Effects of different thinning intensities on soil carbon storage in *Pinus laricio* forest of Apennine South Italy

Giovanna Settineri[1], Carmelo Mallamaci[1], Miroslava Mitrović[2], Maria Sidari[1], Adele Muscolo[1*]

[1]Agriculture Department, "Mediterranea" University, Feo di Vito -89122- Reggio Calabria, Italy; [2] Department of Ecology, Institute for Biological Research "Siniša Stanković", University of Belgrade, Bulevar Despota Stefana 142, 11060 Belgrade, Serbia

*Corresponding author: Adele Muscolo, Dipartimento di Agraria, Università "Mediterranea" Feo di Vito - 89122- Reggio Calabria Italy. email: amuscolo@unirc.it

Tel. 003909651654364

Fax 00390965 1694344

Acknowledgements This study was supported by the Ministry of Education, Science and Technological Development of Serbia, Grant no. 173018 and by PhD scholarship DIBAF, UNITUS Viterbo, Italy. Authors thank two anonymous reviewers for their stimulating comments on the manuscript.

Abstract

This study investigated in a *Pinus laricio* forest of south Italy, how systematic thinning of different intensities (intense thinning, T45; moderate thinning, T25; clear cut, CC and no thinning, T0) affected soil biological properties, organic matter trend and carbon (C) storage in soil and plants. Soil carbon content and carbon/nitrogen (C/N) ratio were significantly higher in the T45 than in control, T25 and CC. Under T45 the soils had also the highest enzymatic activities, microbial biomass carbon (MBC) and colonies of fungi and bacteria. The humification parameters (humification ratio, HR; the degree of humification, DH; humification index, HI) indicated T45 as the best silvicultural practice-approach-method to manage *Pinus laricio* forest for increasing soil carbon storage. The dendrometric parameters evidenced that T45 caused the greatest increment in wood growth (diameter and height), showing that the positive effect of the intense systematic thinning (T45) on the mechanical stability of plantation was related to the ability of trees to accumulate large amounts of carbon in their wood-tissues. These data were confirmed by wood density value that was the highest in pine trees under the T45. This study showed that in *Pinus laricio* forest under T45 C stock increased in soil and plant, already 4 years after thinning.

Keywords: Pine forest- Carbon storage- Clear cut- Dendrometric parameters- Thinning intensity- Soil biological activity

Introduction

Global warming and climate change concerns have heightened the interest in terrestrial carbon sequestration, in order to explore opportunities for climate change mitigation (Whorf and Keeling 1998; Leung et al. 2014). Forests play a prominent role in the global carbon cycle, contributing to store more than 80% of terrestrial aboveground and 40% of terrestrial belowground carbon storage (Kirschbaum 1996; Six et al. 2002; Alemu 2014). The possible rapid change in the status of forests – from a steady state of minimal CO_2 emission/sequestration to major CO_2 emitter

– may offer a cautionary tale of how quickly the source/sink status of large-scale forest C stocks can change (Birdsey et al. 2006). Our understanding of how forest management influences standing C stocks, however, is limited because many of forest carbon studies were focused on quantifying trends in forests in the natural state (Gough et al. 2008; Krug et al. 2012). In unmanaged forests total ecosystem C stocks generally increase with stand age as pools of living biomass, forest floor material (organic soil horizons) and mineral soil C accumulate through stand development before ultimately leveling off in older stands (Pregitzer and Euskirchen 2004; Bradford et al. 2009). Forest management activities have a number of potential influences on these general trends in C stock dynamics (Johnson 1992; Muscolo et al. 2007a, b; Kim et al. 2009; Muscolo et al. 2010, 2017).

Among silvicultural practices, thinning, which removes some trees from a forest in order to redistribute tree growth onto fewer and consequently with more valuable stems, is a crucial practice in the forest ecosystem management (Weiskittel et al. 2011). It is generally used for a variety of purposes, across both public and private ownerships in many types of forests and regions, representing the physiological basis for tree productivity and a key driver of ecosystem productivity (Chapin et al. 2011). Thinning has significant influence on forest soil (affecting root density, microbial communities, organic matter turnover and nutrient budgets), tree growth and even on the whole forest ecosystem (Tian et al. 2010; Qiu et al. 2011). The decreased density in and below forest canopies, generated by thinning, leads to improved light intensity conditions in forest stands, which in turn speed up litter decomposition rate (Zhao et al. 2014). Thinning is expected to change soil environments, the allocation of aboveground and belowground productivity, root density and turnover (Keith et al. 1997; Bowden et al. 2004). Thus, thinned forests might be expected to have lower rates of C accumulation than unmanaged forests. Increases in soil temperature and moisture following thinning are correlated with increased heterotrophic respiration rates, but reduced live root biomass leads to lower autotrophic respiration rates (Ryu et al. 2009). Thus, total soil CO_2 efflux may be higher (Selig et al. 2008) or lower (Sulligan et al. 2008) in thinned stands than unmanaged stands when comparing the same environmental conditions. In practice, these opposing

factors can contribute to either increase in soil C following thinning (Selig et al. 2008) or have little impact on soil C (North et al. 2009). Direct removal of live tree biomass during harvesting generally reduces total ecosystem C stocks (North et al. 2009; Davis et al. 2009) and affects soil microclimate (Jassal et al. 2009). Findings of Achat et al. (2015) clearly demonstrated that using the intensive harvest strategy at its maximum level decreased soil carbon storage. Besides SOC losses, the removal of logging residues had other negative effects on forest soils, such as a decrease in nutrient availability (mainly due to increased exportation of nutrients) which could lead to a reduction in site fertility and tree growth, thereby reducing, in the long term, carbon sequestration rate in the biomass of trees. Clarke et al. (2015) showed that the reduction in SOC stock after harvesting, observed in the organic layer of forests, depended on soil types, climatic conditions and tree species. For all these reasons, forest thinning may decrease carbon stocks in forest soil and in vegetation, increasing soil CO_2 efflux at different degree (Houghton et al. 2003; Mäkipää et al. 2014). Some studies, however, reported little differences in live tree C when stands that were thinned from below were compared with unmanaged stands (Hurteau et al. 2009). These contrasting results demonstrated the importance of ecosystem-specific studies to examine the impact of a variety of silvicultural options on total ecosystem carbon stocks.

In Italy, thinning of pine forests is the most effective silvicultural treatment to enhance the economic value of these stands (Cantiani and Chiavetta 2015). Therefore, this research aims to understand how thinning affects the dynamic of total carbon as well as each of its component pools in *Pinus laricio* forest ecosystem in Aspromonte Mountain (Calabria). We estimated carbon stocks in *Pinus laricio* stands, evaluating carbon pool dynamics in forest subject to different thinning (no thinning, moderate and intense thinning) and clear cut over two contrasting seasons (winter and summer) in order to verify if environmental conditions affected in a short term soil carbon pools. Our aim was also to identify the silvicultural practice that best increased and/or maintained carbon storage in pine forest. Our hypothesis was that by increasing thinning intensities, the properties of

soil related to fertility and quality could decrease, while the stand stability, wood quality, diameter and volume growth of the remaining stand could improve.

Materials and Methods

Study area

The study area, located in 60-years-old natural regenerated *Pinus laricio* stands in Aspromonte Mountain (Zervò, Calabria) (38°24'24" N; 16°01'82" E) at an elevation of 1100 m a.s.l. covers 190 ha. A typically Mediterranean climate prevailed in the study area, with an annual mean temperature of around 10 °C, with minimum and maximum monthly means of 3 °C (coldest month) and 17 °C (warmest month). The annual rainfall is 1838 mm, with minimum precipitation in summer. Rainfalls are unevenly distributed over the year. According to Pavari's phytoclimatic classification (Pavari 1959) the stands belong to the *Castanetum* zone. The soils, developed from high rank metamorphic rocks, such as schist and biotitic gneisses, were classified according to the IUSS WRB (2006) as Humic Cambisols, with a xeric soil regime moisture. In 2010 started the forest management of this stand. A study area of approximately 45 ha was set up for the plot investigation. The 45 ha were split as follow among the four treatments: i) 15 ha for the no thinning (T0: control, 1935 Nha^{-1}); 10 ha for the moderate thinning (T25: 25% basal area (BA) removal 1354 Nha^{-1},); 10 ha for the intense thinning (T45: 45% BA removal 780 Nha^{-1}) and 10 ha for clear cut (CC: 100% thinning, 0 Nha^{-1}). The experimental design was randomized and consisted of five blocks in each site. Each block in the T0 was 3 ha, while in the T25, T45 and CC was 2 ha. Thinnings were designed to reduce stand density, removing all of the trees present in the stand. Residues were removed after the different levels of thinning to reduce summer fire risks and the decay rate of biomass to mitigate environmental pollution in Mediterranean areas.

Soil sampling

Soil sampling was carried out in two different seasons (summer and winter 2014), which differ in soil moisture, soil temperature, soil microbial biomass, etc. Soil samples (0-30 cm) were randomly taken using a soil borer from 3 points within 20x20m quadrat (5) of each block, after removing the litter layers. A total of 120 samples were collected. Physicochemical properties of soil were detected on air-dried, sieved (2 mm mesh) soils. Soil water content (WC) and microbial biomass were detected in fresh soil samples within 24 h of sample collection.

Soil chemical and physical analysis

Particle size analysis was carried out by the hydrometer method, using sodium hexametaphosphate as a dispersant (Boujoucos 1962); pH was measured in distilled water and 1 M KCl using a 1:2.5 (soil:water) suspension; soil total nitrogen (N) was determined by Kjeldahl's procedure (Bremner and Mulvaney 1982) and cation exchange capacity (CEC) was determined by using the barium chloride-triethanolamine method (Mehlich 1953). Electrical conductivity (EC) was detected according to the method described by Blakemore et al. (1987): 10 g of dry soil were put into a glass beaker and mixed with 50 mL deionized H_2O, the suspension was shaken for 30 min and, after decantation, the conductivity was measured by using a conductometer. The water content was determined by drying the soil to constant weight in oven at temperature 105 °C and measuring the soil sample mass after and before drying. The water mass (or weight) is the difference between the weights of the wet and oven dry samples.

Total soil organic carbon (TOC) was determined according to Springer and Klee (1954). The content of organic carbon was calculated by back-titration with a solution of 0.2 N $FeSO_4$.

For total extractable organic carbon (TEC) determination, each soil sample (5 g) was extracted by adding 100 mL of 0.1 N NaOH/0.1 N $Na_4P_2O_7$ solution at 65 °C for 48 h, under N_2 atmosphere. TEC was fractioned into humified (humic acid, HA + fulvic acid, FA) and non-humified (NH) fractions (Ciavatta et al. 1990). Humic and fulvic acid carbon (HA+FA)

determination was performed as reported above, on 10 mL of 0.5 N NaOH solutions (Ciavatta and Govi 1993).

Humification parameters, such as humification index (HI), humification rate (HR) and degree of humification (DH), were calculated as shown in equations 1-3 (Ciavatta et al. 1990):

1) HI=NH/(HA+FA)
2) HR%=100(HA+FA)/TOC
3) DH%=100(HA+FA)/TEC

"HI" represents the ratio between not humified and humified extracted carbon, while HR is the percent of humification rate and DH is the percent of humified carbon in the extract. Humification index is normally 0.5 or more and may even reach 1 with slightly humified extracted organic matter. The HR % parameter is proportional to the state of humification of the soil organic matter. The DH % is 100% when the extracted organic carbon is completely humified (NH=0) and 50% if HI=1. However, when the extracted organic carbon is very humified, the index is close to zero (Gigliotti et al. 1999).

Water soluble phenols (WSP) were extracted with distilled water (Kaminsky and Muller 1978) and determined by using the Folin-Ciocalteau reagent, following the method of Box (1983). Tannic acid was used as a standard and the concentration of water-soluble phenolic compounds was expressed as tannic acid equivalents (μg TAE g^{-1} dry soil).

Soil biochemical analysis

Microbial biomass C was determined by the chloroform fumigation-extraction procedure (Vance et al. 1987). The filtered soil extracts of both fumigated and unfumigated samples were analyzed for soluble organic C using the method of Walkley and Black (Nelson and Sommers 1982).

Microbial activity was determined by the hydrolysis of fluorescein 3,6-diacetate (FDA) into fluorescein, according to Adam and Duncan (2001). The enzyme activity was expressed in μg fluorescein g^{-1}soil h^{-1}. Dehydrogenase (DH) activity was determined by the method of von Mersi

and Schinner (1991). Protease (PRO) activity was determined on 1 g (fresh weight) according to the method of Nannipieri et al. (1980). Catalase activity (CAT) was measured by the method of Beck (1971). Results were expressed as μmol O_2 g^{-1} soil min^{-1}.

Soil microbial analysis

Soil bacteria, fungi and actinomycetes were extracted following the method of Elliott and Des Jardin (1999). Total bacteria, fungal and actinomycetes colonies were estimated following the methods of Picci and Nannipieri (2003) and Eaton et al. (2005).

Tree analysis

We analyzed the trees within each 20x20m quadrat plot for all the silvicultural treatments that provided any trees for sampling.

In detail, the following dendrometric parameters: stand density (SD, Nha^{-1}); diameter at breast height (DBH, cm); height (H, m), dominant height (DH, m); basal area (BA, m^2ha^{-1}); volume (V, m^3ha^{-1}); arithmetic DBH (cm); arithmetic height (m); quadratic mean diameter (QMD, cm); ratio H/D (adimensional) and wood density (WD, gcm^{-3}) were measured immediately after thinning (2010) and 4 years after thinning (2014).

The dendrometric analyses were carried out with standard methods. The DBH was measured by caliper, while the total height of all the trees was measured with Vertex ultrasonic measuring device. By using these dendrometric parameters it was possible to determine the H/D ratio indicating population mechanical stability.

Wood density analysis

Wood samples were collected by using an increment borer to extract a section of wood tissue from a living tree with relatively minor injury to the plant itself at a steam breast height. The wood samples were stored in a conditioning chamber (12 h, 20 °C, 50% RH). The wood density

was measured with a X-ray densitometer to make a noninvasive analysis. The radiographic image was obtained by using LabVIEW v. 6i (National Instruments) software. Wood X-ray densitometry is a radiation detection method in which X-ray attenuation is converted to wood density (Fellin 2005).

Statistical analysis

All sets of experiments were repeated five times. All statistical analyses were performed using Systat v. 8.0 software package (SPSS Inc., Evanston, Ill, USA). All datasets were tested for normality using Shapiro-Wilk and Jarque-Bera tests. Treatment means were compared using Tukey's test (Sokal and Rohlf, 1981) to determine which means differed significantly at $p \leq 0.05$. One-way analysis of variance (one-way ANOVA) was used to test the differences in soil and plant quality under different thinning. Two-way ANOVA was used to test relationships between treatments and seasons. Significant differences and effects were determined as $p \leq 0.05$.

Results

Soil physical features

All the soils analyzed belong to the sandy-loam textural class, with 10% silt, 8 % clay and 82% sand. Our results showed that soil texture did not change over seasons and over treatments (data not shown).

pH measured in H_2O was slightly acid in all treatments and ranged between 4.99 and 5.25 in summer, and 5.53 and 5.68 in winter. Treatments did not significantly affect soil pH ($p=0.4$); however, significant differences were detected between the two seasons, with higher pH values ($p<0.05$) in winter than in summer (Figure 1).

Water content significantly differed among the treatments and between the seasons ($p<0.05$), with the highest values in winter, in the CC treatment. Electrical conductivity significantly differed ($p<0.05$) among treatments in both summer and winter seasons, with the

highest values in winter (Figure 1). Analysis of variance showed that seasonal variations affected EC and WC more than treatments; the interactions of the two factors, treatment and season, had the lowest effect with respect to the factors individually considered (Table 1).

Soil chemical features

The analyzed soil chemical properties in each thinned stand had the highest values in the summer season, except for N and WSP. Conversely, in unthinned forest soil, the values of OM, CAT and CEC were significantly lower in summer than in winter (Table 2).

The greatest amount of OM was detected in the T45 with values of 24.21% in summer and 15.54% in winter, respectively (Table 2). Analysis of variance showed that treatments affected OM more than seasons and the interactions of the two factors (treatment and season) had the lowest effect compared to the factors individually considered (Table 3). Similar behavior was observed for microbial biomass C. The maximum MBC amount was recorded in the T45 in summer (7997 μg C g^{-1}dry soil), and the lowest one was detected in T0 in winter (6027 μg C g^{-1}dry soil) (Table 2). Soil C/N value ranged from 9 to 19.5; the highest values were found in summer in the T45, and the lowest one in winter in CC. The interactions of the two factors (treatment and season) were not significant compared to the factors individually considered ($p=0.1$). The highest FDA activity was detected in the T45, both in summer and in winter seasons, followed by the T25 (Table 2). The lowest FDA activity was found in T0 in both seasons. A similar behavior was observed for PROT, CAT and DH. Analysis of variance showed that treatments affected FDA and CAT more than seasons; on the contrary, seasons affected PROT and DH more than treatments; the interactions of the two factors, treatment and season, had less impact than the factors individually considered (Table 4). WSP differed significantly between the two seasons ($p<0.05$) but not among the treatments ($p=0.4$); WSP amount was higher in winter than in summer (Table 2). Regarding TOC, TEC and C_{HA+FA} the ranking was in the order T45>T25>CC>T0, both in summer and winter

seasons. HA/FA ranking was as follow T25≥CC>T0>T45. In the T45 the HR and DR were the highest in summer and winter, while HI was the lowest (Table 5).

Soil microbial features

In all sites, microbial population was significantly higher in summer than in winter, bacteria were more abundant than fungi and no actinomycetes were found. T45 was the most effective treatment that significantly increased the colonies of bacteria in summer and in winter, the ranking was as follow: T45>T25>CC>T0 (Figure 2). Analysis of variance showed that seasons affected fungal and bacterial populations more than treatments; the interactions of the two factors, treatment and season, had the highest effect than the factors individually considered on fungal population, and the lowest ones on bacterial population with respect to the factors individually considered (Table 6).

Tree parameters

Volume, basal area, DBH and height increased but H/D ratio decreased within the 4 year after thinning at all sites. The greatest increment was observed in T45. The H/D ratio in the T45 decreased most followed by T25 and T0, although it already started with the lowest value of all stands (Table 7). Additionally, wood density increased 4 year after thinning in T45 and T25 more than T0.

Discussion

Carbon storage in soils is a dynamic balance between input of organic matter and output mostly in the form of CO_2 efflux (Tian et al. 2010). The overall response of forest ecosystems, as C source and/or sink, is variable according to forest type, development stage and silvicultural management (Johnson 1992; Nilsen and Strand 2008). Many studies reported that silvicultural management practices may affect directly and/or indirectly C dynamics in forest ecosystems (Johnson 1992; Neary et al. 1999; Nilsen and Strand 2008). Recent studies synthesized evidence regarding forest

management effects on soil organic carbon (Lal 2005; Jandl et al. 2007), but quantitative information are limited to specific management issues. The ability of forest soils to sequester C is due to the deposition and accumulation of a resistant slowly decomposable C pool that was estimated to represent approximately 65% of the organic matter in soil. Forest management can change this belowground process (Tang et al. 2005), with consequent negative effects on nutrient concentrations (Ashagrie 2007), water retention (Resck 2008) and carbon storage (Lal 2006). Roscoe and Buurman (2003) quantified the effects of forest management on soil estimating only total organic carbon, but often the changes management-induced are not duly reflected in TOC values; thus, parallel determination of stable and labile fractions of soil organic matter (SOM) would be very useful as already suggested by de Figueiredo et al. (2010). Simultaneous examination of changes in SOM fractions and in soil biological properties can give important information about the impact that forest management have on C cycle (Sicardi et al. 2004; Sidari et al. 2005). It has well been demonstrated that soil ecosystems with large microbial communities improve soil carbon sequestration more than soil ecosystem with reduced biodiversity (de Graaff 2015). Increasing evidence in literature indicates that taxonomic and functional compositions of microbial communities are strong drivers of SOM processes (Hooper et al. 2005). Fungal hyphae and polysaccharides microbial-originated produce organic polymers, which form and stabilize aggregates, playing an important role in building and conserving soil structure (Darbyshire et al. 1993). Additionally, soil bacteria have been identified as a functional group in driving soil organic matter dynamic (Nannipieri et al. 2003; Fierer et al. 2007). Soil bacterial communities are strongly linked to the extracellular enzymes involved in carbon transformation, whereas fungi are associated with activities of extracellular enzymes driving carbon oxidation (You 2014). Under T45 the soils had the highest amount of microbial biomass and bacteria. These results qualitatively demonstrated a microbial diversity-SOM dynamics relationship. These data were also confirmed by the results of enzymatic activities. Soil enzymes play an important role in organic matter decomposition and nutrient cycling. Several studies showed that enzyme activities can be used as early indicators of

changes in soil properties originated by management practices and consequences of global changes (Deng and Tabatabai 1994; Kandeler 1999; Ajwa et al. 1999; Alvear et al. 2005; Muscolo et al. 2014, 2015). Our results showed an increase in all enzymatic activities in managed soils, mostly in the T45, supporting previous findings of Jiménez et al. (2016) indicating that soil with great diversity and amount of ground vegetation had greater enzymatic activity. The seasonality influenced soil carbon sequestration and in summer time, in agreement with previous results of Rowland et al. (2014), we observed the greatest amount of OM, TOC, TEC and the highest values of HI, HR and DR in each forest treatment. The ranking effects of treatments on soil was instead the same in summer and winter. Our results evidenced that seasons affected soil carbon storage less than treatments, suggesting that the lower density of trees in the T45 resulted in different regimes of light, temperature and humidity at the ground and in the soil, increasing herbaceous vegetation and easily degradable litter, which in turn promoted an increase in soil microbial biomass and overall in bacteria amount. Our results are in agreement with the findings of Bardgett et al. (2003), Lee and Jose (2003), Allison (2006), De Deyn et al. (2009), Billings et al. (2010), showing that microbes are largely responsible for the cycling of plant-available soil nutrients and for the decomposition of organic matter and soil carbon sequestration, affecting the ratio of carbon converted to carbon dioxide or to soil organic carbon. We found that T45 was the practice that mostly increased the stable fraction of SOM and the humification ratio, the degree of humification, while decreased the humification index. All together these humification parameters indicated that the T45 was the silvicultural practice to adopt for increasing soil carbon storage.

Pinus growth (diameter and height) and wood density changed with the treatments. H/D ratio in T45 was lower than in T25 and T0, suggesting that the positive effect of the T45 on the mechanical stability of the trees is due to a large accumulation of wood distributed more in the diameter rather than in the height. Additionally, an increment in basal area and wood density suggested that T45 was the best practice for pinus growth. In short, we can suggest that the T45 is able to increase C concentration both in *Pinus laricio* tree and in soil, with evident effects 4 years after thinning.

Conclusions

Our study provides scientific information for establishing the role of pine forest soils in carbon sequestration programs, predicting the consequences of current management practices for future forest productivity, and understanding how ecological processes interact with human interventions to influence soil carbon storage. These results are important for land managers policymakers, carbon accountants and scientists working on a variety of forest-related issues. Our challenge is now to expand our knowledge to other forests so that we can predict the dynamic behavior of soil processes and the impact of management practices on carbon storage. Ability to meet this challenge will play a key role in determining the sustainability of forest management activities.

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Figure Captions

Figure 1. Physical soil analysis: water content (WC%), pH (H_2O), pH (KCl), and electrical conductivity (EC μs cm^{-1}) under *Pinus laricio* plantation differently managed: intense thinning, T45; moderate thinning, T25; no thinning, T0; and clear cut, CC. Treatments marked with the same letter are not significantly different ($P > 0.05$)

Figure 2. Colonies of fungi and bacteria (CFU g^{-1} dry soil) in soil under *Pinus laricio* plantation differently managed: intense thinning, T45; moderate thinning, T25; no thinning,T0 and clear cut, CC. Treatments marked with the same letter are not significantly different ($P > 0.05$)

Table 1 Analysis of variance of the effects of treatments, seasons and their interactions on physical and chemical soil parameters: water content (WC); pH (H_2O); pH (KCl); electrical conductivity (EC)

		WC	pH (H_2O)	pH (KCl)	EC
R^2		0.937	0.206	0.129	0.969
	Treatments	87.381*	0.745	0.405	219.838*
F ratio	Seasons	128.571*	7.168*	4.494*	502.219*
	Interaction	66.667*	0.329	0.073	25.903*

*$p<0.05$

Table 2 Chemical and biochemical soil analysis: organic matter (OM%), total nitrogen (N%), C/N ratio, fluorescein diacetate (FDA, µg fluorescein g^{-1}soil h^{-1}) protease (PROT, µg tyrosine g^{-1}dry soil/2h), catalase (CAT, µmol O_2 g^{-1} soil min^{-1}), dehydrogenase (DHA, µg TTF g^{-1} h^{-1}), microbial biomass C (MBC, µg C g^{-1}dry soil), water soluble phenols (WSP µg TAE g^{-1} dry soil) and cation exchange capacity (CEC, meq $100g^{-1}$ dry soil) under *Pinus laricio* plantation differently managed: intense thinning, T45, moderate thinning, T25, no thinning, T0 and clear cut, CC

Season		OM	N	C/N	FDA	PROT	CAT	DHA	MBC	WSP	CEC
	T45	24.21^{a}	0.72^{a}	19.5^{a}	71.92^{a}	90.90^{a}	1.88^{a}	11.15^{a}	7997^{a}	200^{a}	39.2^{a}
	T25	18.35^{b}	0.63^{c}	16.5^{b}	58.52^{b}	80.35^{b}	1.69^{b}	7.36^{b}	7574^{b}	200^{a}	34.3^{b}
Summer	T0	7.68^{d}	0.37^{d}	12^{c}	45.86^{d}	68.22^{d}	0.74^{d}	5.89^{d}	6378^{d}	200^{a}	18.5^{c}
	CC	16.86^{c}	0.62^{b}	15.8^{b}	53.18^{c}	76.07^{c}	1.13^{c}	6.23^{c}	6810^{c}	195^{a}	33.5^{b}
	T45	15.54^{a}	0.70^{b}	13^{a}	61.80^{a}	63.01^{a}	1.41^{a}	4.40^{a}	7550^{a}	222^{a}	31.3^{a}
	T25	14.49^{b}	0.69^{b}	12^{b}	53.25^{b}	59.86^{b}	1.32^{b}	3.77^{b}	6800^{b}	233^{a}	31.4^{a}
Winter	T0	12.32^{d}	0.67^{b}	11^{c}	42.85^{d}	52.79^{d}	0.94^{d}	1.93^{d}	6027^{d}	229^{a}	28.0^{b}
	CC	13.48^{c}	0.83^{a}	9^{d}	50.10^{c}	56.81^{c}	1.03^{c}	2.24^{c}	6352^{c}	228^{a}	30.0^{a}
Replicates		5	5	5	5	5	5	5	5	5	5
Factors		*P-value*	*P-value*	*P-value*	*P-value*	*P-value*	*P-value*	*P-value*	*P-value*	*P-value*	*P-value*
Results of ANOVA											
Season		<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Treatment		<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	=0.4	<0.05
Interactions		<0.05	<0.05	=0.1	<0.05	<0.05	<0.05	<0.05	<0.05	=0.4	<0.05

Different letters in the same column indicate, within each season, significant differences (Tukey's test, $p \leq 0.05$)

Table 3 Analysis of variance of the effects of treatments, seasons and their interactions on chemical soil parameters: organic matter (OM), water soluble phenols (WSP) and total nitrogen (N)

		OM	WSP	N
R^2		0.923	0.391	0.424
F ratio	Treatments	90.167*	0.228	45.373*
	Seasons	55.566*	24.304*	24.999*
	Interaction	31.332*	0.236	14.217*

*$p<0.05$

Table 4 Analysis of variance of the effects of treatments, seasons and their interactions on biological soil parameters: fluorescein diacetate (FDA), protease (PROT), catalase (CAT), dehydrogenase (DHA) and microbial biomass C (MBC)

		FDA	PROT	CAT	DHA	MBC
R^2		0.892	0.950	0.763	0.874	0.653
F ratio	Treatments	97.761*	19.957*	34.420*	74.902*	21.497*
	Seasons	23.271*	203.537*	8.479*	504.224*	7.616*
	Interactions	4.899*	11.125*	5.592*	5.040*	1.069

$p < 0.05$

Table 5 Effect of intense thinning, T45, moderate thinning, T25, no thinning, T0, and clear cut, CC on total organic carbon (TOC), total extractable carbon (TEC), humic acid (HA), fulvic acid (FA), humic acid plus fulvic acid carbon $C_{HA+FA,}$ humic acid/fulvic acid (HA/FA), humification index (HI), humification rate (HR), humification degree (DR)

Season		TOC %	TEC %	C_{HA+FA} %	HA/FA	HI	HR %	DR %
	T45	14.07^{a}	12.6^{a}	10.83^{a}	1.17^{c}	0.16^{d}	77.0^{a}	85.9^{a}
	T25	10.66^{b}	8.4^{b}	6.77^{b}	1.42^{a}	0.24^{c}	63.5^{b}	80.5^{b}
Summer	T0	4.46^{d}	3.3^{d}	2.61^{d}	1.25^{b}	0.26^{b}	58.5^{c}	79.1^{b}
	CC	9.80^{c}	7.6^{c}	5.56^{c}	1.43^{a}	0.37^{a}	56.7^{c}	73.1^{c}
	T45	9.03^{a}	7.8^{a}	6.85^{a}	1.22^{d}	0.14^{d}	75.3^{a}	87.5^{a}
	T25	8.42^{b}	6.6^{b}	4.95^{b}	1.96^{a}	0.33^{b}	58.8^{c}	75.2^{c}
Winter	T0	7.16^{d}	5.3d	3.85^{c}	1.58^{c}	0.38^{a}	53.5^{d}	72.1^{c}
	CC	7.83^{c}	6.2^{c}	5.0^{b}	1.67^{b}	0.24^{c}	63.3^{b}	80.7^{b}
Replicates		5	5	5	5	5	5	5
Factors		*P-value*	*P-value*	*P-value*	*P-value*	*P-value*	*P-value*	*P-value*
Results of ANOVA								
Season		<0.05	<0.05	<0.05	<0.05	<0.05	=0.4	=0.4
Treatment		<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Interaction		<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

Different letters in the same column indicate, within each season, significant differences (Tukey's test, $p \leq 0.05$)

Table 6 Analysis of variance of the effects of treatments, seasons and their interactions on fungi and bacteria colonies in soil under *Pinus laricio*

		Fungi	Bacteria
R^2		0.985	0.978
	Treatments	263.438*	326.250*
F ratio	Seasons	408.437*	540.000*
	Interaction	1020.938*	97.500*

*$p<0.05$

Table 7 Dendrometric parameters: stand density (SD, Nha^{-1}); diameter at breast height (DBH, cm); height (H, m), dominant height (DH, m); basal area (BA, $m^2\ ha^{-1}$); volume (V, m^3ha^{-1}); arithmetic DBH (cm); arithmetic height (m); Quadratic mean diameter (QMD, cm); ratio H/D (adimensional); wood density (WD, g cm^{-3}) of *Pinus laricio* trees differently managed: intense thinning, T45, moderate thinning, T25, no thinning, T0 and clear cut, CC

Year	Thinning intensities	SD	DBH	H	DH	BA	V	Arithmetic DBH	Arithmetic H	QMD	H/D	WD
2010	T45	780	25.6^{a}	20.2^{a}	21.0^{a}	46.5^{c}	532.4^{c}	27.5^{a}	19.1^{a}	27.55^{a}	78.9^{b}	0.53^{a}
	T25	1354	23.1^{b}	20.0^{a}	20.7^{a}	60.3^{b}	698.3^{b}	23.4^{b}	18.9^{a}	23.81^{b}	86.6^{a}	0.51^{ab}
	T0	1935	21.4^{c}	18.0^{b}	19.1^{b}	77.3^{a}	748^{a}	22.2^{c}	17.1^{b}	22.55^{c}	84.1^{a}	0.49^{b}
2014	T45	780	30.3^{a}	$20,8^{a}$	22.2^{a}	54.1^{c}	562.4^{b}	31.3^{a}	19.2^{a}	29.71^{a}	68.6^{b}	0.58^{a}
	T25	1354	26.9^{b}	20.9^{a}	21.4^{a}	68.9^{b}	728.4^{a}	27.3^{b}	19.3^{a}	25.45^{b}	77.6^{a}	0.53^{b}
	T0	1935	23.6^{c}	18.4^{b}	20.1^{b}	84.5^{a}	774.8^{a}	24.4^{c}	16.7^{b}	23.57^{c}	77.9^{a}	0.52^{b}

Different letters in the same column indicate, within each season, significant differences (Tukey's test, $p \leq 0.05$)

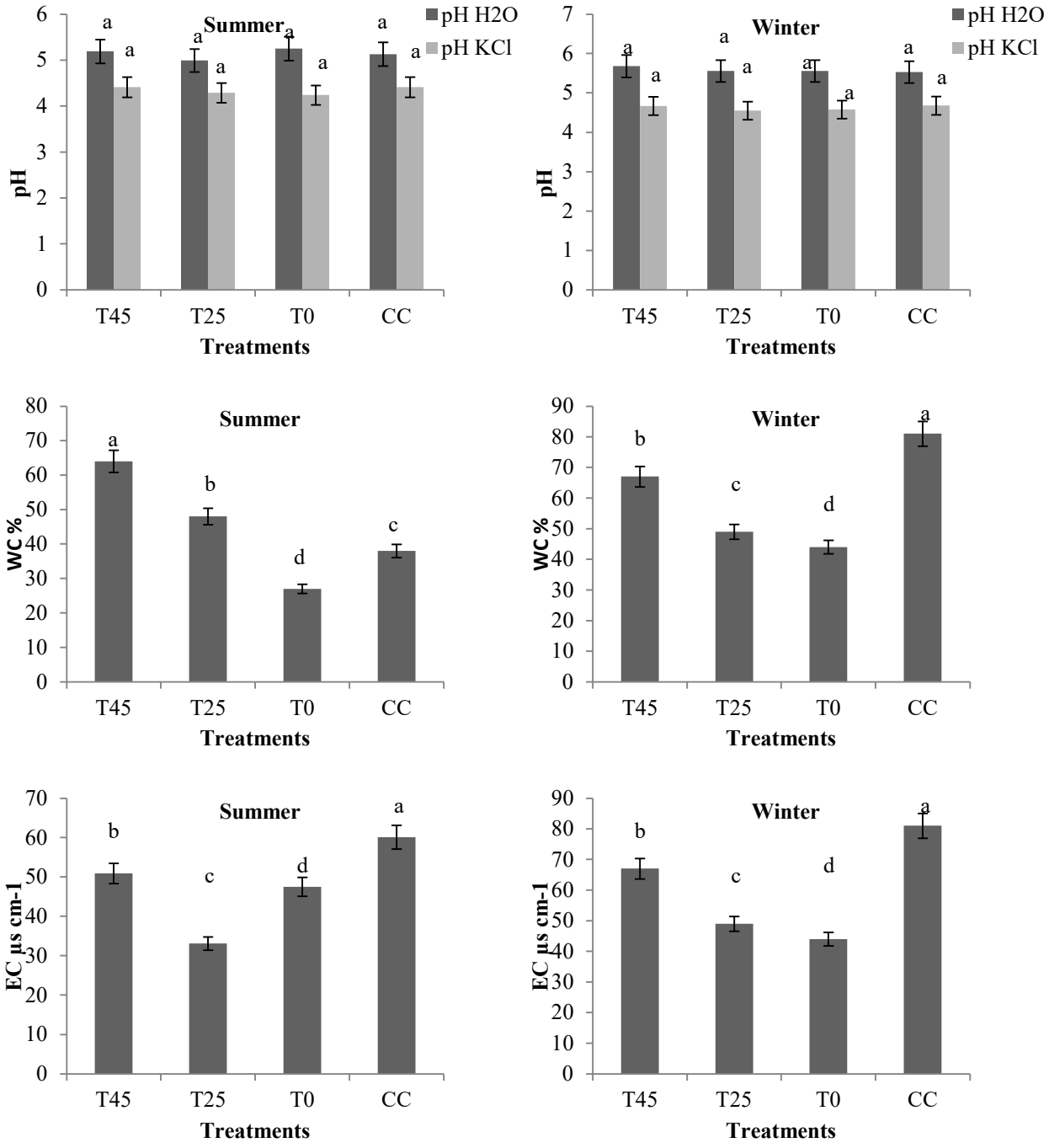
Summer
pH H2O
pH KCl
pH
T45
T25
T0
CC
Treatments
Winter
WC %
EC µs cm-1

Figure 1

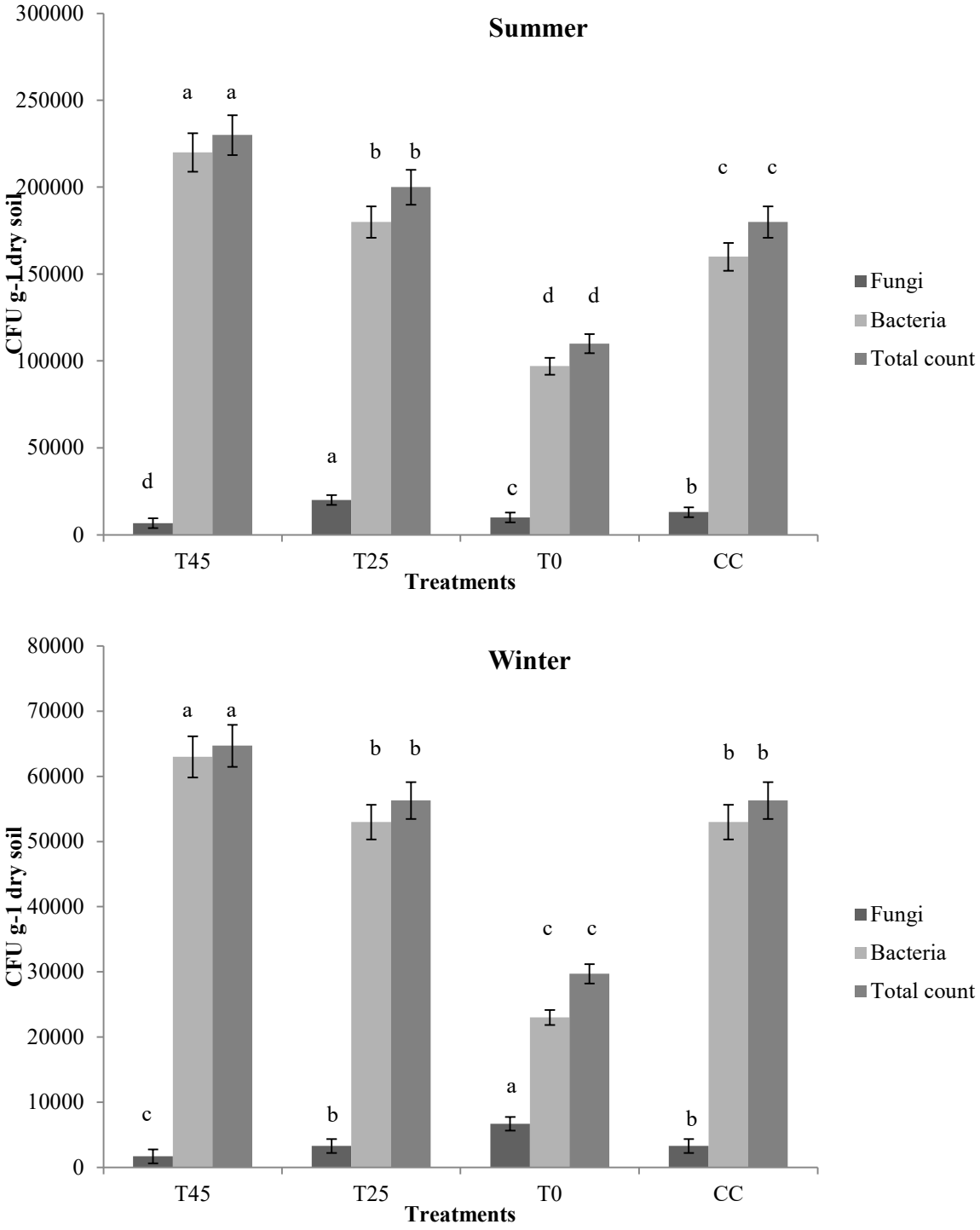
Summer
CFU g-1dry soil
300000
250000
200000
150000
100000
50000
0
a a
b b
c c
d d
a
c
b
d
T45
T25
T0
CC
Treatments
Fungi
Bacteria
Total count
Winter
CFU g-1 dry soil
80000
70000
60000
50000
40000
30000
20000
10000
0
a a
b b
b b
c c
a
c
b
b
T45
T25
T0
CC
Treatments
Fungi
Bacteria
Total count

Figure 2