further investigation for improving our understanding of the long-term management effects on soil C dynamics.

Key words: Agroforestry systems, carbon mineralization, enzyme activity, long-term management, tree effect, vineyards

1. Introduction

Multiple effects of trees in agropastoral systems have been described (Moreno et al., 2007), as well as a positive role of trees on soil fertility through improved utilization and recycling of soil elements (Dhillon et al., 2008). Trees affect the soil carbon cycle, mainly by supplying organic matter through the dead leaves that fall from canopy (Andivia et al., 2010) and rhizodeposition (Grayston and Campbell, 1996), by influencing the soil water regime through the interception of precipitation (Sun et al., 2013), and by generating specific microclimate conditions that affect chemical, biochemical, and biological processes (Di Bene et al., 2011).

In the Mediterranean wooded grassland landscapes, such as the Portuguese montado and Spanish dehesa environments, oaks generate a favorable microclimate for soil C sequestration (Howlett et al., 2011; Gómez-Rey et al., 2012). Agricultural landscapes that include perennial woody crops, such as vineyards, represent a major source of potential C storage or release (Williams et al., 2011). Almagro et al. (2009) showed, under Mediterranean conditions, different soil CO_2 effluxes between land uses and, within uses, between beneath- and intercanopy sites. These findings have been weakly explained by soil temperature regime or soil water content, suggesting that microbial activity, as with other factors, could play a role in the soil C dynamic.

Soil microbial properties are known to exhibit high space–time variability (Cavigelli et al., 2005). Understanding the spatial variability of soil properties and its functions is important in determining soil constraints to plant nutrition and appropriate management of soil resources (Keil et al., 2011).

Studies carried out in natural systems showed that trees' distribution affects soil organic matter mineralization (Chatterjee and Jenerette, 2011) and soil microbial community seasonal dynamics (Waldrop et al., 2006). However, the combined effects of long-term management and trees' spatial distribution on C cycling and microbial activity have not been sufficiently investigated so far.

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We hypothesized that soil systems generated by long-term management and, more specifically, their positions under or out of the tree canopy projections represent different soil microenvironments that may play a distinct role in providing ecosystem services associated to the soil C cycling and functional activity. In a previous paper, Lagomarsino et al. (2011) showed that land use and management practices in such environments influenced C cycling in terms of organic C storage, microbial biomass pool, C mineralization, enzyme activity, and related functional diversity. In this paper we focused on the variation in soil C content and microbial functions across microenvironments in the context of Mediterranean wooded grasslands and vineyards under different soil management regimes.

2. Materials and methods

2.1. Site description

The study was carried out in the Berchidda long-term observatory, in Sardinia (Italy), 40°46′N, 9°10′E, mean altitude 285 m a.s.l. The soil type in the area is a Haplic Endoleptic Cambisol, Dystric (WRB, 2006), with sandy loam texture. Soil pH varies from moderately to slightly acid, i.e. from 5.2 to 6.4. The climate is typically Pluviseasonal oceanic low meso-Mediterranean low subhumid (Worldwide Bioclimatic Classification System, 1996–2009) with a mean annual rainfall of 630 mm (70% from October to March), mean annual temperature of 14.2 °C, and aridity index (rain / reference evapotranspiration on an annual basin) of 0.53. The area is representative of the climate, vegetation type, and management of some of the most common agroforestry systems in the Mediterranean basin (Bacchetta et al., 2009; Bagella et al., 2013). The potential vegetation in the sampled areas would be a *Quercus suber* forest (Bagella and Caria, 2011) and the dominant landscape of the area is a mosaic of vineyards for the production of Vermentino DOCG wine and wooded hay-land or pasture (dehesa-like landscape). Tree cover in the grasslands ranges between fewer than 10 to more than 50 trees per hectare.

In this observatory, 4 different land uses, stable for at least 15 years, were chosen across increasing human management intensity: wooded pasture, wooded hay-land, grass-covered vineyard, and tilled vineyard, all already described by Francaviglia et al. (2012). In the tilled vineyard, pruning residues are exported and organic fertilizer is added, while in the grass-covered vineyard, pruning residues are cut and left on the soil. Two microenvironments were considered in the pasture and hay-land: hay-land (HAO) and pasture (PAO) both out of trees' influence and hay-land (HAU) and pasture (PAU) both under the projection of the tree canopy. In the case of hay-land, the soil under the tree canopy projection was tilled prior to seeding the hay forage crop. Two microenvironments were also considered in the 2 vineyards: tilled vineyard (VTR) and grass-covered vineyard (VGR) along the rows and tilled vineyard (VTI) and grass-covered vineyard (VGI) between the rows.

2.2. Soil sampling and storage

The top 0–20 cm of soil was sampled after removal of the litter layer (Conant et al., 2001). The soil cores (approximately 1000 g each) were sampled in October 2009. Each sampling point was identified taking into account geomorphology, exposure, slope, and previous sampling schemes (Lagomarsino et al., 2011; Seddaiu et al., 2013). Overall, 20 bulk soil samples, each represented by a pooled sample of 3 random subsamples, were collected considering 3 replicates in each vineyard microenvironment and 2 in each grassland microenvironment. The collected soil samples were immediately sieved at 2 mm and stored at 4 °C prior to analysis.

Our study was focused on characterizing spatial variability across microenvironments, assuming homogeneous ecological conditions of the sampling sites, as done by Seddaiu et al. (2013) in the same long-term observatory. Accordingly, we chose to intensively study 1 site from each land use type rather than attempting to replicate sites across the ecosystem. Even though this experimental design was a case of "simple pseudoreplication" (Hurlbert, 1984), our approach has been considered valid to compare land uses in the same site (Lagomarsino et al., 2011) and for comparing between positions with respect to the tree crown projections (Almagro et al., 2009; Gómez-Rey et al., 2012).

2.3. Soil organic C, microbial biomass, and respiration

Total soil organic C (TOC) was estimated after oxidation with $K_2Cr_2O_7$ and subsequent titration of unreduced $Cr_2O_7^-$ with $Fe(NH_4)_2(SO_4)_2$, according to the Springer and Klee method as illustrated by Nelson and Sommers (1996). Microbial biomass carbon (MBC) was estimated following the fumigation extraction method: 2 aliquots of moist soil (20 g of oven-dried soil) were weighed and the first (not fumigated) was immediately extracted with 80 mL of 0.5 M K_2SO_4 for 30 min by oscillating and shaking at 200 rpm and then filtered (Whatman No. 42). The second aliquot was fumigated for 24 h at 25 °C with ethanol-free $CHCl_3$ and then extracted as described above. Organic C in the extracts was determined after oxidation with 0.4 N $K_2Cr_2O_7$ at 100 °C for 30 min (Vance et al., 1987). Microbial biomass was calculated as follows: Biomass C = EC / k_{EC}, where EC is the difference between organic C extracted from fumigated soils and organic C extracted from nonfumigated soils, and k_{EC} = 0.38. The sum of EC + MBC represents the labile fraction (LC) according Dumontet et al. (2001).

For measuring microbial respiration 20 g (oven-dried basis) of moist soil was placed in 1-L stoppered glass jars.

The CO_2 that evolved (MR) was trapped, after 1, 2, 4, 7, 10, 14, 17, 21, and 28 days of incubation, in 2 mL of 1 M NaOH and determined by titration of the excess NaOH with 0.1 M HCl (Badalucco et al., 1992). The CO_2 that evolved during the 28th day of incubation was used as the basal respiration value (MR_{basal}). The C that evolved during the 28 days of incubation (Cm) was used to calculate the potentially mineralizable C (C_0) and the rate constant (k), using the first order kinetics model $Cm = C_0 \times (1 - e^{-kt})$ as reported by Riffaldi et al. (1996), where t is the time of incubation. The mineralization quotient qM was calculated as μg C – CO_2 cum / μg TOC (Pinzari et al., 1999).

2.4. Enzyme activities and functional diversity

Enzyme activity was measured according to the methods of Marx et al. (2001) and Vepsäläinen et al. (2001), based on the use of fluorogenic methylumbelliferyl (MUF) substrates. Soil samples were analyzed for N-acetyl-β-glucosaminidase (NAG), α-glucosidase (αG), β-glucosidase (βG), butyrate esterase (BUT), acid phosphatase (AP), arylsulfatase (ARYL), xylosidase (XYL), and acetate esterase (EST) activity using MUF-conjugated surrogate substrates (Sigma, St Louis, MO, USA). The respective substrates were 4-MUF-N-acetyl-β-glucosaminide, 4-MUF- β-D-glucoside, 4-MUF-β-D-glucoside, 4-MUF-butyrate, 4-MUF-phosphate, 4-MUF-sulfate, 4-MUF-7-β-D-xyloside, and 4-MUF-acetate. A moist soil sample (equivalent to 1 g of oven-dried material) was weighed into a sterile jar and 50 mL of Na-acetate buffer, pH 5.5, was added. A homogeneous suspension was obtained by homogenizing with UltraTurrax at 9600 rpm for 3 min. Aliquots of 100 μL were withdrawn and dispensed into a 96-well microplate (3 analytical replicates/sample/substrate). Finally, 100 μL of 1 mM substrate solution was added, giving a final substrate concentration of 500 μM. Fluorescence was measured after 0, 30, 60, 120, and 180 min of incubation at 30 °C. Fluorescence (excitation 360 nm; emission 450 nm) was measured with an automated fluorometric plate reader (Fluoroskan Ascent). From the enzyme activities, the Simpson–Yule index (SYI) was calculated following the equation $E = 1 / \Sigma pi_2$, as indicated by Bending et al. (2004), where pi is calculated as respiration / enzymatic response to a substrate as a proportion of respiration / enzymatic responses summed across all substrates for a soil. The order of magnitude of the values obtained for the different respiration / enzymatic responses varies considerably depending on the specific activity being determined, thus leading to some enzymes / substrate having more weight than others. To solve this problem, the percentage of the maximum value found for that specific respiration / enzymatic response was used for the calculation of the SYI (Rodríguez-Loinaz et al., 2008).

2.5. Statistical analysis

All variables were tested for normality with Shapiro–Wilk statistics and transformed into log prior to assessing the differences between land uses by one-way ANOVA. The post hoc mean comparisons were performed using Fisher's protected least significant difference (LSD) method ($P < 0.05$). Pearson's correlation coefficients and associated significances were calculated between enzyme activities and indicators of soil organic C. To assess the enzyme activity, a hierarchical clustering of the 8 microenvironments was performed. The matrix used was made of 8 microenvironments × 8 variables.

All statistical analyses were performed with the SAS System (SAS Institute, 1999) except for the hierarchical clustering, which was performed with the SYN-TAX 2000 program package (Podani, 2001).

3. Results

The only significant differences between microenvironments were observed in the wooded grassland sites, while no significant differences were observed between vineyard microenvironments. In particular, wooded grasslands under tree canopy showed significantly higher values of TOC, with MR_{basal} and C_0 being significantly higher just in pasture (Table 1). At the end of the incubation period, MR was significantly higher under the PAU than the PAO, whereas HAU showed an intermediate behavior between PAU and the other microenvironments (Figure 1). LC and MBC were significantly higher in pasture than in hay-land, which was more similar to vineyard (Table 1). There were not any significant differences in k between microenvironments (Table 1), while qM was significantly lower in PAU than in the HAO and vineyard microenvironments (Table 1).

Hydrolytic enzymes (βG, αG, ARYL, and NAG) were higher in PAU than in PAO, while no significant differences were observed between HAO and HAU microenvironments and also within vineyards (Table 2). AP activity was higher in the pasture and similar in hay-land and vineyards, with a decreasing trend in the sequence of HAU ≥ HAO ≥ VTI = VTR ≥ VGR = VGI (Table 2). XYL activity was higher in grassland sites and grass-covered vineyard than in tilled vineyard (Table 2). In the VGR and VGI we observed lower activity of EST than was observed in PAO and PAU (Table 2). βG was the only enzymatic activity that differed between grass-covered vineyard and tilled vineyard; moreover, no differences were found within land use, and between row and interrow microenvironments (Table 2).

The SYI in the PAU was higher than in the PAO, while it did not differ between the hay-land microenvironments or among the 4 vineyards microenvironments (Table 3).

The correlation analysis showed that TOC was significantly correlated with all the enzymatic activity indicators (Table 4). LC and MBC were not significantly correlated with βG, αG, and EST (Table 4). MR_{basal} and

Table 1. Total organic carbon (TOC), labile carbon (LC), microbial biomass carbon (MBC), basal microbial respiration (MR_{basal}), potentially mineralizable carbon (C_0), rate constant (k), and mineralization quotient (qM) in the 8 soil microenvironments.

	TOC, %	LC, μg C g^{-1}	MBC, μg C g^{-1}	MR_{basal}, μg C – CO_2 g^{-1} h^{-1}	C_0, μg C – CO_2 g^{-1}	k, day^{-1}	qM
PAO	2.2 bc	251 a	294 a	0.15 b	219 b	0.088 a	0.97 dc
PAU	2.8 a	251 a	293 a	0.32 a	384 a	0.075 a	0.74 d
HAO	1.4 dc	127 c	186 b	0.16 b	202 b	0.103 a	1.51 ab
HAU	2.3 ab	180 bc	214 b	0.22 ab	269 ab	0.090 a	1.04 bcd
VGI	1.6 cd	153 bc	218 b	0.14 b	214 b	0.080 a	1.39 abc
VGR	1.5 bcd	175 bc	237 ab	0.16 b	244 ab	0.081 a	1.54 abc
VTI	1.3 d	196 ab	210 b	0.11 b	133 b	0.074 a	1.61 a
VTR	1.4 dc	197 ab	177 b	0.11 b	161 b	0.093 a	1.27 ab

Different letters within each column indicate differences at $P < 0.05$ (Fisher's protected LSD method). PAO: pasture out of tree canopy; PAU: pasture under tree canopy; HAO: hay-land out of tree canopy; HAU: hay-land under tree canopy; VGI: grass-covered vineyard, interrow; VGR: grass-covered vineyard, along the row; VTI: tilled vineyard, interrow; VTR: tilled vineyard, along the row.

C_0 were particularly highly related with βG, ARYL, and NAG (Table 4). Significant correlations were not observed between k and enzyme activities, while qM was negatively correlated with all enzyme activities considered (Table 4).

The cluster analysis grouped the microenvironments into 3 main clusters: 1) PAU; 2) HAO, HAU, and PAO; and 3) VGI, VGR, VTI, and VTR (Figure 2).

4. Discussion

Oak canopies generated high variation in terms of soil organic C content within land use. No variation in soil organic C associated with grapevine trees was observed within the vineyards. The high TOC observed in soil under oak canopies might be the result of the long-term transfer to the soil of high amounts of organic C deriving from the aboveground tree biomass (Moreno et al., 2007) and tree rhizodeposition (Pausch et al., 2013), as well as from dejections of grazing sheep that, particularly in the summer, rest in the shade under the tree canopies. According to what was observed by Gómez-Rey et al. (2012), PAU showed a content of C_0, MR_{basal}, and MR higher than that in PAO, indicating a larger soil microbial activity and a positive trend for C stock potential. These findings might suggest, given the similar MBC values between PAO and PAU, the presence of 2 structurally distinct microbial communities in the open grassland or below the tree canopy. However, additional data would be required to support this interpretation.

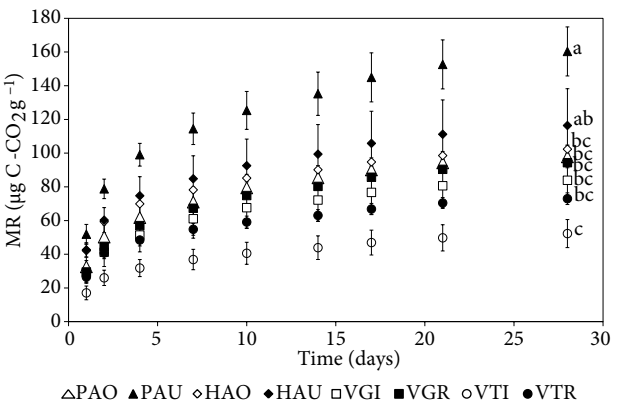

Figure 1. Soil microbial respiration (MR) kinetic during 28 days of incubation. Bars represent standard errors. Different letters for means at the end of the incubation period indicate differences at $P < 0.05$ (Fisher's protected LSD method). HAO: hay-land out of tree canopy; HAU: hay-land under tree canopy; PAO: pasture out of tree canopy; PAU: pasture under tree canopy; VTI: tilled vineyard, interrow; VTR: tilled vineyard, along the row; VGI: grass-covered vineyard, interrow; VGR: grass-covered vineyard, along the row.

The variation in terms of MR, C_0, and MR_{basal}, with values always significantly lower in tilled vineyard than under the oak canopies, was interpreted as the effect of the different quantitative and qualitative C inputs, as it was observed comparing the vineyard with oak forest soils (Carlisle et. al., 2006). This trend, in the case of wooded grazing systems, may be explained also by animal dejections that increase soil organic matter mineralization (Lai et al., 2012). LC and MBC values were consistent to what was observed by Lagomarsino et al. (2011). The differences in terms of qM suggested a greater C sequestration potential generated by trees and pasture management, as was observed comparing forest and pasture with agricultural systems (Lagomarsino et al., 2011).

The correlation analysis confirmed the relevance of the enzyme activities considered in this work in the study of C dynamics. The variation in terms of soil organic C was consistent to the SYI, higher in PAU than in PAO, and the

Table 2. Activities of β-glucosidase (βG), α-glucosidase (αG), acid phosphatase (AP), arylsulfatase (ARYL), N-acetyl-β-glucosaminidase (NAG), xylosidase (XYL), acetate esterase (EST), and butyrate esterase (BUT) in the 8 soil microenvironments.

	βG	αG	AP	ARYL	NAG	XYL	EST	BUT
nmol MUF g^{-1} h^{-1}								
PAO	665 b	57 b	4235 ab	234 b	751 bc	280 abc	16772 a	8699 abc
PAU	1879 a	143 a	5528 b	532 a	1669 a	430 a	15357 ab	10178 a
HAO	1006 ab	66 b	3407 bc	203 bc	893 b	288 abc	11251 abc	7158 abc
HAU	1481 ab	85 ab	3829 b	264 b	897 b	389 ab	12466 abc	9196 ab
VGI	1176 ab	41b	2252 cd	182 bc	447 bc	211 bc	7495 c	6081 c
VGR	1198 a	53 b	1924 d	182 bc	528 bc	275 abc	6027 c	6927 bc
VTI	770 b	55 b	3007 bcd	122 c	318 c	173 c	9478 abc	6554 bc
VTR	691 b	55 b	2788 bcd	130 c	270 c	202 bc	8200 bc	6779 bc

Different letters within each column indicate differences at $P < 0.05$ (Fisher's protected LSD method). PAO: pasture out of tree canopy; PAU: pasture under tree canopy; HAO: hay-land out of tree canopy; HAU: hay-land under tree canopy; VGI: grass-covered vineyard, interrow; VGR: grass-covered vineyard, along the row; VTI: tilled vineyard, interrow; VTR: tilled vineyard, along the row.

clustering analyses that clearly separated PAU from the other microenvironments. The strong correlations among MR_{basal}, C_0, and βG confirmed the importance of cellulose mineralization on C cycling assessment (Mariscal-Sancho et al., 2010). High values of these variables under the tree canopy might indicate the important role of saprophytic fungi on C cycling, in relation to their role as sources of β-glucosidase (Hayano and Katami, 1977; Hayano and Tubaki, 1985). However, further studies are worthwhile to validate this hypothesis.

Table 3. Simpson–Yule index (SYI) in the 8 soil microenvironments.

	SYI
PAO	6.94 bc
PAU	7.76 a
HAO	7.57 ab
HAU	7.54 ab
VGI	6.73 bc
VGR	6.77 bc
VTI	7.06 abc
VTR	6.54 c

Different letters within each column indicate differences at $P < 0.05$ (Fisher's protected LSD method). PAO: pasture out of tree canopy; PAU: pasture under tree canopy; HAO: hay-land out tree canopy; HAU: hay-land under tree canopy; VGI: grass-covered vineyard, interrow; VGR: grass-covered vineyard, along the row; VTI: tilled vineyard, interrow; VTR: tilled vineyard, along the row.

Higher αG under the oak canopies was attributed to the input of acorn biomass (Lee et al., 2011). The lack of significant differences in terms of AP between microenvironments of the 2 grassland management types and the tilled vineyard was associated with sheep dejections and the addition of poultry droppings as organic fertilizer in the tilled vineyard (Okur et al., 2009). Higher ARYL and NAG activities observed under the oak canopy in the pasture were associated with macrofungal richness, as these saprophytic organisms benefit from nutrients' availability and hyphae if soils are preserved from tillage (Santos-Silva et al., 2011). In fact, Castillo-Monroy et al. (2011) observed a high covering of soil biological crust under perennial plant canopy if soil is undisturbed. Moreover, ARYL and NAG were linearly correlated with TOC, MR_{basal}, C_0, and qM; therefore, the activities of these enzymes were associated with both high soil metabolic activity and TOC. This finding was consistent with the expected positive effect of biodiversity on nutrient utilization efficiency and on soil carbon sequestration capacity (Maestre et al., 2012). The PAU microenvironment seems to generate such favorable conditions.

Within vineyards, the higher βG was associated with a greater cellulose availability under grass cover, due to the persistent input of grass growing and chopped pruning residuals that are removed from the field in the tilled vineyards.

The grouping of HAO, HAU, and PAO in the same cluster was interpreted as the effect of recurrent tillage which, in the long term, may have contributed to determining homogeneous characteristics in terms of microbial communities and their equilibrium with organic matter pools (Schmidt et al., 2011). According

Table 4. Correlation matrix between total organic carbon (TOC), labile carbon (LC), microbial biomass carbon (MBC), basal microbial respiration (MR_{basal}), potentially mineralizable carbon (C_0), rate constant (k), mineralization quotient (qM), and activities of β-glucosidase (βG), α-glucosidase (αG), acid phosphatase (AP), arylsulfatase (ARYL), N-acetyl-β-glucosaminidase (NAG), xylosidase (XYL), acetate esterase (EST), and butyrate esterase (BUT).

	TOC	LC	MBC	MR_{basal}	C_0	k day^{-1}	qM
βG	0.48*	ns	ns	0.70***	0.77***	ns	−0.44*
αG	0.59**	ns	ns	0.54*	0.47*	ns	−0.53*
AP	0.73***	0.63**	0.55*	0.59**	0.50*	ns	−0.67**
ARYL	0.75***	0.45*	0.61**	0.84***	0.78***	ns	−0.68***
NAG	0.78***	0.47*	0.67**	0.85***	0.79***	ns	−0.74***
XYL	0.64**	ns	0.53*	0.69***	0.67**	ns	−0.64**
EST	0.47*	ns	ns	ns	ns	ns	−0.48*
BUT	0.70***	0.61**	0.62**	0.67*	0.66**	ns	−0.68***

*: P < 0.05; **: P < 0.01; ***: P < 0.001; ns: not significant; n = 20.

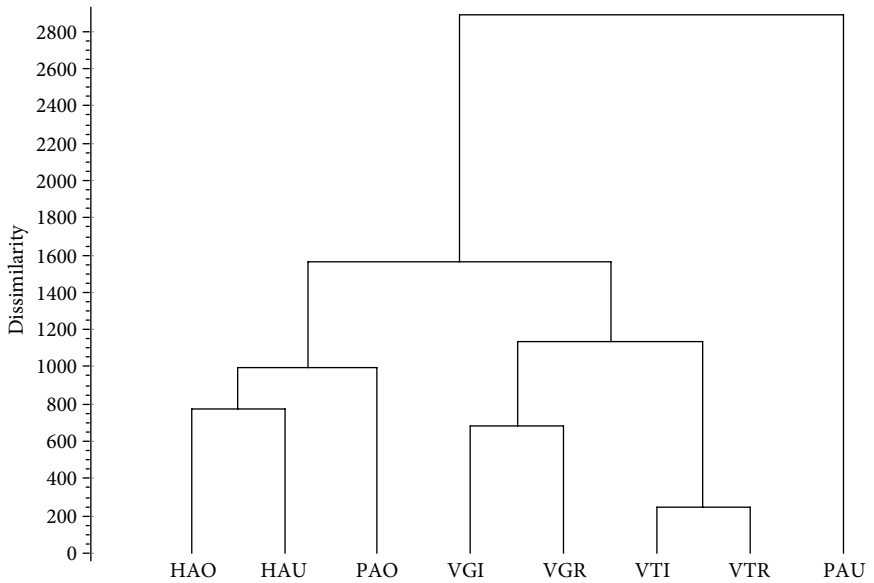

Figure 2. Clustering by enzyme activities of the 8 microenvironments. HAO: hay-land out of tree canopy; HAU: hay-land under tree canopy; PAO: pasture out of tree canopy; VGI: grass-covered vineyard, interrow; VGR: grass-covered vineyard, along the row; VTI: tilled vineyard, interrow; VTR: tilled vineyard, along the row; PAU: pasture under tree canopy.

to what was observed by Lagomarsino et al. (2011), the 2 vineyard management types appeared not to be influenced by tillage, as the 4 microenvironments formed a single cluster characterized by the lowest functional diversity index. Thus, it is evident that the soil functional diversity was flattened by the increased anthropic pressure (e.g., deep inversion tillage at the vineyard establishment) when compared to that of the grassland habitats.

In conclusion, wooded grazed grasslands were more effective in producing spatial variation of topsoil organic C than vineyards. Under tree canopy in the grassland we found the highest soil organic C content, which was correlated to a high enzymatic activity. The soil microbial functional diversity increased with decreasing level of anthropic pressure in different microenvironments. According to these results, it appears relevant to consider spatial variability when assessing the C sink capacity and the C dynamics of wooded grasslands; this variability appears less important in the case of woody systems with higher levels of anthropization. However, further studies are needed for a more in-depth understanding of spatial microgradients associated with isolated tree canopies in wooded grasslands and the role of these latter factors as drivers of soil functional variability.

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References

Almagro M, López, J, Querejeta, J, Martínez-Mena M (2009). Temperature dependence of soil CO_2 efflux is strongly modulated by seasonal patterns of moisture availability in a Mediterranean ecosystem. Soil Biol Biochem 41: 594–605.

Andivia E, Fernández M, Vázquez-Piqué J, González-Pérez A, Tapias R (2010). Nutrients return from leaves and litterfall in a Mediterranean cork oak (*Quercus suber* L.) forest in southwestern Spain. Eur J Forest Res 129: 5–12.

Bacchetta G, Bagella S, Biondi E, Farris E, Filigheddu R, Mossa L (2009). Forest vegetation and serial vegetation of Sardinia (with map at the scale 1:350,000). Fitosociologia 46: 3–82.

Badalucco L, Grego S, Dell'Orco S, Nannipieri P (1992). Effect of liming on some chemical, biochemical and microbiological properties of acid soil under spruce (*Picea abies* L.). Biol Fert Soils 14: 76–83.

Bagella S, Caria MC (2011). Vegetation series: a tool for the assessment of grassland ecosystem services in Mediterranean large-scale grazing systems. Fitosociologia 48: 47–54.

Bagella S, Satta A, Floris I, Caria MC, Rossetti I, Podani J (2013). Effects of plant community composition and flowering phenology on honeybee foraging in Mediterranean sylvo-pastoral systems. Appl Veg Sci 16: 689–697.

Bending GD, Turner, MK, Rayns F, Marx MC, Wood M (2004). Microbial and biochemical soil quality indicators and their potential for differentiating areas under contrasting agricultural management regimes. Soil Biol Biochem 36: 1785–1792.

Carlisle EA, Steenwerth KL, Smart DR (2006). Effects of land use on soil respiration: conversion of oak woodlands to vineyards. J Environ Qual 35: 1396–1404.

Castillo-Monroy AP, Maestre FT, Rey A, Soliveres S, García-Palacios P (2011). Biological soil crust microsites are the main contributor of soil respiration in a semiarid ecosystem. Ecosystems 14: 835–847.

Cavigelli MA, Lengnick LL, Buyer JS, Fravel D, Handoo Z, McCarty G, Millner P, Sikora L, Wright S, Vinyard B et al. (2005). Landscape level variation in soil resources and microbial properties in a no-till corn field. Appl Soil Ecol 29: 99–123.

Chatterjee A, Jenerette GD (2011). Changes in soil respiration Q10 during drying-rewetting along a semi-arid elevation gradient. Geoderma 163: 171–177.

Conant RT, Paustian K, Elliott ET (2001). Grassland management and conversion into grassland: effects on soil carbon. Ecol Appl 11: 343–355.

Dhillon KS, Dhillon SK, Thind HS (2008). Evaluation of different agroforestry tree species for their suitability in the phytoremediation of seleniferous soils. Soil Use Manage 24: 28–216.

Di Bene C, Pellegrino E, Tozzini C, Bonari E (2011). Changes in soil quality following poplar short-rotation forestry under different cutting cycles. Ital J Agron 6: 28–35.

Dumontet S, Mazzatura A, Casucci C, Perucci P (2001). Effectiveness of microbial indexes in discriminating interactive effects of tillage and crop rotations in a Vertic Ustorthens. Biol Fert Soils 34: 411–416.

Francaviglia R, Coleman K, Whitmore AP, Doro, L, Urracci G, Rubino M, Ledda L (2012). Changes in soil organic carbon and climate change – Application of the RothC model in agro-silvo-pastoral Mediterranean systems. Agr Syst 112: 48–54.

Gómez-Rey MX, Garcês A, Madeira M (2012). Soil organic-C accumulation and N availability under improved pastures established in Mediterranean oak woodlands. Soil Use Manage 28: 497–507.

Grayston SJ, Campbell CD (1996). Functional biodiversity of microbial communities in the rhizospheres of hybrid larch (*Larix eurolepis*) and Sitka spruce (*Picea sitchensis*). Tree Physiol 16: 1031–1038.

Hayano K, Katami A (1977). Extraction of β-glucosidase activity from pea field soil. Soil Biol Biochem 9: 349–351.

Hayano K, Tubaki K (1985). Origin and properties of β-glucosidase activity of tomato-field soil. Soil Biol Biochem 17: 553–557.

Howlett DS, Marcose MG, Mosquera-Losada MR, Nair PKR, Nair VD (2011). Soil carbon storage as influenced by tree cover in the Dehesa cork oak silvopasture of central-western Spain. J Environ Monitor 13: 1897–904.

Hurlbert SH (1984). Pseudoreplication and the design of ecological field experiments. Ecol Monogr 54: 187–211.

Keil D, Meyer A, Berner D, Poll C, Schützenmeister A, Piepho HP, Vlasenko A, Philippot L, Schloter M, Kandeler E et al. (2011). Influence of land-use intensity on the spatial distribution of N-cycling microorganisms in grassland soils. FEMS Microbiol Ecol 77: 95–106.

Lagomarsino A, Benedetti A, Marinari S, Pompili L, Moscatelli MC, Roggero PP, Lai R, Ledda L, Grego S (2011). Soil organic C variability and microbial functions in a Mediterranean agro-forest ecosystem. Biol Fert Soils 47: 283–291.

Lai R, Seddaiu G, Gennaro L, Roggero PP (2012). Effects of N fertilizer sources and temperature on soil CO_2 efflux in Italian ryegrass crop under Mediterranean conditions. Ital J Agron 7: 196–201.

Lee JW, Ha MG, Yi YB, Chung CH (2011). Chromium halides mediated production of hydroxymethylfurfural from starch-rich acorn biomass in an acidic ionic liquid. Carbohyd Res 346: 177–182.

Maestre FT, Quero JL, Gotelli NJ, Escudero A, Ochoa V, Delgado-Baquerizo M, García-Gómez M, Bowker MA, Soliveres S, Escolar C et al. (2012). Plant species richness and ecosystem multifunctionality in global drylands. Science 335: 214–218.

Mariscal-Sancho I, Santano J, Mendiola MA, Peregrina F, Espejo R (2010). Carbon dioxide emission rates and β-glucosidase activity in Mediterranean Ultisols under different soil management. Soil Sci 175: 453–460.

Marx MC, Wood M, Jarvis SC (2001). A microplate fluorimetric assay for the study of enzyme diversity in soils. Soil Biol Biochem 33: 1633–1640.

Moreno G, Obrador JJ, Garcìa A (2007). Impact of evergreen oaks on soil fertility and crop production in intercropped dehesas. Agr Ecosyst Environ 119: 270–280.

Nelson DW, Sommers LE (1996). Total carbon, organic carbon, and organic matter. In: Sparks DL, Page AL, Helmke PA, Loeppert RH, Soltanpour PN, Tabatabai MA, Johnston CT, Sumner ME, editors. Methods of Soil Analysis. Part 3. Chemical Methods. Book Series No. 5. Madison, WI, USA: Soil Science Society of America, pp. 961–1010.

Okur N, Altındışlı A, Çengel M, Göçmez S, Kayıkcıoğlu HH (2009). Microbial biomass and enzyme activity in vineyard soils under organic and conventional farming systems. Turk J Agric For 33: 413–423.

Pausch J, Tian J, Riederer M, Kuzyakov Y (2013). Estimation of rhizodeposition at field scale: upscaling of ^{14}C labelling study. Plant Soil 364: 273–285.

Pinzari F, Trinchera A, Benedetti A, Sequi P (1999). Use of biochemical indices in the Mediterranean environment: comparison among soils under different forest vegetation. J Microbiol Meth 36: 21–28.

Podani J (2001). SYN-TAX 2000. Computer Programs for Data Analysis in Ecology and Systematics. User's Manual. Budapest, Hungary: Scientia.

Riffaldi R, Saviozzi A, Levi-Minzi R (1996). Carbon mineralization kinetics as influenced by soil properties. Biol Fert Soils 22: 293–298.

Rodríguez-Loinaz G, Onaindia M, Amezaga I, Mijangos I, Garbisu C (2008). Relationship between vegetation diversity and soil functional diversity in native mixed-oak forests. Soil Biol Biochem 40: 49–60.

Santos-Silva C, Gonçalves A, Louro R (2011). Canopy cover influence on macrofungal richness and sporocarp production in montado ecosystems. Agroforest Syst 82: 149–159.

SAS Institute (1999). SAS/STAT User's Guide, Version 8.2. Cary, NC, USA: SAS Institute.

Schmidt MWI, Torn MS, Abiven S, Dittmar T, Guggenberger G, Janssens IA, Kleber M, Kögel-Knabner I, Lehmann J, Manning DAC et al. (2011). Persistence of soil organic matter as an ecosystem property. Nature 478: 49–56.

Seddaiu G, Porcu G, Ledda L, Roggero PP, Agnelli A, Corti G (2013). Soil organic matter content and composition as influenced by soil management in a semi-arid Mediterranean agro-silvo-pastoral system. Agr Ecosyst Environ 167: 1–11.

Sun X, Wang G, Lin Y, Liu L, Gao Y (2013). Intercepted rainfall in *Abies fabri* forest with different-aged stands in Southwest China. Turk J Agric For 37: 494–504.

Vance ED, Brookes PC, Jenkinson DS (1987). An extraction method for measuring soil microbial biomass C. Soil Biol Biochem 19: 703–707.

Vepsäläinen M, Kukkonen S, Vestberg M, Sirviö H, Niemi RM (2001). Application of soil enzyme activity test kit in a field experiment. Soil Biol Biochem 33: 1665–1672.

Waldrop MP, Firestone MK (2006). Seasonal dynamics of microbial community composition and function in oak canopy and open grassland soils. Microb Ecol 52: 470–479.

Williams JN, Hollander AD, O'Geen AT, Thrupp LA, Hanifin R, Steenwerth K, McGourty G, Jackson LE (2011). Assessment of carbon in woody plants and soil across a vineyard-woodland landscape. Carbon Balance Manag 6: 11.

Worldwide Bioclimatic Classification System (1996–2009). S. Rivas-Martinez & S. Rivas-Saenz, Phytosociological Research Center, Spain, http://www.globalbioclimatics.org.

WRB (2006). World Reference Base for Soil Resources World Soil Resources Report 103. Rome: IUSS, ISRIC, FAO.