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Temporal effects of thinning on soil organic carbon pools, basal respiration and enzyme activities in a Mediterranean Holm oak forest

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Abstract

Soil organic carbon pools have an important role in the maintenance of ecosystems as a source of energy for soil microorganisms. Soil biological and biochemical properties are essential for the decomposition of organic matter. These soil properties can be affected by thinning, which is considered sustainable when the soil properties are maintained or improved. We studied the effects of selective thinning and shrub clearing, performed with an ecohydrological approach, in a marginal Holm oak forest in a semiarid area on soil properties. The effects of thinning (T) were compared with an untreated area (control, C). Fine woody debris was ground into mulch onto the thinned area. Forest floor and mineral soil properties were analyzed between five months and seven years after the thinning. In the forest floor, gravimetric water content (GWC_{ff}) and water soluble organic carbon ($WSOC_{ff}$) were analyzed and compared between T and C. In mineral soil GWC_{ms}, soil organic carbon (SOC), $WSOC_{ms}$, soil basal respiration (BR), soil microbial biomass carbon (MBC) and soil enzymes (acid phosphatase (Acid PA) and urease (URE)) were analyzed. In the early stage, the results showed slightly higher SOC and $WSOC_{ms}$ in T likely due to fine woody debris left on the forest floor. However, seven years after the thinning the effects of the thinning on all the studied variables were negligible. All variables showed high spatial-temporal variability. Our results suggest that selective thinning and shrub clearing in the studied site do not affect negatively soil properties when woody debris is left on the forest floor.

Keywords

Adaptive forest management; silvicultural treatments; *Quercus ilex*; woody debris; organic carbon pools.

1. Introduction

Holm oak (*Quercus ilex* L.) is a broadleaf evergreen sclerophyllous tree, widely distributed in the Mediterranean Basin. According to Ducrey (1992), *Q. ilex* L. forests cover more than 6 million ha in the Mediterranean Basin, mostly in its western part. Spain has 18.4 million hectares of forest and the most representative species in terms of surface cover is *Q. ilex*, which covers 2.8 million ha as oak grove (15.4% of the total area of Spanish forests), besides being the dominant species in 88% of “dehesas”, accounting for a further 2 million ha (MAGRAMA, 2017). Grosso et al. (2018) state that Holm oak forest at medium altitudes (800-1400 m) can replace beech forest in response to climate change. Fernández-Alonso et al. (2018) based on different studies stated that on the Iberian Peninsula several species from the genus *Quercus* spp. may replace Scots pine (*Pinus sylvestris* L.) due to both the predicted changes in temperature, precipitation and changes in land use and forestry. On the other hand, Cabon et al. (2018) alerts of the rising vulnerability of oak coppices of Southern Europe to drought as a result of the ongoing climate change and the lack of forest management that generates a structural aging of the stands, and reported that thinning has been widely regarded as a means to improve the resistance of evergreen oak forests to climate change by decreasing the competition for water between the remaining stems. The main goal of thinning treatments is to remove mainly weaker trees to increase the growth, health, and value of the remaining ones, by increasing the growing space of standing individuals and decreasing plant competition.

Soil is an important component of forest ecosystems as it is involved in many important ecosystem processes, such as organic matter decomposition, water and nutrient availability, etc. As reported by Johnson and Curtis (2001) the effects of forest management on soil C are important to understanding the consequences not only because is a variable determining soil fertility but also because of the role of soils as a source or sink for C on a global scale. Of equal importance is the study of C content and its dynamics in the forest floor (Currie et al., 2002). One of the key points in the effects of thinning on the forest floor and mineral soil C and other soil properties (i.e. enzyme activities) is the management of woody debris including retention on the soil, incorporation into soil or removal (Adamczyk et al., 2015; Wan et al., 2018). Moreover, because of the environmental importance of C sequestration, many studies have aimed to elucidate the effects of woody debris on the forest C sink. According to Johnson and Curtis (2001), the positive effect on soil C and N of leaving residues on site seems to be restricted to coniferous species, although some studies in coniferous forests also show little or no effect of residues on soil C or N. They reported that several studies have clearly shown that residues had little or no effect on soil C or N in either hardwood or mixed forests. Thinning can decrease forest floor C contents only for some time through reducing litter production from remaining trees until the canopy grows and accelerating litter decomposition (Gliksman et al., 2018). Conversely, thinning can increase forest floor C contents by enhancing the development of understory vegetation (Lee et al., 2018; Son et al., 2004) and leaving unharvested residues on the forest floor (Hytönen and Moilanen, 2014). Nave et al. (2010) carried out a meta-analysis to study harvest impacts on forest floor and mineral soil C storage in temperate forests, and reported that harvesting caused forest floor C storage to decline but losses were significantly smaller in coniferous/mixed stands than in hardwoods. It is difficult to detect significant rapid changes in soil organic C under different management practices (Li et al., 2013; Wang et al., 2013a). The study of different soil organic C fractions can provide information on soil conditions under forest management (Cheng et al., 2017; de Moraes Sá et al., 2018).

WSOC and MBC are part of the active soil organic matter and as active fractions are usually more affected than total soil organic C by management practices and can be used as early indicators of change in soil organic matter (SOM) status (Qing-kui et al., 2005).

According to Fierer (2017), soil microorganisms are clearly a key component of both natural and managed ecosystems. Microorganisms have a fundamental role in the biogeochemical cycles of the elements and it is widely accepted that a high level of microbial activity is necessary for the maintenance of an adequate soil quality (Bastida et al., 2007). Microbial biomass acts as a source and sink of available nutrients. Thinning potentially modifies microbial biomass and enzyme activities due to changes in microclimate and substrate availability (Kim et al., 2019). An increase in microbial biomass after thinning could assure the retention of soil C but at the same time the loss of soil C through soil microorganisms’ respiration. Thinning practices can potentially influence soil respiration (autotrophic and heterotrophic respiration) by modifying root activity, inputs of labile organic C, substrate availability, soil temperature and soil water content (López-Serrano et al., 2016). Basal respiration rate (microbial soil respiration rate without organic substrate addition, BR) is commonly accepted as a key indicator for measuring changes to soil quality (ISO, International Organization for Standardization, 2002, 2012; Creamer et al., 2014). The assessment of BR is used to quantify changes in the activity of soil microbial community and has been applied in thinning research studies.

Other key parameters contributing to soil fertility and quality, and consequently impacting plant growth and forest productivity are soil enzyme activities (Sardans and Peñuelas, 2005). Thinning may impact the activity of extracellular enzymes due to the changes produced in microclimate and soil properties. Increase (Chen et al., 2016), decrease (Geng et al., 2012) or no variation (Geng et al., 2012; Hedo et al., 2016; Kim et al., 2016; Kim et al., 2018) in enzyme activities have been reported in forest soils. Elucidation of the changes in soil enzyme activity can provide insight into the nutrient dynamics and ecosystem functioning (Sinsabaugh et al., 2008).

Upon the above studied literature, it can be concluded that the effects of thinning on soil properties are controversial, and this might be due to plant species, stand ages, the intensity of thinning, the management of coarse and fine woody debris, the type of soil, soil depth, thinning site conditions, the different climatic conditions, time since treatment, time of the year, spatio-temporal heterogeneity, etc. (Fig. 1).

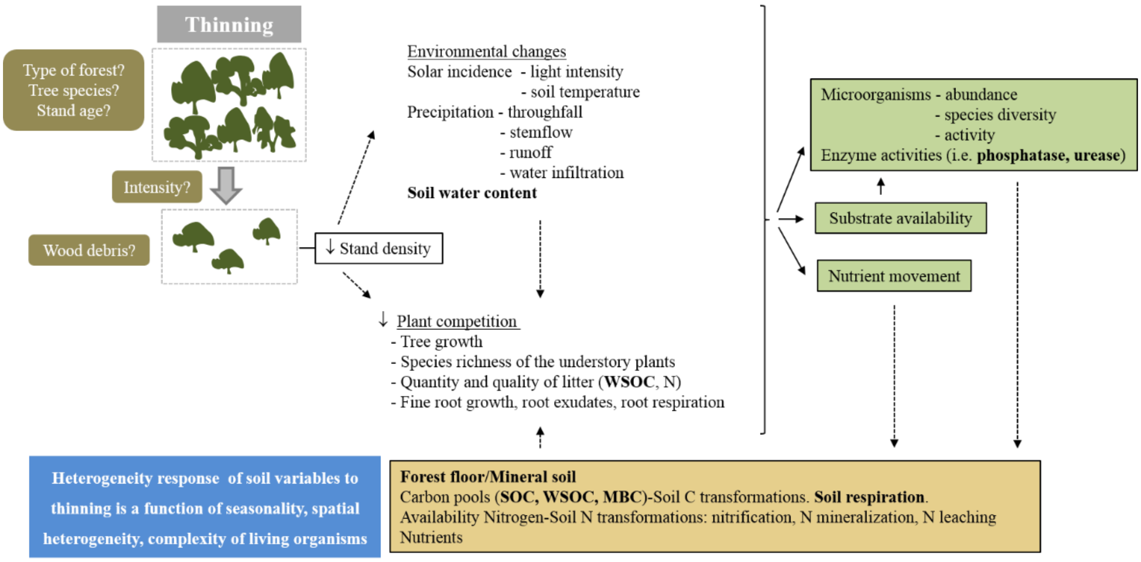

Fig. 1. Overview of the forest components and processes that can be affected by thinning. In bold the variables measured in this study.

It is important to emphasize that presented study was carried out in a marginal semiarid forest where selective thinning was studied as an adaptive measure to compensate for rainfall reduction (del Campo et al., 2019). Whilst the effects of adaptive forest management on tree growth, tree-climate sensitivity and tree-water use have been sufficiently addressed, their impact on soil biology has been much less studied, especially in semiarid forests where silviculture traditionally has aimed full canopy cover for soil protection, as opposed to canopy opening in adaptive treatments.

Jandl et al. (2019) reported that among scientists and practitioners is widely agreed that global change alters site properties such as climate, water supply, and the nutrient supply from soils, and the need for adaptive management of forests. The adaptive forest management is urgent in Mediterranean drylands where the scenario predicted is greater drought frequency and severity (IPCC, 2014).

This study was framed within an overall project aiming to evaluate the effects of adaptive forest management on water fluxes, growth dynamics, field CO_2 flux and soil properties on *Quercus ilex* stands in a dry-subhumid environment in south-eastern Spain for an integrated assessment of the ecohydrology of the ecosystem. The hypothesis of the presented research herein was that thinning can impair soil properties in a marginal semiarid ecosystem dominated by Holm oak. We hypothesized that, after thinning, C labile pools will increase in the thinned area, at least temporarily, due to increased substrate supply through harvest woody debris left onto the thinned area and possibly through root activity; this increase would entail an increase in MBC, BR, and enzyme activities. Therefore the objectives were to: 1) examine whether GWC and WSOC in forest floor, and GWC, WSOC, MBC, BR and enzyme activities in mineral soil were affected by thinning along the studied period (five months to 7 years after thinning); 2) find out the effects of microclimatic conditions on the above-mentioned variables. The effects of thinning were studied in eight sampling periods with contrasting temperature and precipitation.

2. Materials and methods

2.1. Study site and experimental design

The study was carried out in “La Hunde” public forest, being one of the few well-preserved Holm oak forests of the Valencian Community and the largest in the province of Valencia (39°04′50″ N; 1°14′47″ W, 1080-1100 m a.s.l.). The average annual precipitation is 466 mm, the mean annual temperature is 12.8°C, and the mean annual potential evapotranspiration is 749 mm (García-Prats et al., 2018). The area is defined as semiarid area according to the definition of drylands by precipitation, and as dry-subhumid area according to the aridity index definition (Huang et al., 2016). The dominant species is Holm oak (*Quercus ilex* subsp. *ballota* (Desf.) Samp.), but other species found in the experimental area were *Pinus halepensis*, *Juniperus phoenicea*, *Q. faginea, and J. oxycedrus*. According to the World Reference Base, the soil of the study area is Kastanozem Calcic. For the last five decades, no silvicultural intervention was done due to its marginality and the protective role assigned to this forest type.

The experimental design was a complete block design with two treatments (thinning and untreated control), three blocks of similar size and 3 samples per each block-treatment combination. In May 2012, thinning with shrub clearing treatment was executed by a contractor of the Valencian Forest Service in a rectangular area of about $1800\,m^2$ (T) (Figure 2). The thinned area was split into three plots of similar size from upslope to downslope. Coarse woody debris were removed from the thinned area and fine wood debris (< 6 cm) were piled and ground into mulch onto T. Adjoining the thinned area, a control area (C) of similar size was established and also split into three plots. Total basal area removed in the thinned area was 41% and density reduction was 73%. Forest structure metrics of each plot has been described by del Campo et al. (2018). Both areas have similar climatic characteristics, the same slope (31%) and aspect (NW). Soil characteristics in the thinning and control areas (0-15 cm depth) were similar (Table 1). The soil in the study site is relatively shallow (10-40 cm) and has high stoniness (>50%). All samples were taken at least 2 m away from the limits to avoid edge effects.

(A) (B)

Fig. 2. Experimental site (A) thinned area (T) and (B) control area (C).

Areas	pH 1:2.5	EC 1:2.5† ($dS\ m^{-1}$)	$CaCO_3$ (%)	WHC†† (%)	Texture
C	7.90±0.12	0.66±0.24	36.69±12.88	115.1±10.5	Loam
T	7.84±0.16	0.67±0.23	38.09±9.08	129.2±22.8	Loam

Table 1. Soil characteristics in both experimental areas (C, control, not treated and T, treated cleared/thinned) (0-15 cm depth). Values are mean ± standard deviation (n=9). †EC: electrical conductivity; ††WHC: water-holding capacity.

Details of the experimental site and treatment design have been described in previous papers (Di Prima et al., 2017; del Campo et al., 2018; Garcia-Prats, 2018).

2.2. Soil sampling and environmental variables

Sampling was carried out bi-monthly between October 2012 (5 months after the thinning treatment) and June 2013. The following sampling dates were November 2016, June 2017 and May 2019 (4-7 years after the thinning treatment). Samples of forest floor and mineral soil were taken randomly from the field in 3 points per plot. Forest floor was defined as the organic material above the mineral soil. Sampling was done carefully in order to avoid contamination with the mineral material. Forest floor varied from one point to another, some of them had decaying litter and a thin fermentation layer. Mineral soil samples were taken from the upper 10–15 cm layer once the litter had been removed.

Precipitation was continuously measured in an open area apart from the experimental site, air temperature was monitored in the experimental site in the buffer zone between both thinned and control areas. These measurements were carried out as described by del Campo et al. (2019).

2.3. Analyses of soil and forest floor samples

Fresh forest floor samples were passed through a 4-mm sieve whereas fresh mineral soil through a 2-mm sieve. Samples were stored at 4°C prior to the analyses. GWC_{ms}, $WSOC_{ms}$, MBC, BR and enzymes activities were analyzed in fresh mineral soil, and GWC_{ff} and $WSOC_{ff}$ were analyzed in fresh forest floor. An air-dried sub-sample of mineral soil was sieved through a 500-µm sieve to determine SOC. For all analytical assays, the average value of two or three replicates per sample was used, and data have been expressed on an oven dry-weight basis and when required on organic carbon basis or microbial biomass carbon.

2.3.1. Soil physical and chemical properties

Soil and forest floor moisture was calculated gravimetrically by drying soils at 105 °C 48 h and subsequently expressing water content as a percentage of the dry weight. Electrical conductivity (EC) and pH were measured in a 1:2.5 (w/v) aqueous solution, in a conductivimeter (Crison GLP31, Spain) and pH meter (Crison micropH 2000, Spain), respectively. Carbonate content was determined with a Bernard's calcimeter. The texture was determined by the Bouyoucos method; samples were pretreated with hydrogen peroxide to remove organic matter. The water-holding capacity (WHC) was assayed by the method of Forster (1995). SOC was determined by wet oxidation with 1N potassium dichromate in acidic medium and evaluating the excess of dichromate with 0.5N ferrous ammonium sulphate, as described by Walkley and Black (1934). WSOC was determined in the aqueous extract of soils (1:2.5) and forest floor (1:8), obtained after 30 min of mechanical shaking, centrifugation at 2500 rpm for 5 min and filtration through a Whatman 42 paper filter. WSOC in the extracts was assessed by $K_2Cr_2O_7$ oxidation in concentrated H_2SO_4 (Yakovchenko and Sikora, 1998).

2.3.2. Microbial biomass, basal respiration, and soil enzyme activities

MBC was determined using the chloroform fumigation-extraction procedure (Vance et al., 1987), and the 0.5 M K_2SO_4 extracted carbon was measured in the same way as for WSOC. The difference in C concentration between fumigated and non-fumigated extracts was expressed as microbial biomass-C by multiplying by a factor (Kc) of 0.38 (Vance et al. 1987). BR was determined on 10 g of fresh soil samples, incubated in hermetically sealed flasks in the dark at 25°C for 4 days. The respiration rate in that period was calculated from the increment in % CO_2 in the headspace volume of the flask, which was measured with a CO_2 sensor (Checkpoint, PBI Dansensor, Ringsted, Denmark). Potential Acid PA was evaluated by spectrophotometry as the amount of p-nitrophenol (*p*-NP) released from 1 g soil after incubation at 37 °C for 1 h with the substrate p-nitrophenyl phosphate in MUB buffer (pH 6.5). Then 0.5 M $CaCl_2$ was added and the *p*-NP released was extracted with 0.5 M NaOH and filtered (Filter-Lab 1246) (Tabatabai and Bremner, 1969). Potential URE was determined as the amount of NH_4^+-N released from 2 g soil after incubation for 1.5 h with urea (6.4%) at 37 °C in 0.2 M phosphate buffer (pH 7)

(Nannipieri et al., 1980); the released NH_4^+-N was determined in a flow injection analyzer (FIAStar 5000, Foss 15 Tecator, Höganäs, Sweden).

2.4. Data analysis

Thinning effects were assessed by comparing T and C for soil and forest floor water content, and water soluble organic carbon, and for soil biological and biochemical properties in each sampling date. Differences between treatments (thinning and control) were analyzed with two-way ANOVA (treatment and block as fixed factors). Tukey's HSD test was used for post hoc means separation. Differences at $P<0.05$ were regarded as statistically significant. Non-normal data were log, square- transformed or transformed using the Statgraphics power transformation tool to stabilize the variance prior to calculation. When assumptions of normality or equality of variances were not met, the nonparametric Kruskal-Wallis test was used. As it was aimed to find out how forest thinning affects forest floor and soil variables at different recovery stages, the recovery time was divided into three stages: early stage (≤2 year after thinning), medium stage (> 2 and ≤5 year) and late stage (7 years after thinning) (Zhang et al., 2018). The relationships between the variables were assessed using Spearman's correlation coefficients. Principal component analysis (PCA) was used to interpret relationships between the studied variables. Statistical analyses were performed using the Statgraphics XVII software package for Windows.

3. Results

3.1. Soil moisture

No difference in GWC_{ff} between treatments was found except for one sampling date, October 2012 (Fig. 3A), in which GWC_{ff} was higher in C. GWC_{ms} was not affected by the treatment except for higher values in T in February 2013 and April 2013. Supplementary Table S1 provides a summary of the two-way ANOVA performed on soil properties. GWC_{ff} ranged from 12.17 ± 3.10 to 95.78 ± 29.63% of fine forest floor mass in T, and from 13.28 ± 5.60 to 121.53 ± 39.37% in C. GWC_{ms} ranged from 14.23 ± 3.46 to 51.22 ± 8.82% in T, and from 14.42 ± 4.04 to 43.00 ± 5.60% in C. The lowest values for GWC_{ff} and GWC_{ms} were obtained in the warmer months studied (June 2013 and 2017, and May 2019) (Fig. 3). Climatic conditions (air temperature and precipitation) are provided as Supplementary Material (Table S2). GWC_{ff} and GWC_{ms} showed high correlation between them (r= 0.771, P<0.0001). Moreover, GWC_{ms} correlated with many of the studied variables (see epigraph 3.5).

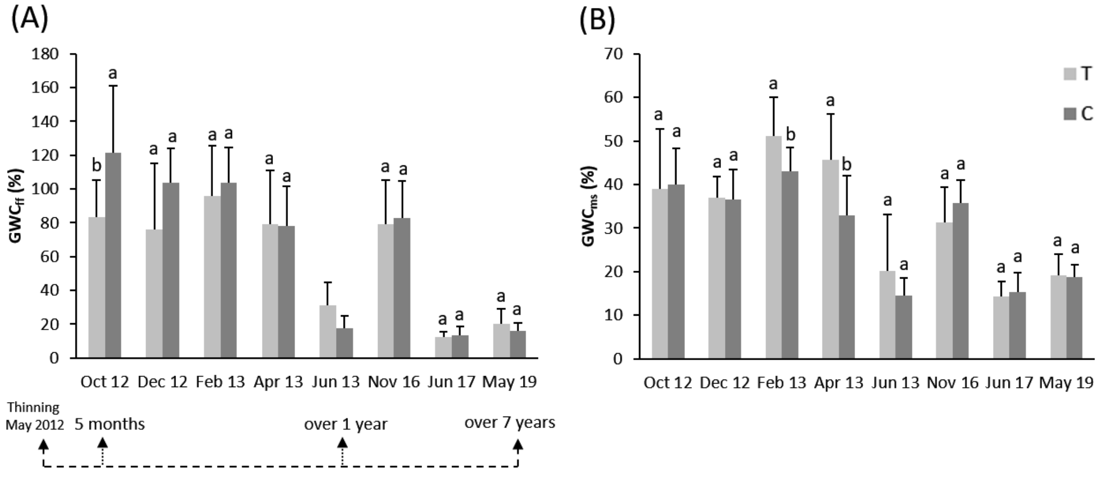

Fig. 3. Gravimetric water content of forest floor (GWC_{ff}) (A) and mineral soil (GWC_{ms}) (B) along the studied period. Bars represent means ± standard deviation (n = 9). Lower case letters indicate significant difference between treatments ($P < 0.05$). T: thinning treatment; C: control. In June 2013, the statistical analysis was not performed for GWC_{ff}

due to absence of forest floor material in the sampled points taken at the upper thinned plot.

3.2. Soil organic carbon

SOC content in the C five months after the thinning treatment was 10.88 ± 2.56% and this value remained constant throughout the 7 years of study (Fig. 4). In T a slightly higher content was observed at 5 months (13.86 ± 3.26%), and it remained higher at least until the first year after the thinning treatment, however by November 2016 (4.5 years after the thinning treatment) SOC content was similar in the thinned and control areas. No significant differences appeared along the study period in single date analyses except for June 2013; although grouping data by early, mid and late date effects, there was a significant difference between treatments in the early stage but not in the medium and late stages (Tables S3 and S4).

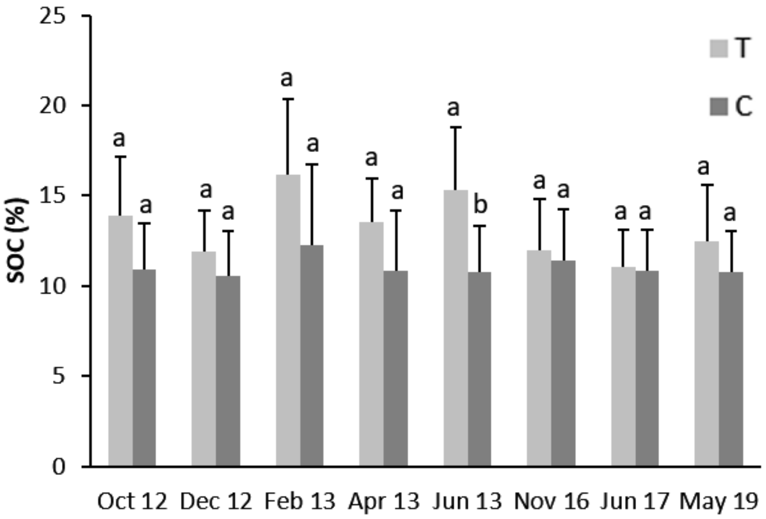

Fig. 4. Soil organic carbon for the studied treatments during the sampling dates. Bars represent means ± standard deviation (n = 9). Lower case letters indicate significant difference between treatments ($P < 0.05$). T: thinning treatment; C: control.

3.3. Water soluble organic carbon

WSOC together with MBC are considered indicators of the amount of available soil C substrate. WSOC$_{ff}$ varied little between the treatments (Fig. 5A). During the first year after the thinning treatment, the forest floor and the mineral soil had a slightly higher WSOC concentration in T, however, there were only statistically significant differences in the first sampling date for WSOC$_{ff}$ and for WSOC$_{ms}$ (Fig. 5). In C, WSOC$_{ms}$ remained constant in the first four sampling dates, and we obtained similar concentrations in June 2013 and in the last three sampling dates. WSOC$_{ff}$ showed high spatial-temporal variability. WSOC$_{ff}$ ranged from 422.3 ± 328.3 to 1294.9 ± 499.8 mg kg^{-1} in T, and from 234.8 ± 76 to 1245.9 ± 399.4 mg kg^{-1} in C along the studied period. WSOC$_{ms}$ varied between 100.7 ± 48.0 and 247.1 ± 148.9 mg kg^{-1} in T, and from 80.0 ± 29.1 to 202.5 ± 68.4% in C contributing with 0.08–0.19% of the SOC. We obtained a higher value of WSOC in forest floor than in mineral soil.

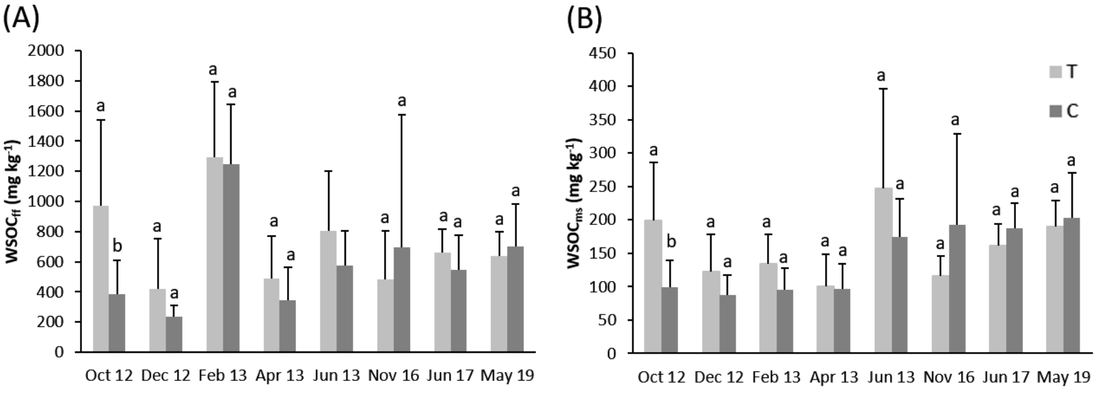

Fig. 5. Water soluble organic carbon of forest floor (WSOC$_{ff}$) (A) and mineral soil (WSOC$_{ms}$) (B) along the studied period. Bars represent means ± standard deviation (n = 9). Lower case letters indicate significant difference between treatments ($P < 0.05$). T: thinning treatment; C: control. In June 2013, the statistical analysis was not performed

for $WSOC_{ff}$ due to absence of forest floor material in the sampled points taken at the upper thinned plot.

3.4. Microbial carbon biomass, basal respiration, and soil enzyme activities

No difference in MBC between treatments was found except for two sampling dates, November 2016 and June 2017 (Fig. 6A), in which MBC was higher in C. MBC varied between 605 ± 289 and 1511 ± 331 mg kg^{-1} in T, and from 724 ± 388 to 1137 ± 528 in C along the studied period, and contributed 0.50–1.05% of the SOC. According to Sparling (1992), MBC and the microbial quotient (MBC in relation to SOC) are useful measures to monitor SOM and both provide a more sensitive index than organic C measured alone. Microbial quotient was similar in both treatments until June 2013 (Table S5). In June 2013, November 2016, and June 2017, microbial quotient was higher in C, and this was accompanied by more Acid PA specific activity (Acid PA/SOC) in November 2016 and June 2017. In May 2019, there were no differences between treatments. In the studied area, the microbial quotient varied from 5.26 ± 2.63 to 10.01 ± 2.44 mg C g^{-1} SOC. No significant differences were found in BR between treatments except for June 2017 (Fig. 6B). BR varied between 16.50 ± 5.77 and 82.50 ± 45.69 mg CO_2-C kg^{-1} d^{-1} in T, and from 19.40 ± 12.24 to 67.96 ± 55.08 in C along the studied period. The lower values were obtained in June 13 and June 2017, and the highest in February 2013 and April 2013, in complete concordance with the samples which showed the lowest and highest GWC_{ms} respectively. There were no differences in the microbial metabolic quotient (qCO_2=BR/MBC) between treatments in any of the sampling dates (Table S5), and this index varied between 21.64 ± 14.66 and 116.30 ± 93.95 mg C-CO_2 g^{-1} C d^{-1}.

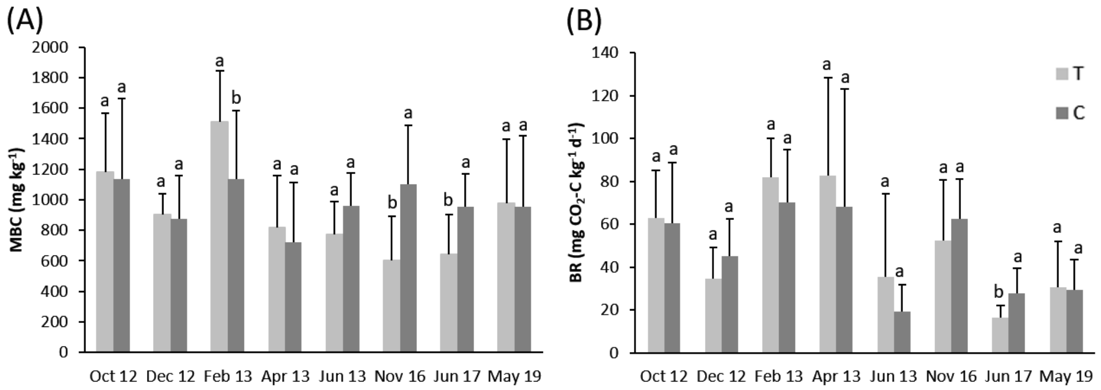

Fig. 6. Microbial biomass carbon (A) and basal respiration (B) along the studied period. Bars represent means ± standard deviation (n = 9). Lower case letters indicate significant difference between treatments ($P < 0.05$). T: thinning treatment; C: control.

We measured the potential activity of Acid PA and URE and, in general, we found that the treatment had no effect on the activity of both enzymes (Fig. 7). Throughout the study, Acid PA varied between 3.22 ± 0.74 and 7.16 ± 3.00 µmol g^{-1} h^{-1}, and URE between 1.45 ± 0.50 and 9.79 ± 2.77 µmol g^{-1} h^{-1}. Enzyme activities responded differently to environmental changes. URE was much lower in the warmer months studied (June 2013 and June 2017) but we did not find a clear pattern in Acid PA. Normalizing enzymatic activity levels to MBC may be a qualitative metric of the microbial community function in response to specific treatments (Shi et al., 2018 and references therein), and can help to minimize the impact of spatial variability in MBC between samples. AcidPA/MBC and URE/MBC had similar values for T and C in all sampling dates except for June 2013 and November 2016 when it was higher in T (Table S5).

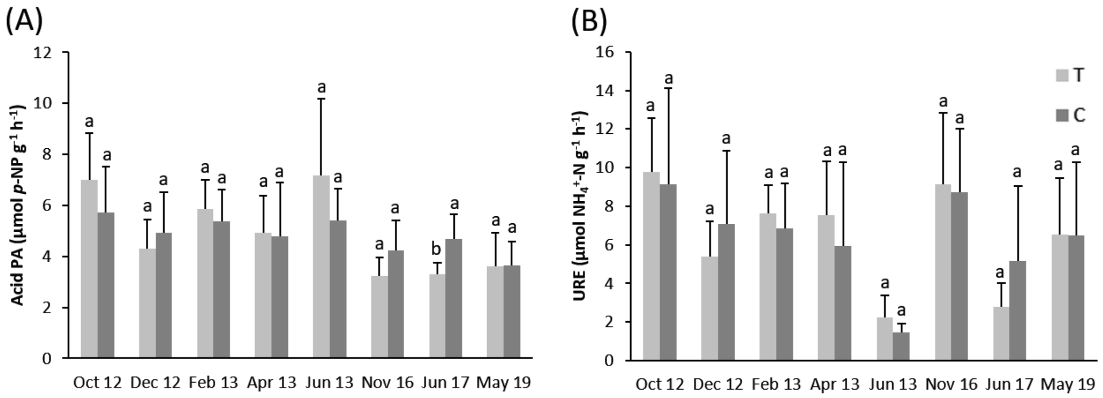

Fig. 7. Acid phosphatase activity (A) and urease activity (B) along the studied period. Bars represent means ± standard deviation (n = 9). Lower case letters indicate significant difference between treatments ($P < 0.05$). T: thinning treatment; C: control.

3.5. Multivariate analysis of soil properties and environmental variables

Spearman correlation matrix revealed that a large number of soil variables were significantly correlated to each other (Table 2). When pooling all the data we found that GWC$_{\mathrm{ff}}$ highly correlated with GWC$_{\mathrm{ms}}$. GWC$_{\mathrm{ms}}$ correlated positively with all the variables except with WSOC$_{\mathrm{ms}}$, that correlated positively with air temperature. The higher correlations were obtained between BR and GWC$_{\mathrm{ms}}$, and BR and URE (Fig. 8). There were positive significant correlations between SOC and labile carbon fractions (MBC, WSOC$_{\mathrm{ms}}$, WSOC$_{\mathrm{ff}}$), and with BR and both enzymatic activities. BR correlated positively with all the variables except WSOC$_{\mathrm{ms}}$. As it was expected MBC positively correlated with BR, and with the two measured enzymes. Although this correlation is not always obtained in nature due to the two enzymes studied are extracellular and can remain adsorbed to the humic clay complex. MBC was positively related to substrate availability (SOC and WSOC$_{\mathrm{ms}}$). Mean air temperature of the 7 days prior to the sampling dates were highly correlated with the GWC_{ff} and GWC_{ms} showing the influence of evaporation with the soil water content.

Variables^{a,b}	GWC_{ff}	GWC_{ms}	SOC	$WSOC_{ff}$	$WSOC_{ms}$	MBC	BR§	Acid PA	URE
GWC_{ms}	**0.771^{***}**								
SOC	0.188^{*}	0.345^{***}							
$WSOC_{ff}$	ns	ns	0.395^{**}						
$WSOC_{ms}$	-0.364^{***}	-0.322^{***}	0.457^{***}	0.381^{***}					
MBC	0.265^{**}	0.384^{***}	0.465^{***}	0.293^{***}	0.302^{***}				
BR	**0.748^{***}**	**0.830^{***}**	0.423^{***}	0.245^{**}	ns	0.461^{***}			
Acid PA	0.420^{***}	0.408^{***}	**0.562^{***}**	0.299^{***}	0.300^{***}	**0.552^{***}**	**0.560^{***}**		
URE	**0.581^{***}**	**0.586^{***}**	0.399^{***}	0.182^{*}	ns	0.478^{***}	**0.745^{***}**	0.376^{***}	
T_{a_7days}	**-0.717^{***}**	**-0.707^{***}**	ns	ns	0.489^{***}	ns	-	ns	-0.443^{***}

Table 2. Spearman correlation coefficients between forest floor and mineral soil moisture, organic carbon fractions, enzymes activities and air temperature (n = 135-143).

[a] GWC, gravimetric water content; SOC, soil organic carbon; WSOC, water soluble organic carbon mineral soil; BR, basal respiration; MBC, microbial biomass C; Acid PA, acid phosphatase activity; URE, urease activity; T_{a_7days}, mean air temperature of the 7 days prior to the sampling date. Period analysed: October 2012 – May 2019.

[b] ff, forest floor; ms, mineral soil.

[§] Basal respiration was not analysed for correlation with soil temperature because it was determined in the laboratory at 25° C and with its own GWC.

Significant correlations: ns: non-significant; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

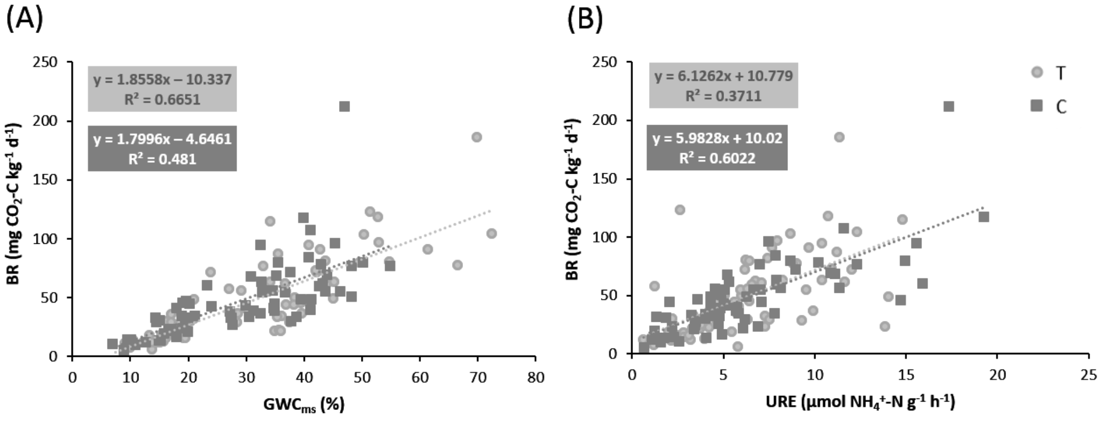

Fig. 8. Linear regression between (A) basal respiration and gravimetric water content (n = 140), (B) basal respiration and urease activity (n = 140).

Through a principal component analysis with all the studied data, we retained two components. The first and second components explained 47.11% and 22.71% of the total variance (Fig. 9). PC1 was mainly weighted by BR, GWC_{ms}, GWC_{ff}, enzyme activities, and MBC and PC2 by $WSOC_{ms}$ and air temperature of the 7 days prior to the sampling date.

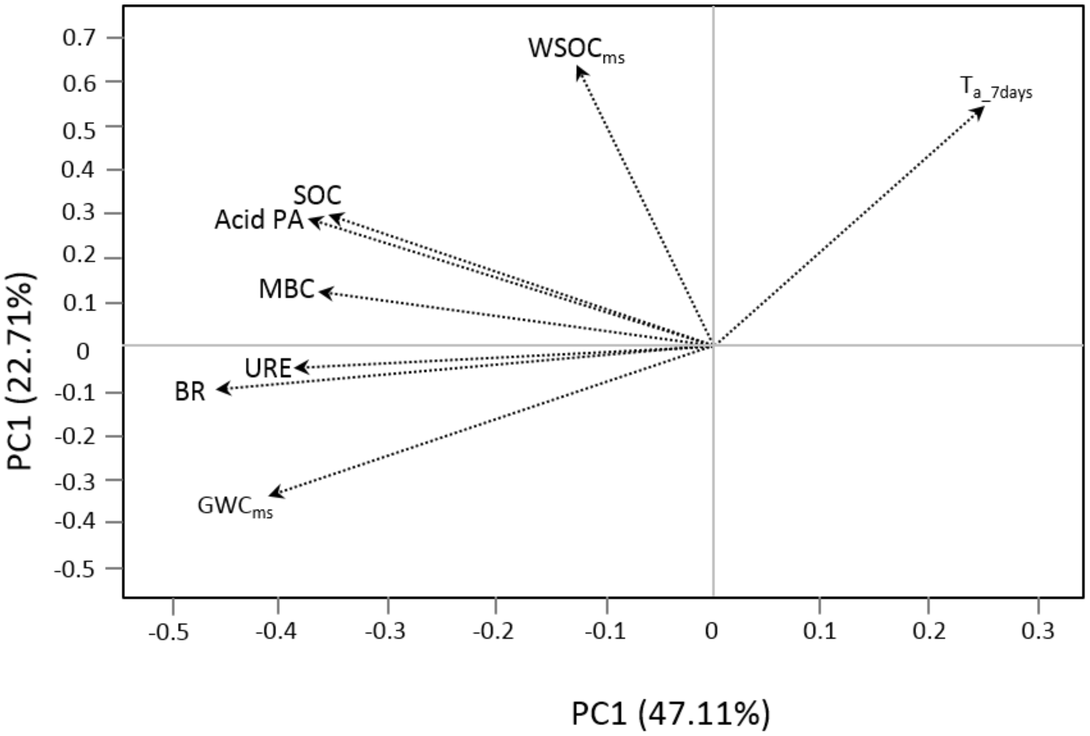

Fig. 9. PCA-ordination biplot (PC1 vs PC2) of soil samples from thinned and control treatments (n = 143). GWC_{ff}, gravimetric water content forest floor; GWC_{ms}, gravimetric water content mineral soil; SOC, soil organic carbon; $WSOC_{ff}$, water soluble organic carbon forest floor; $WSOC_{ms}$, water soluble organic carbon mineral soil; BR, basal respiration; MBC, microbial biomass C; Acid PA, acid phosphatase activity; URE, urease activity; T_{a_7days}, mean air temperature of the 7 days prior to the sampling date.

4. Discussion

Results showed that in the studied site thinning treatment slightly influenced soil properties but in a different way depending on time elapsed since the treatment. Forests are an important reservoir of soil organic carbon that is in equilibrium with its environment. SOC represents an important source of nutrients and energy for soil microorganisms. The constant value of SOC in the control area confirms the persistence of SOC in ecosystems (Schmidt et al. (2011). The SOC values obtained fell within the range of SOC in a Holm oak forest soil reported by Grosso et al. (2018). Our study demonstrated that SOC was not affected by thinning practices 7 years after the thinning treatment. However, during the first year after the treatment, T had slightly higher SOC content than C, likely because the decomposition of woody debris can create a pulse of organic matter, particularly as in our case that debris was ground to accelerate its decomposition. Johnson and Todd (1998) reported that residues left in warmer hardwood forests rapidly decompose. Bastida et al. (2019) reported that after thinning, *Q. ilex* roots can survive and regrow and that roots of *Q. ilex* are an important source of SOM. Therefore, the slightly increase in SOC may be due to both woody debris and root activity. Some researchers found that thinning had no significant impact on SOC in the mineral soil during the short-term period, and others concluded that thinning effects were not large in mineral soils (Johnson and Curtis, 2001; Kim et al., 2009; Yang et al., 2011). Zhao et al. (2019) did not detect a significant effect of thinning on either SOC or MBC five years after thinning but in their case, all thinning residuals were removed from the plots. However, Chen et al. (2016) found that seven years after thinning without residue retention, SOC increased in fall but decreased in spring.

According to Cheng et al. (2018), most works have previously focused on total soil organic C changes, therefore, less experimental evidence is available for the effect of thinning on SOC fractions in the mineral soil. WSOC is likely the most labile and mobile form of soil organic carbon (Boyer and Groffman, 1996). It is also considered the most reactive soil carbon source (Scaglia and Adani, 2009), and it has been commonly used as an indicator for microbial activities as it is readily available for microbes. We obtained a higher value of WSOC in forest floor than in mineral soil as reported by other authors (i.e. Huang and Schoenau, 1996). The $WSOC_{ms}$ values were similar to those obtained by Bastida et al. (2019) in a Mediterranean Holm-oak forest. The correlation obtained between $WSOC_{ms}$ and $WSOC_{ff}$ indicates that $WSOC_{ms}$ is not only related to root exudates but also to WSOC from the upper horizon, and therefore to litter decay and woody debris. $WSOC_{ms}$ likely was derived from the organic layer by leaching. As reported by various authors (Leinemann et al., 2018; Michalzik et al., 2001) organic topsoil layers are important sources of dissolved organic matter transported to below the soil organic layer, and therefore this source of labile carbon can be consumed by microorganisms in the upper centimeters of the mineral soil (Fröberg et al., 2007; Lee et al. (2018)). $WSOC_{ms}$ correlated positively with temperature. Since $WSOC_{ms}$ is considered as being generated from SOM decomposition and litter leaching, $WSOC_{ms}$ production could be expected to increase in the warmer months (Jiang and Xu, 2006).

The mean values of MBC fell within the range of MBC presented by Flores-Rentería et al. (2015) for *Quercus ilex* forest. There were no differences in MBC between treatments along the study except for two sampling dates (medium stage), however seven years after the treatment there were no differences between treatments. Wang et al. (2013b) studied the effect of a long-term thinning on MBC in a Larch **(***Larix gmelinii***)** forest between eleven and thirteen years after the last thinning, and reported that MBC at the thinned site was 8% lower than at the un-thinned site. However, Kim et al. (2018) showed opposite results seven years after the treatment. They studied the effects of thinning on microbial biomass in the soil of *Pinus densiflora* Sieb. et Zucc. forests and found that, in one of the studied sites, thinning promoted accumulation of microbial biomass and, this effect tended to increase with thinning intensity. However, in the other site no difference in MBC between treatments was found. As microorganisms are involved in the decomposition of organic matter, it would be expected that any influence on the soil content of organic C and N would influence their amount and activity. Thus, it is unexpected that in our thinned area in the first year of study a slight increase in SOC is not accompanied by an increase in MBC.

We obtained basal respiration in laboratory incubations. There were no differences in BR between treatments throughout the study. Bolat (2014) studied the effect of thinning on BR in a black pine forest two years after the thinning, and reported that thinning decreased BR but without significant effect. Because BR was obtained at constant soil temperature, BR depended on soil moisture, the availability of substrate and the number of active microorganisms. The high correlation between BR and GWC_{ms} and the lack of correlation with $WSOC_{ms}$ may indicate that the labile organic carbon fraction is not a limiting factor for microorganisms' development that is highly influenced by GWC_{ms} content. Soil water content is one of the most important factors explaining the variation in the effect of thinning over soil biological and biochemical properties as it follows from the Spearman correlation coefficients.

Trasar-Cepeda et al. (2008) reported that enzymatic activity is highly sensitive to external agents and biochemical properties and usually display a high degree of both spatial and temporal variability. Regarding Acid PA and URE, no significant differences between T and C were found along the studied period. Geng et al. (2012) did not find significant differences between treatments for phosphatase activity under pitch pine in the 0-10 cm soil depth. Likewise, Kim et al. (2016) did not find significant influences of thinning on acid phosphatase activity in *Larix kaempferi* forest soils in the short term. In the same way, Kim et al. (2018) reported that thinning did not alter enzyme activities in the soil of *Pinus densiflora* forests after 7 years. Conversely, Ntoko et al. (2018) reported lower acid phosphatase activity in heavily-thinned plots compared to control and explained that it could be attributed to the lower plant available P in those plots. In our case, the enzyme activity was consistent with the lack of increase in the levels of microbial biomass under the thinning treatment. A similar result was obtained by Kim et al. (2018). We would have expected higher levels of enzymes in T due to woody debris left on the soil because soil enzyme activities are believed to be associated with the availability of C and N and their ratio. Soil nutrient enrichment might enhance enzyme activity for microbial nutrient acquisition, however, enzyme activities often decline under conditions of high nutrient availability (Allison and Vitousek, 2005). The relationship between enzyme activity and soil organic matter was particularly clear for the Acid PA activity which positively correlated with SOC and $WSOC_{ms}$ for all dates taken together. Both enzymes also had a different seasonal behavior, Acid PA did not exhibit high seasonal changes, however, the URE presented a more seasonal behavior, with minimum in the warmer months. Sardans and Peñuelas (2005) reported that enzyme activities can vary depending on the sampling date in zones with a seasonal climate (i.e. Mediterranean area) and that the highest activities occur in spring together with the most active growth of plants and microbial activity and that autumn is the second most active season in activity in those Mediterranean areas. They explained that this could be because in spring, temperatures could be optimal as well as water availability and the higher quantity of litter in a Holm oak forest. Moreover, Sardans and Peñuelas (2005) found that the activities of the enzymes involved in the nitrogen cycle (i.e. urease), were the most affected by drought, decreasing their activity in a Mediterranean *Quercus ilex* L. forest and that this could be explained by the limiting role of N in the Holm oak forest of the Prades mountains reported by Mayor et al. (1994). In our case, we did not measure URE in spring but URE was highest in autumn 2012 and 2016, followed by winter 2013 and the lowest value were obtained in summer 2013 and 2017. However, Wic-Baena et al. (2013) reported that season had no significant influence on urease and phosphatase activities. Moreover, the urease activity has not always been reported as correlated with soil water availability (Sall and Chotte, 2002). Acid PA values obtained in this study were lower in the first 15 cm layer of soil profile in comparison with the values reported by other authors (Sardans and Peñuelas, 2005; Wic-Baena et al., 2013). However, the activity of URE was similar than the one obtained by Hedo et al. (2016) in a semiarid forest. All the forest floor and mineral soil variables analyzed exhibited great spatial and temporal variability.

5. Conclusions

The results of this study revealed that in the early stage, there were a slightly higher soil organic carbon and water-soluble organic carbon in the mineral soil in the thinned treatment likely due to fine woody debris left on the forest floor. However, seven years after the thinning the treatment does not affect the soil carbon pools, basal respiration and enzyme activities, endorsing the sustainability of this kind of management from the point of view of the studied carbon pools and biological and microbiological properties in the studied site, in spite of the intensity of the thinning. Successful forest management should be associated with the maintenance or improvement of soil biological and biochemical properties due to their importance in nutrient cycling. In the study site, it is clear that woody debris is a significant source of soil organic carbon in the short-term. It is a good practice to leave the fine wood debris on the forest floor to counteract the reduction of litter inputs that entails likely a reduction of carbon availability for microbial growth and plant nutrition.

Continuous research on forest ecosystems is suggested to bridge gaps in our understanding of soil organic carbon, microbial activity, and enzyme activity, given their significance in forest health and quality. In addition, this study should be completed with the study of the root distribution, biomass and activity after thinning as well as the effect on soil N and its transformations.

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