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Do improved pastures affect enzymatic activity and C and N dynamics in soils of the *montado* system?

As pastagens melhoradas alteram a actividade enzimática e a dinâmica do C e do N nos solos do sistema montado?

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ABSTRACT

Vast *montado* areas are threatened by degradation, as the result of a long history of land use changes. Since improved pastures have been installed aiming soil quality improvement and system sustainability, it is crucial to evaluate the effects of these management changes on soil organic matter status and soil biological activity, as soil quality indicators. Therefore, a 35-yr old improved pasture and a natural pasture were studied, considering areas beneath tree canopy and in the open. Total organic C, total N, hot water soluble (HWS) and particulate (POM) C, microbial biomass C (MBC) and N (MBN), C mineralization rate (CMR) and net N mineralization rate (NMR) were determined. In addition, for a 1-yr period, soil β-glucosidase, urease, proteases and acid phosphomonoesterase were periodically determined. Improved pasture promoted the increase of soil C and N through POM-C increment, particularly beneath the trees canopies. The two study pastures did not show differences regarding soil microbial biomass, but variations in CMR, HWS-C and N availability (proteases and urease activities) suggest divergent soil microbial communities. Tree regulator role on C, N and P transformation processes in soil was confirmed.

Keywords: C and N mineralization rates; evergreen oak woodland management; organic matter fractions; soil enzymes; soil microbial biomass.

RESUMO

Muitas áreas de montado estão em risco de degradação como resultado de um longo historial de modificações de uso. Nalgumas áreas foram instaladas pastagens melhoradas, visando melhoria da qualidade do solo e a sustentabilidade do sistema. Os efeitos desta alteração de gestão foram avaliados comparando uma pastagem melhorada com 35 anos e uma pastagem natural, considerando-se áreas sob e fora da influência das copas. Determinaram-se os teores de C orgâncio e N total, de C das fracções solúvel em água quente (HWS) e particulada (POM) e de C e de N na biomassa microbiana (MBC e MBN), e as taxas de mineralização de C e N (CMR e NMR). Determinaram-se ainda periodicamente as actividades das enzimas β-glucosidase, urease, proteases e fosfomonoesterase ácida do solo. A pastagem melhorada promoveu o aumento de C e N do solo através do aumento do C da POM, principalmente em áreas sob a copa das árvores. A biomassa microbiana do solo não distinguiu as duas pastagens, mas diferenças na CMR, no HWS-C e na disponibilização de N (actividade das proteases e da urease) sugerem comunidades microbianas do solo divergentes. Confirmou-se o papel regulador das árvores sobre os processos de transformação do C, do N e do P no solo.

Palavras-chave: biomassa microbiana do solo; enzimas do solo; fracções de matéria orgânica; gestão do montado; taxas de mineralização de C e N.

Introduction

Mediterranean agroforestry systems have a renewed interest, not only for their economic value, either for forest and agriculture productivity, as for the associated environmental, social, and cultural values (Rigueiro-Rodríguez *et al*., 2009). The *montado* system is the most extensive agroforesty system in the Iberian Peninsula, where it occupies about 3 million hectares (Eichhorn *et al*., 2006). Oak trees, especially *Quercus suber* L. and *Quercus rotundifolia* Lam., are associated with agricultural crops, grassland or shrubs, forming a savannah-like landscape. This ecosystem supports large production diversity - cork, acorns, aromatic and medicinal plants, extensive pasture, fuel (wood and charcoal), cinegetic species, among others - and significant environmental services, like carbon sequestration and biodiversity conservation.

Until the 1970 decade, Portuguese *montado* systems were overexploited with cereal crops, which contributed to strong soil degradation. Nowadays, the majority of these areas are used for permanent pastures under an increased grazing pressure. Consequently, problems of soil erosion, compaction and decline of organic matter, as well as ecosystem biodiversity losses and deficient tree regeneration are threatening the *montado* long term sustainability (Moreno and Pulido, 2009). Additionally, natural climate variability in the Mediterranean region and future climate change scenarios (Miranda *et al*., 2002) entail risks for the *montado* system.

Improved pastures, rich in legumes, have been recently installed in some of these agro-ecosystems, aiming increased stocking rates and soil quality enhancement (Haynes and Williams, 1993; Gómez-Rey *et al*., 2012). It is known that trees in the *montado* form patches ("islands") with different soil fertility levels, nutrient turnover rates, microclimatic conditions and energy availability, thus creating a mosaic landscape (Moreno and Pulido, 2009). In this context, it is essential to understand the form and extension of biogeochemical processes changes arising from recent management changes, the inherent heterogeneous systems structure, and climatic conditions variability.

A study was developed under the hypothesis that improved pasture could modify soil organic matter status both in quantity and quality, the associated biological activity, and that these effects might also be altered by tree canopy cover. Soil carbon and nitrogen dynamics - total, labile fractions, mineralization rates and cycle-related enzymes activities - from two adjacent *montado* areas with different management practices (natural and sown pastures) were quantified and compared. Since phosphorus is a key nutrient for legume-rich pasture establishment and maintenance, soil extractable P and phosphatase activity were also evaluated. Given the relevant effects of soil water content and temperature on soil biological activity, soil enzymatic activity was determined seasonally in an attempt to monitor soil organic matter transformation processes throughout the year.

Materials and Methods

Study areas

Study sites were located in two adjacent cork oak (*Quercus suber* L.) *montados* at *Herdade dos Esquerdos* (Vaiamonte; 39°07′-39°08′ N, 7°29′-7°30′ W), in the NUT III Portuguese region of Alto Alentejo. Both areas are characterized by gentle undulated topography and soils are developed over gneiss, with sandy loam texture and classified as *Leptic Regosols* associated with *Leptosols* with dystric characteristics (IUSS, 2006).

The climate is Mediterranean mesothermic dry sub-humid, therefore, presenting hot and dry summers (Figure 1). Mean annual rainfall is 620 mm, 80% of which occurs between October and May. Mean annual temperature is 15 °C, varying from 8.4 °C in January to 23.5 °C in August (INMG, 1991). Rainfall and air and soil temperatures were daily recorded from November 2011 to October 2012 by a local automatic weather station. When compared with climate normals (1951-1980) for the Portalegre meteorological station, rainfall from December 2011 to April 2012 was abnormally low.

Improved pasture area (IP) was sown thirty five years ago, after a long period of traditional rotation including cereals and grazing pasture. Seed mixture included mainly *Trifolium* spp., *Ornithopus* spp. and *Lolium* spp. Trees cover about 35% of ground area, corresponding to 30-40 trees ha^{-1}. Every two years, 300 kg ha^{-1} of natural rock phosphate (26.5% P_2O_5, 35% CaO, 3.2% SO_3 and 0.8% MgO) were applied to the soil. The area has been grazed by sheep and pig herds, with an annual stocking rate of 5 to 8 animal ha^{-1}.

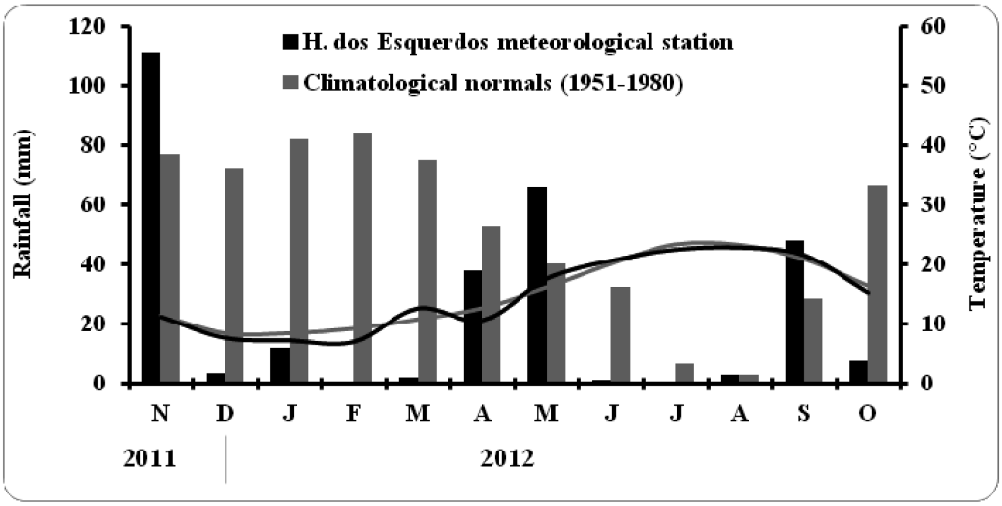


Figure 1 - Average monthly air temperature (°C) and cumulative average monthly rainfall (mm) at Portalegre meteorological station from 1951 to 1980 and in *Herdade dos Esquerdos* (HE) metereological station from November 2011 to October 2012.

The adjacent area managed as a natural pasture was assumed as a control treatment (NP). This area has been grazed by 0.9 sheep ha^{-1} year^{-1}. Shrubs, mostly *Quercus coccifera* L., *Cistus* spp. and *Crataegus monogyna* Jacq., were controlled every four to six years by soil tillage (disking). Natural herbaceous vegetation included composite plants and legumes such as *Chamaemelum mixtum* (L.) All., *Leontodon taraxacoides* (Vill.) Mérat, *Trifolium* spp., *Ornithopus* spp. and *Biserrula pelecinus* L. (STRAW, 2014).

Soil sampling

In each area, six 100×100 m plots were delimitated parallel to and 20 m away from the boundary between the two systems. Each plot was divided in four 50×50 m sub-plots in the center of which a circular 1256 m^2 area (40 m radius) was considered, following the official Portuguese forestry inventory procedures (DGF, 2001). Two of the resulting four circles were randomly selected and two representative trees (similar crown and breast height diameters) were marked in each one, totalizing 12 trees for each pasture management system. Soil samples were taken beneath each selected tree canopy (about half distance from the trunk) and in open area (approximately twice the crown radius away from the trunk). At these sampling points, three undisturbed soil samples were collected at 0-10 cm depth for bulk density determination.

For chemical characterization, disturbed soil samples were collected at 0-10 cm depth approximately at the four cardinal points direction. These four soil samples were mixed to form a composite sample for each tree and position (beneath and out of tree canopy), which were then air dried and passed through a 2 mm sieve.

The same scheme was followed to collect samples from the top 5 cm soil layer. These samples were randomly combined two by two, and resulting composite samples were sieved at 5 mm and kept refrigerated (approximately 4 °C) until all biochemical determinations were completed.

For enzymatic activity determinations, from autumn 2011 to summer 2012, soil samples (0-5 cm) were seasonally collected (2 December 2011, 2 March 2012, 20 April 2012 and 20 August 2012) as previously described. Fresh soil samples were randomly paired, mixed, sieved at 2 mm and kept refrigerated at about 4°C. Deviations in rainfall distribution along the study period made impossible to collect samples in the middle of annual seasons; for example, autumn sampling took place in December, due to a delay in the beginning of the rainy season, while winter sampling was postponed to March, also owing to the absence of expected season rainfall (see Fig. 1).

Laboratory procedures

Undisturbed soil samples were oven-dried at 105 °C until constant weight for soil bulk density calculation.

The 0-10 cm air dried soil samples were used to determine total organic C by the potassium dichromate oxidation method and total N by the Kjedahl method (Póvoas and Barral, 1992). In the same samples, hot water soluble C was extracted in a soil suspension on hot water (85 °C) after one hour at equilibrium (Khanna *et al.*, 2001), and measured using an autoanalyzer. Carbon in particulate organic matter (POM) fraction (corresponding to the non-humified OM), obtained by wet sieving at 50 μm, was also determined by the dichromate oxidation method. Soil characteristics, such as pH in a 1:2.5 soil:water suspension, non acid cations (Ca^{2+}, Mg^{2+}, Na^+ and K^+) and Egnér-Riehm extractable potassium and phosphorous were also quantified.

The autumn (first sampling date) wet soil samples collected from the top 5 cm soil layer were used in the fumigation-extraction method proposed by Vance *et al.* (1987) to measure the microbial biomass C and N. Also, 50 g sub-samples were incubated at about 60% of water field capacity and 25°C for 120 days for C mineralization rate assessment. Samples were placed in hermetic jars with a 0.5 M NaOH solution to absorb the released CO_2. Other sub-samples (approximately 1 kg) were used for a 16-week incubation period (25 °C, 60% water field capacity) to determine net N mineralization rate. Soil was kept in plastic bags and sub-samples (10 g) were periodically extracted with 50 mL of 2M KCl solution for mineral N (NO_3^--N and NH_4^+-N) determinations using an autoanalyzer (García *et al.*, 2003).

Soil enzymatic activity, namely β-glucosidase, urease, protease and acid phosphomonoesterase were measured in seasonally collected 0-5 cm wet soil samples using methods described by Tabatabai (1982), Kandeler *et al*. (1999), Ladd and Butler (1972) and Tabatabai and Bremner (1969), respectively.

Results were expressed at 105ºC oven-dry basis and soil water content was determined for all samples and dates.

Statistical analysis

Total C and N content, C and N fractions and mineralization rates were subjected to analysis of variance (ANOVA) at a significant probability of 5%, with all combinations of management (improved and natural pasture) and position (beneath canopy or open-field) as a single factor with four levels. Means, standard deviation and Tukey test significant separations (α=0.05) are presented.

Enzymatic activity data were analysed by ANOVA with management and position as factors. Given the great influence of timing of sampling on the variability of results, each date was analysed separately. When necessary, means were separated using Tukey test (α=0.05). Correlation coefficients were calculated for enzyme activity and soil water content, as well as for soil and air temperature.

Results

Physical and chemical soil status

Improved pasture soil showed lower soil bulk density when compared with natural pasture, although only significantly so beneath the trees canopies (Table 1). Higher sum of non acid cations and a significant increase in extractable P content were observed in improved pasture related to the natural pasture, either beneath tree canopy or in the open. Extractable K was lower, although not significantly, in improved than in natural pasture soils.

Improved pasture induced significant increases in soil total organic C and total N concentrations, comparatively with natural pasture area (Table 2). This effect was evident in the open (49% increase in organic C, and 39% in N), as well as under tree

Table 1 - Soil bulk density (BD), soil reaction (pH-H_2O), sum of exchangeable non acid cations (Ca^{2+}, Mg^{2+}, Na^+ and K^+), extractable phosphorus (P) and potassium (K) in the 0-10 cm soil layer of improved (IP) and natural (NP) pasture, beneath tree crown (BC) and in open area (OA) (mean±standard deviation; n=12)

		BD	**pH-H_2O**	**Non acid cations**	**P**	**K**
		g cm^{-3}		$cmol_c$ kg^{-1}	μg g^{-1}	
OA	IP	1.36±0.06**a**	5.57±0.27**a**	5.81±2.32**b**	50.9±20.0**b**	96.6±45.3**c**
	NP	1.48±0.09**a**	5.56±0.20**a**	3.36±0.72**c**	4.4±2.4**d**	156.4±43.7**bc**
BC	IP	1.15±0.14**b**	5.67±0.25**a**	6.52±2.33**a**	74.9±29.0**a**	204.7±116.6**ab**
	NP	1.36±0.21**a**	5.66±0.21**a**	5.31±1.13**b**	8.4±3.5**c**	258.5±57.5**a**

Values in the same column followed by the same letter are not significantly different at α = 0.05.

canopy (67 and 72% increases in total organic C and total N, respectively).

C and N dynamics

Soil particulate organic matter carbon (POM) in open areas was nearly the double (but not significantly different) in the improved pasture, compared with the natural pasture (Table 2). Tree cover exhibited the main differentiating effect regarding this parameter, resulting in a 16% increase in POM proportion to total organic C in the improved pasture, compared with the natural pasture.

The same pattern of variation was shown by the hot water soluble carbon concentration, with 106 and 158% increase in improved compared with natural pasture soils, beneath tree canopy and in the open, respectively (Table 2). Differences between management systems were more evident in the open, with hot water soluble C fraction (as a percentage of total C) being 1.5% higher in improved than in natural pasture.

No significant differences were observed in microbial biomass C and N concentrations between pastures (Table 3). Proportions of these fractions to total organic C and total N were significantly higher in the natural pasture, in areas beyond tree influence. Beneath tree crown, relative proportion of microbial biomass C and N were comparatively lower and did not distinguish the two pasture management systems.

After 120 days of soil incubation, carbon mineralization rate was higher for the natural pasture (5.8 and 4% in the open and beneath tree crown, respectively) compared with the improved pasture (about 3% for both positions) (Table 3). In contrast, Net N mineralization rate was not significantly different between improved and natural pastures both beneath tree crown (about 11% for both pastures) and in the open (6.0 and 3.7% in improved and natural pastures, respectively).

Enzymatic activity

As a result of the abnormal rainfall during the study period, soil water content was relatively low in all sampling dates, the highest values being lower than 30% water content (w/w), obtained in autumn 2011 (Figure 2).

Soil enzymes activities associated with C, N and P cycles were clearly influenced by tree canopy but did not respond consistently to pasture management (Figure 3).

The β-glucosidase activity did not differ at any sampling date in soils with different pasture

Table 2 - Total organic C (C), total N (N), particulate C (POM), hot water soluble C (HWS) and labile C percentages in total C (POM/C and HWS/C) in the 0-10 cm soil layer of improved (IP) and natural (NP) pasture, under (BC) and out (OA) of tree canopy influence (mean±standard deviation; n=12)

		C	N	POM	HWS	POM/C	HWS/C
		g kg^{-1}				%	
OA	IP	21.19±5.83**b**	1.74±0.45**b**	8.44±4.05**b**	1.03±0.45**b**	38.1±8.1**ab**	4.8±1.3**a**
	NP	12.89±3.24**c**	1.03±0.24**c**	4.83±2.08**b**	0.40±0.15**c**	38.8±19.8**ab**	3.3±1.6**b**
BC	IP	35.47±10.23**a**	2.54±0.53**a**	17.20±5.80**a**	1.63±0.68**a**	48.1±7.6**a**	4.5±1.2**ab**
	NP	23.77±3.59**b**	1.82±0.23**b**	7.65±1.98**b**	0.79±0.18**bc**	32.6±7.8**b**	3.3±0.7**b**

Values in the same column followed by the same letter are not significantly different at α = 0.05.

Table 3 - Microbial biomass C and N (MBC, MBN), relative percentages of total C and N (MBC/C, MBN/N), C mineralization rate (CMR) and net N mineralization rate (NMR) in the 0-5 cm soil layer of improved (IP) and natural (NP) pastures, under (BC) and out (OA) of tree canopy influence (mean±standard deviation; n=6)

		MBC	MBN	MBC/C	MBN/N	CMR	NMR
		mg kg^{-1}		%		mg g^{-1}	
OA	IP	354.0±82.8**a**	72.1±23.6**ab**	1.68±0.36**b**	4.16±1.20 **b**	29.6±6.1**b**	60.4±37.5**a**
	NP	344.2±97.3 **a**	77.1±23.9**a**	2.71±0.68**a**	7.54±2.16**a**	58.3±19.3**a**	36.5±32.5**a**
BC	IP	236.2±53.3 **ab**	41.3±11.0**b**	0.68±0.17**c**	1.62±0.33**c**	31.2±8.7**b**	110.9±58.4**a**
	NP	193.6±107.2 **b**	40.3±25.8**b**	0.84±0.50**c**	2.31±1.65**bc**	40.9±9.6**ab**	109.1±57.9**a**

Values in the same column followed by the same letter are not significantly different at α = 0.05.

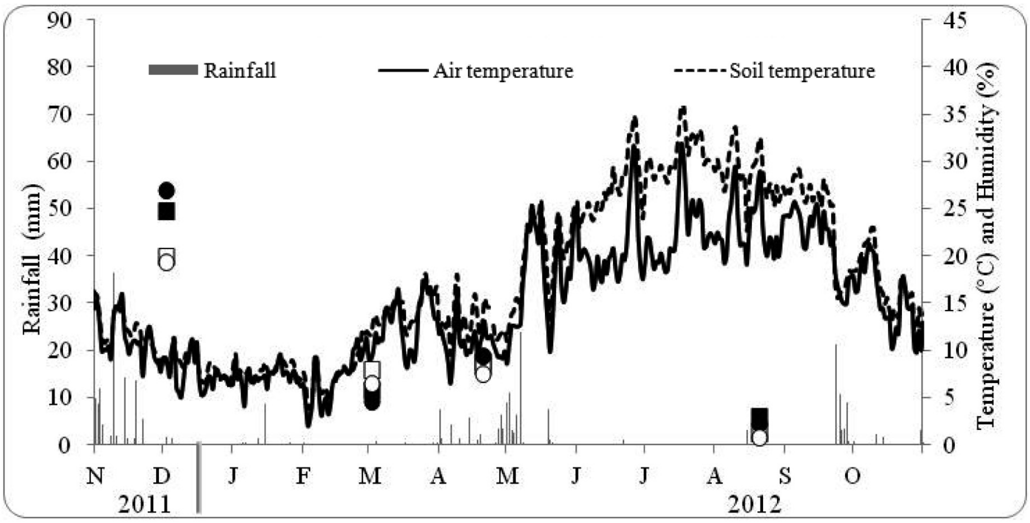


Figure 2 - Daily rainfall (mm) and average daily air and soil (4 cm depth) temperatures (°C) recorded by the *Herdade dos Esquerdos* meteorological station and soil water content (w/w) determined in collected soil samples (n=6). IP BC - ■; IP OA - □; NP BC - ●; NP OA - ○.

management (Figure 3-a), whereas tree canopy potentiated a higher activity throughout the year, although only significantly in summer in the natural pasture.

Natural pasture presented a greater urease activity in three sampling dates, the exception being summer time, when there was no significant difference between pastures (Figure 3-b). On the other hand, soil proteases activity was higher in the improved pasture during winter (Figure 3-c). N cycle-related soil enzymatic activity was positively enhanced under the tree canopy during the whole study period.

Natural pasture showed a higher acid phosphomonoesterase activity than improved pasture, but differences were only significant in the autumn (Figure 3-d). Tree canopy has negatively affected this enzymatic activity throughout the year, except for summer when no significant differences were found.

The highest β-glucosidase activity in summer resulted in a strong and positive correlation with soil and air temperatures, but a negative correlation was found with soil water content (Table 4). Urease activity was also positively correlated with air and soil temperatures and negatively correlated with soil water content, while proteases activity showed a reverse correlation with these environmental factors. Acid phosphomonoesterase activity showed strong and positive correlation with soil water content, whereas no correlation was found with air and soil temperature.

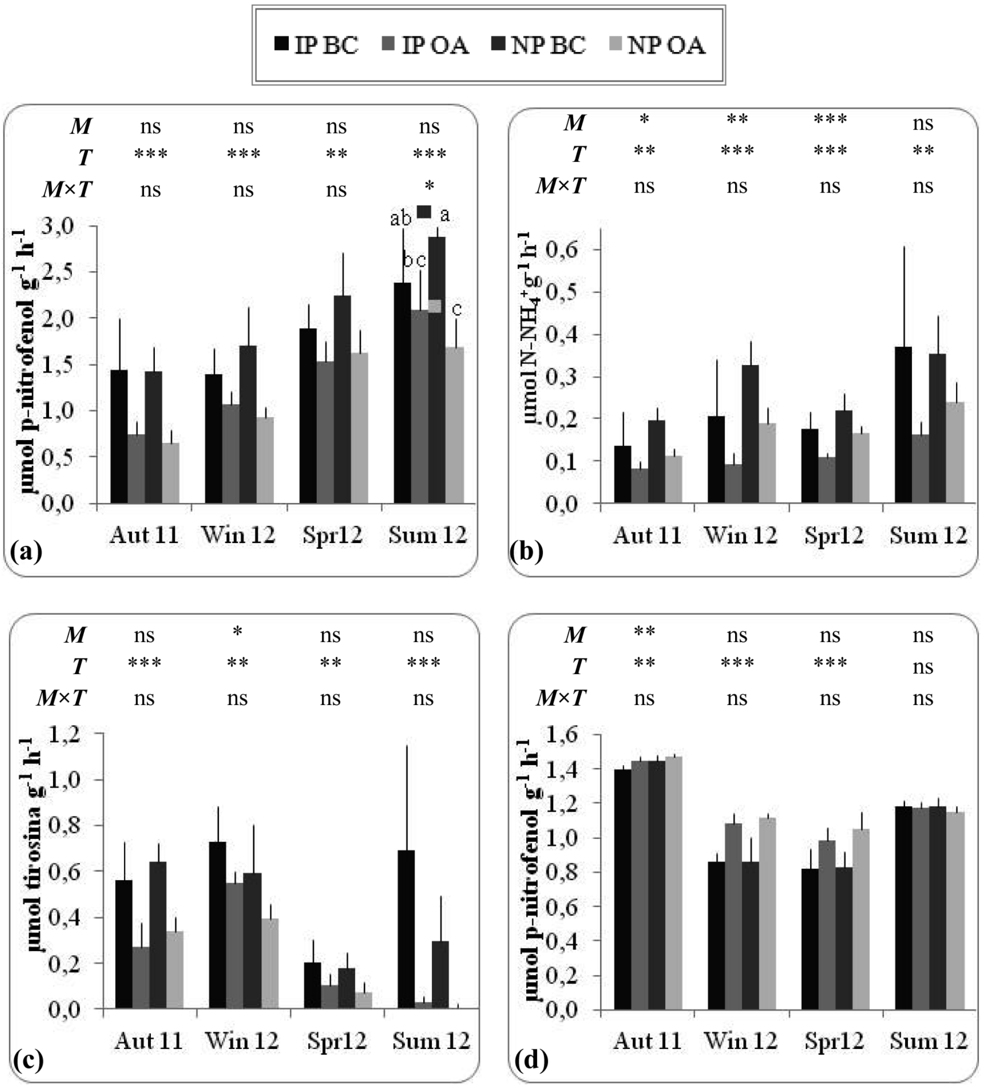
Discussion

Physical and chemical soil status

Although the two studied pastures showed similar soil reaction, differences were found in soil chemical characteristics both beneath and out of tree canopy. Variations in soil extractable P were the most relevant difference between pasture management areas, as the result of frequent phosphate fertilizer application in the improved pasture area. Exchangeable non-acid cations followed the same trend, mainly due to an increase in exchangeable Ca^{2+} (data not shown) driven by fertilizer applications.

Decreasing concentration of soil extractable K observed in improved pasture may be associated with the higher pasture productivity and consequent higher extraction by pasture root system. In fact, soil extractable K in improved pastures of open areas is already below 125-150 mg K kg^{-1}, the threshold recommended for satisfactory permanent pasture development in the Alentejo region (Serrano *et al.*, 2014). Thus, future K fertilizer needs should be considered for such area to warrant this nutrient availability at an adequate level. Furthermore, our results highlight K demands by improved pastures as an important issue that should be addressed before their installation, especially in soils that are naturally poor in this nutrient (Haynes and Williams, 1993).

In open areas, greater herbaceous biomass production in sown than in natural pasture



ns - not significant ($p \geq 0.05$); * - significant for $\alpha<0.05$;
**** - significant for $\alpha<0.01$; *** - significant for $\alpha<0.001$.**

Figure 3 - ANOVA results for each sampling date for pasture (P), tree (T) and M×T interaction. Mean activity (± standard deviation) of soil β-glucosidase (a), urease (b), proteases (c) and acid phosphomonoesterase (d) in the study areas from autumn 2011 to summer 2012 (n=6). Graphic bars in the same group (date) with different letters are significantly different at α=0.05.

Table 4 - Correlation coefficients (r) between soil enzyme activity and soil water content and mean daily soil and air temperatures

Enzyme activity	Soil water content	Air temperature	Soil temperature
β-glucosidase	-0.45**	0.62**	0.62**
Urease	-0.38**	0.45**	0.43**
Proteases	0.26**	-0.24*	-0.27**
Fosfatase	0.60**	0.01 ns	0.05 ns

ns - not significant (p>0.05); * - significant for α<0.05; ** - significant for α<0.01.

resulted in higher total soil organic C and N contents, partially confirming the formulated hypothesis for the present study. This pattern was less pronounced under the tree canopies, expressing the balancing effect of tree cover that enhances soil C and N accumulation (Waldrop and Firestone, 2006), which gives some insight to the second component of the hypothesis under study.

The observed soil organic C increase in improved pasture area, compared with the natural pasture, may represent the potential of those pastures for soil organic matter accumulation in the study site. Our results showed that, for a 35-yr period, improved pasture added about 1 kg C m^{-2} (ca. 27 g C m^{-2} $year^{-1}$) to the 0-10 cm top soil layer, both beneath (0.9 kg C m^{-2}) and out of tree canopy (1.0 kg C m^{-2}). Nevertheless, it should be emphasized that this increment in study sites did not take into account spatial heterogeneity beneath tree crown (Nunes, 2004) or tree effect beyond tree crown vertical projection (Simón *et al*., 2012).

The decrease of soil bulk density in improved pasture area might be explained by the above mentioned accumulation of organic matter associated with greater amounts of herbaceous residues returned to the soil, both above (Cubera *et al*., 2009) and below-ground (STRAW, 2014).

C and N dynamics

The highest particulate organic matter concentration in improved pasture, compared with the natural pasture in open area, is in line with the highest total organic C concentration in the former. However, the relative proportion of particulate organic matter to total organic C was similar in the two studied pastures (about 38%), reflecting this fraction high dependency on total organic C (McLauchen and Hobbie, 2004).

In contrast to particulate organic matter, hot water soluble C proportion to total organic C was positively influenced by the improved pasture, indicating potential higher microbial activity, as soluble C is known to be the most important substrate to support microorganism activity (Marschner and Bredow, 2002). It should be noticed that hot water soluble C expressed more markedly soil organic matter changes due to improved pasture than total or particulate organic C did. This easy, reliable, and reproducible soil parameter seems to meet the needs for a good soil quality indicator to assess management changes in *montado* systems (Haynes, 2000; Schloter *et al*., 2003).

Despite the higher total organic C and similar relative proportion of particulate organic C, soil from improved pasture showed lower C mineralization rate than soil from the natural pasture, suggesting that different biogeochemical processes intensity may be enhancing the recalcitrant or protected organic matter fraction, thus mediating organic C accumulation in sown pastures soils.

Although N net mineralization rate did not clearly distinguish the two studied pastures, our results apparently follow Gómez-Rey *et al.* (2012) findings, who reported higher potential N availability in improved pastures associated with N-enriched legume species debris. The contrasting trends regarding C and N mineralization rates changes by improved pastures suggest that potentially higher N availability is promptly counterbalanced by lower C mineralization, thus keeping C:N ratio nearly unchanged (about 13 in both study areas).

Microbial biomass C and N were similar for the two studied pasture soils, and therefore their proportions to total soil C and N were different (higher in the natural than in the improved pasture). Nevertheless, given the comparatively lower hot water soluble C content and higher C mineralization rate determined in natural pasture soils, microbial population may be energetically less efficient (Anderson and Domsch, 1990), and thus differ from the improved pasture soil microbial community in structure, composition and functionality. This is supported by Waldrop and Firestone (2006) results in Mediterranean oak and grassland agroforestry systems, where different plant communities determined different soil microbial population compositions, and by Goméz-Rey *et al.* (2013) results in shrub-encroached *montado* systems, who suggested that diverse organic substrates from shrubs would facilitate C turnover.

The role of trees as biogeochemical processes regulators in *montado* soils has been widely acknowledged, being commonly associated with the effect of tree litterfall deposition (Moreno *et al*., 2007; Gallardo *et al*., 2000). Results of the present study regarding net N mineralization rate and microbial biomass C and N are in agreement with this balancing effect, as under tree crown they were independent on pasture management type. In contrast, particulate organic matter accumulation under the trees was surprisingly pronounced in the improved pasture management system, following the trend observed by Gómez-Rey *et al.* (2012) in similar sites. The reasons for this pattern are not clear, but the interaction between sown pasture and tree canopy cover may change substrate quantity and quality, decomposers community and associated activity, organic matter protection mechanisms and/or environmental conditions (Brady and Weil, 2008). Management may also exert a key influence on the delay of labile organic matter transformations in this study improved pasture. Higher and regular stocking rate in sown pasture area might accelerate the ecosystem trophic chain, especially beneath the tree canopy, where animals shelter in the shade and deposit most of their faeces (Haynes and Williams, 1993). Comparing grazed with ungrazed areas should be useful to clarify such hypothesis.

It is also noteworthy that tree cover did not affect soil hot water soluble C proportions, and soil C mineralization rate was only diminished by tree cover in the natural pasture system. These findings suggest that trends driven by management (in this case improved pasture establishment) are not always modified by the tree effect. Moreover, improved pastures seemed to exhibit strong effects in soil biological parameters that are closer indicators of immediate microbiological activity status (such as hot water soluble C and C mineralization rate); on the other hand, trees play the main role regulating soil biogeochemical complex processes, revealed by indicators such as microbial biomass and total organic C accumulation.

It should be emphasized that after 35 years of improved pasture management, the major soil organic matter changes were found beneath tree crown, mainly by accumulation of particulate organic matter. It is then crucial to understand how stable is this soil organic matter fraction and how is its C effectively sequestered. Further studies are needed to deepen the understanding on the underlying mechanisms of organic matter mineralization and stabilization processes in *montado* soils, in order to clarify sown pastures potential for long-term C sequestration (Six *et al.*, 2002).

Enzimatic Activity

Soil β-glucosidase activity can be used as a C-cycle indicator (García *et al.*, 2003), but the absence of significant differences among treatments in the present study questions its usage in such heterogeneous systems as *montado*. β-glucosidase activity along the year was not consistent with the previously discussed soil biological activity results (hot water soluble C, microbial biomass C and N and respective mineralisation rates), pointing out how unreliable soil enzymes activity single determinations can be. Tree cover exhibited a major influence on this soil enzyme activity, reflecting its dependency on the substrate availability (Mariscal-Sancho *et al.*, 2010).

Higher proteases activity in soils with improved pasture may be related to the protein-rich substrates available from the higher proportion of legume species present in this area. We admit that shrubs in the natural pasture may have been responsible for soil urease activity increases, as suggested by Gómez-Rey *et al.* (2013) for shrub-encroached *montado* areas. Stronger urease and proteases activities are in accordance with the higher N mineralization rates determined under tree canopy influence, thus confirming the tree effect on N mineralization processes in *montado* soils (Gallardo *et al.*, 2000).

Despite the much higher extractable P content in soil of improved pasture, compared with that of natural pasture, phosphomonoesterase activity seemed to be little affected by the competitive inhibition effect of orthophosphates (Tabatabai, 1982), differing only between management systems in autumn. Acid phosphomonoesterase activity was mostly lower beneath tree canopy than in open areas, where lower soil extractable P may enhance soil phosphatase activity (García *et al.*, 1994). Thus, tree cover might be considered as playing an indirect effect over P transformation cycle.

Mediterranean summer conditions, such as low soil water availability and high temperatures, are associated with low soil biological activity (Sardans and Peñuelas, 2005). However, in the present study, soil β-glucosidase and urease activities were higher in the summer. This may have been caused by a small rainfall event few days before the summer sampling date (see Figure 2). In fact, soil surface rewetting after a long dry period may cause a sudden soil respiration peak, the so called *"Birch effect"* phenomenum (Unger *et al.*, 2012). This highlights the difficulties in interpretation and extrapolation of soil enzymatic activity from single determination results.

Urease activity correlated positively with air and soil temperature, but negatively with soil water content, which is in agreement with Sardans *et al.* (2008) results in a controlled temperature experiment. The influence of climatic conditions was not clear in protease activity, suggesting that no environmental restrictions were imposed to N transformation by these enzymes. The variety of soil microorganism along the year, producing different proteases with different optimal temperature and water requirements, may explain such independent behaviour (Sardans *et al.*, 2008).

The low values of acid phosphomonoesterase activity and their strong positive correlation with soil water content is in agreement with that observed by Sardans and Peñuelas (2005) in induced drought conditions.

The unexpected low available soil water in part of the study period (winter) constrained the data analysis and results interpretation, suggesting the need for broader studies that may include environmental conditions simulations in order to explain the observed soil enzymatic activity patterns. Since soil enzymes analytical determinations are expensive, time consuming and fairly difficult to reproduce due to the high spatial and temporal variability of the results, they seem to fail as adequate soil quality indicators for monitoring changes associated with management changes in *montados* (Brady and Weil, 2008).

Conclusions

Improved pasture establishment determined evident changes in soil C, N and P, as well as in biogeochemical processes, the most evident modifications being observed in the labile organic matter fraction. Trees seemed to play a regulation role in soil organic matter mineralization processes, creating conditions for higher N availability and balancing C and P transformation. The spatial and temporal variability shown by soil enzymatic activity results, suggest great constrains to their use for soil management-induced changes monitoring. Further studies on soil quality changes processes in *montado* systems should take into account environmental and management factors, as well as Mediterranean climate seasonality and inter annual variability.

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