Effect of thinning on biophysical soil properties of *Cupressus lusitanica* stand in Munessa forest, Ethiopia

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ABSTRACT

Forest management practices such as thinning can dramatically change the microenvironment and thereby affect the physicochemical and soil microbiological properties. The aim of this research was to determine the effect of thinning on soil physicochemical properties and microbial biomass under *Cupressus lusitanica* plantation at Munessa forest. The experiment was carried out in a fenced stand treated differently (thinned and unthinned plots) with a completely randomized design. In each plot, 20 sampling spots were randomly selected for soil sample collection from 0 to 10 cm and 10 to 25 cm depth. Independent t-test was used to compare effects of thinning on biophysical properties of the soil. Results showed that soil organic carbon (SOC) and total nitrogen varied significantly between treatments. SOC concentration was generally lower at the soil depth of 0-10 to 10-25 cm. The concentrations of microbial biomarker used as a proxy for microbial biomass assessment were 22% larger in thinned stands. Soils under the thinned stand contained larger concentration of arbuscular mycorrhizal fungi biomarker. The two forest stands were established at the same time with the same stocking and hence differences in soil quality could be possibly resulted from management intervention. These findings showed that *Cupressus* thinning could enhance biomass accumulation and organic C input to the soil.

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INTRODUCTION

The 2015 global forest resource assessment (FRA) report indicated that, during the past thirty years the world's forest area has declined from 4.1 billion ha to just under 4 billion ha, a decrease of 3.1 percent (FAO, 2016). It is the natural forest area that is decreasing. The report specified that planted forest area has increased by over 105 million ha since 1990 and accounted for 7% of the world's forest area. The share of the plantation area in Africa is the lowest among all the continents. Ethiopia has one of the longest forest plantation histories in Africa. The real planting of exotic tree species commenced 110 year ago with the introduction of *Eucalyptus globules* (Pohjonen and Pukkala, 1990). However, large-scale industrial plantations started during the Derge regime in the late 1970s. The current total area estimate is about 1 million ha (MEFCC, 2017). The majority of the plantations are covered by exotic tree species mainly Eucalyptus species (58%) and *Cupressus lusitanica* (29%) of the total area.

Plantation forest needs to be conscientiously managed to enhance stand quality and maintaining ecosystem function and processes. Management to increase overall ecosystem function and accelerate the accumulation of carbon for reserve in a plantation forest requires basic scientific exploration. Forest management practices and other disturbances alter microbial community, size of soil organic carbon, as well as the soil properties, by changing microenvironment condition (Jandl *et al*., 2007). For example, a tending operation such as thinning is typically used to increase the tree-bole volume of usable-sized trees and this can be one of the responsible factors for the largest gain in productivity over unmanaged plots. Such stand management is also expected to influence the environmental condition beneath the existing canopy, soil microclimate, and root dynamics (Da-Lun *et al*., 2010).

Thinning changes the plant community from a high-density forest to an open canopy. The opening of the canopy, due to the removal of a certain number of trees, is an important practice for the management of forests. These activities bring change in stand structure and herbaceous community. It results in important modifications of the microclimatic conditions that influence the ecophysiological functioning of trees and belowground components and it also influences soil-water relationships (Aussenac and Granier, 1988; Grayston and Renneberg, 2006).Thinning

increases the risk of erosion (Carter *et al*., 2006) and changes in soil moisture (Grace *et al*., 2006). Despite the numerous investigations on the impact of thinning on soil properties and microbial biomass, there is inconsistency regarding the impact of thinning on soil biophysical properties. Other studies showed that thinning positively affected soil properties and microbial biomass (Hu and Zhu, 1999; Grady and Hart, 2006; Hwang and Son 2006; Chen *et al*., 2015) or no change in the respective parameters (Tan *et al*., 2008; Jang *et al*., 2016). Other reports show opposite effect of thinning on the soil properties and microbial biomass (Baena et al., 2013; Zhou *et al*., 2015). Thinning improved soil microbial quantity, enzyme activity, total porosity, available nutrients and soil fertility but reduced soil bulk density after 2-year thinning (Zhang *et al*., 2001).

Cupressus lusitanica a fast-growing evergreen conifer with high timber quality, is widely planted species in Ethiopia. The Munessa forest is one of the largest plantation forests established in the 1970s in Ethiopia. It covers about 6973 ha of which *Cupressus lusitanica* was the dominant planted tree species that accounted for 62.5% of the total plantation area (Tesfaye Teshome and Petty, 2000). *Cupressus lusitanica* plantation in the Munessa forest is typically used for industrial timber production. With the exception of weeding there is no other tending operation carried out throughout the rotation periods. Previous studies in Munessa forest have focused on water use efficiency (Masresha Fetene and Beck, 2004), root biomass and soil-plant water dynamics (Fritzsche *et al*., 2006), soil chemistry (Mulugeta Lemenih *et al*., 2004) on *Cupressus lusitanica* plantation. Yeshanew Ashagire *et al*. (2003, 2005) have conducted research on the impact of forest conversion in relation to biogeochemical processes in the same forest. However, knowledge of forest management such as the impact of thinning on the biophysical properties of soil in Munessa forest does not exist. Understanding the variations in soil properties and microbial community is crucial for evaluating the stability of the soil organic pool and the sustainability of forest production following a thinning treatment. Therefore, in the present study, an attempt was made to determine the effects of thinning on soil physical, chemical and microbial properties in a thinned and unthinned control plots of *Cupressus lusitanica* stand found in Munessa forest, Ethiopia.

MATERIALS AND METHODS

Study site
The study area is found in part of the forest stands of the Munessa forest (7°25`44´´N longitude and 38°51´05´´E latitude), located in Oromia Regional State, West Arsi zone, Ethiopia. The study stand was previously planted with *Pinus patula*. The slope is gentle and the elevation of the study plots is 2126 m above sea level. Eight-year meteorological records (2001-2009) in the study area has shown that the mean annual rainfall was 1144 mm and the mean annual temperature was 15 °C (Strobl, 2012). Though the area has been reported to receive a bimodal rain fall (Fritzsche *et al.,* 2006), the data from the past eight years suggest lack of a clear demarcation for the short and long rainy seasons (Figure 1). The soil texture is predominately clay evolved from volcanic parent material and was classified as Mollic Nitisols.

Study design
Two experimental fenced plots were selected in which they were 50 m apart from each other and were uniform with regard to site characteristics. The planted trees are 5 years old. In January 2008, selective thinning has been done in one of the fenced areas by removing the competitors' trees next to trees assigned as a potential crop. No thinning operation was carried out on the other fenced area and was considered as a control. After thinning the number of trees left in the thinned plot was 98 as compared to 144 in the control plot.

Soil sampling and preparation
Core soil samples (cylindrical steel core with 4.0 cm diameter) were obtained separately in triplicate from 0–10 and 10–25 cm depths. Soil sampling was performed within each forest plot at 20 randomly selected sample locations. Samples were obtained from 0-10 cm and 10-25 cm soil depth separately. The soil samples were then mixed to form the composite sample. Three replicates from each composite soil sample were used for further analysis. Immediately after collection the soil samples were sieved with 2 mm mesh size and all visible fine roots were picked out. After homogenization of each soil sample, aliquots (*c*. 2 g) were taken for organic C and total organic N analysis. After air-drying, soil samples were finely ground with a steel ball mill (Mixer Mill, Retsch MM 200) and dried overnight at 105 °C. Likewise, directly after homogenization of the

soil, subsamples of about 10 g were collected separately for phospholipids fatty acid (PLFA) and neutral lipid fatty acid (NLFA) analysis. After removing all visible plant materials, the soil samples were then packed in small glass vials and kept frozen until analyzed.
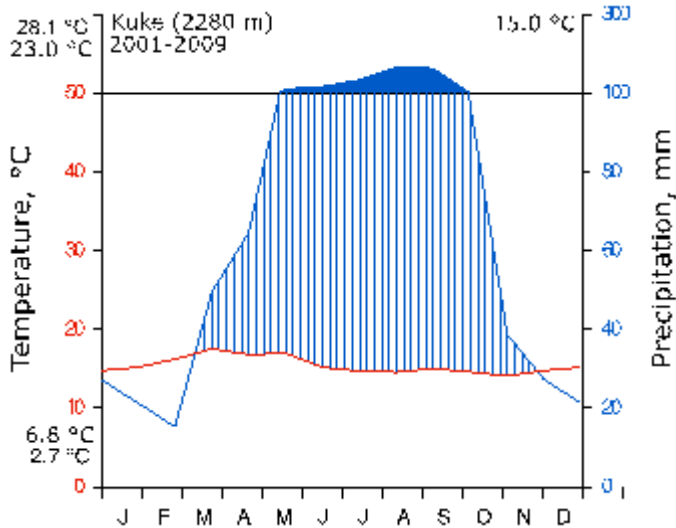

Figure 1. Walter-type climate diagram of the research area (eight-year averages from 2001 to 2009 at Kuke field station).

Laboratory analysis

Air dried soil samples was used to determine texture by the pipette method (Gee and Bauder, 1986). Soil pH was analyzed potentiometrically in 1 M KCl [1:2.5 (m/v)]. Cation exchange capacity (CEC) was determined with the $BaCl_2$ compulsive exchange method (Gillman and Sumpter, 1986). Soil organic carbon and nitrogen contents were analyzed using a Vario EL III elemental analyzer (Elementar Analysensysteme GmbH, Germany). Lipid fatty acid extraction was carried out using frozen soil samples (1.5 g) extracted overnight with a chloroform-methanol citrate buffer mixture (1:2:08) by the modified method described by Bligh and Dyer (1959). The fatty acid 19:0 (nonadecanoic acid methyl ester) was added to the samples as an internal standard. Lipids were separated into neutral, glyco-, and phospholipids using solid phase extraction with silicic acid column (Bond Elut LRC-Si, Varian Agilent Technologies, Santa Clara, CA). Thereafter,

the neutral and phospholipids were subjected to a mild-alkali methanolysis, and the resulting fatty acid methyl esters were separated by gas chromatography using an Agilent 7890A GC-MS (Varian Agilent Technologies, Santa Clara, CA). The fatty acids were quantified by comparison of the peak areas with those of the standard peak. Standard nomenclature was used to refer to the PLFAs according to the designation described in Zelles (1999). Phospholipid fatty acids i15:0, a15:0, i16:0, 18:1ω7c and cy19:0 were used to represent bacterial biomass (Zelles, 1999), while the PLFAs 18:2ω6 and 18:1ω9 were considered to have fungal origin (Zelles, 1997). The neutral lipid fatty acid (NLFA 16:1ω5) was used as a marker fatty acid for AM fungi (Olsson and Johansen, 2000).

Data analysis

Data were expressed as means of three replications for each forest plot and soil depth. Mole percents of individual fatty acids were used as an input values for microbial biomass analysis. The concentrations of each fatty acids (nmol g^{-1} dry soil) were calculated based on the peak areas to an analytical standard peak (19:0, Sigma Chemical Co., St. Louis, MO, USA). These concentrations were used in all statistical analyses. To assess the significance of all data between the thinned and control plots, a mean separation test was used. Normality of treatment group means was assessed using the Shapiro-Wilk test and homogeneity of variance among means using Levene's Test. Differences were considered significant at the $p < 0.05$ level. All graphing and statistical analysis was performed using SigmaPlot version 11 (Systat Software Inc., San Jose, CA, USA).

RESULTS AND DISCUSSION

Soil physicochemical properties

The changes in physicochemical properties at thinned and adjacent control plots are presented in Table 1. The pH was slightly acidic at both experimental plots. Although on average, the change of soil pH between the two plots was not significant, the mean soil pH at a depth of 0-10 cm was lower at the thinned plot (6.0) than control plots (6.3). In contrast, from a depth of 10-25 cm soil pH values tended to be similar in both forest plots (Table 1). Similarly, there was no significant difference in cation exchange capacity (CEC) between the two experimental plots. However,

corresponding values were higher under thinned forest plots which is mainly driven by differences in exchangeable Ca++. Unlike soil pH, there was significant difference in CEC between soil sampling depth. In both forest plots, CEC of the subsoil (10-25 cm) was significantly lower than the topsoil (0-10 cm), with an overall decline of about 30% (Table 1).

From 0-10 to 10-25 cm soil depth the SOC concentration decreased significantly ($P<0.05$). Concurrently to the decline in SOC with soil depth, the CEC decreased as well, showing the impact of soil organic matter on exchangeable nutrients in these forest plot. The results of one-way ANOVA showed that larger SOC concentrations under thinned plot was observed in the 0-10 cm soil layer than in the unthinned plot, but the values were not statistically significant ($p < 0.05$). Furthermore, a similar trend was also observed in TN from the depth (Table 1). However, TN from a soil depth of 10-25 cm was significantly different between the two-forest plots ($P < 0.05$) where the thinned plot soil had higher concentrations than samples taken from the control plot (Table 1).

Forest management intervention can cause significant impact in the soil quality, which can lead to changes in soil physical attributes. After thinning operation, a forest stand produces un-harvested residues such as fallen leaves, shoots, and dying and dead roots that can be used as a nutritive substrate for soil microorganisms. Thinning also creates opening gaps and these gaps promotes solar radiation, soil temperature, and soil moisture (Scharenbroch and Bockheim, 2007) that improves living conditions for soil microbes. Furthermore, thinning treatment could bring change in understory species and functional diversity in gaps (Ares *et al.*, 2010). Therefore, changes in microclimate, soil resource availability as well as plant community composition due to thinning are expected to affect the structure and function of microbial communities (Chatterjee *et al.*, 2008) and consequently affecting microbial regulation on the soil carbon process (Wu *et al.*, 2019). In the present study, the plots were located close to each other and trees were initially planted at the same time and with the same stocking and hence differences in belowground properties could be possibly as the result of management intervention (thinning). This notion emanates from the observation that larger aboveground biomass from individual potential crop trees.

Table 1. Basic characteristic of the soils from 0-10 cm and 10 -25 cm soil depth of thinned and unthinned plots.

Plot/ depth	Sand*	Silt*	Clay*	C‡	N‡	pH§	CEC¶	BS**
	—— g kg⁻¹ ——			– g kg⁻¹ –			mmol₍₊₎ kg⁻¹	%
Thinned plots								
0-10 cm	210±15	395±62	395±67	114±27^a	10.2±2.3^a	6.0±0.5^a	638±92^a	100
10-25 cm	189±8	371±65	440±70	39±15^b	4.3±1.6^b	5.7±0.6^a	449±69^b	100
Unthinned plots								
0-10 cm	203±20	376±20	421±20	90±23^a	8.8±2.2^a	6.3±0.5^a	568±24^a	100
10-25 cm	190±26	329±7	481±27	21±6^b	2.0±0.5^c	5.7±0.7^a	447±74^b	100

*measured by the pipette method (Gee and Bauder 1986); ‡ total carbon and nitrogen measured by dry combustion (Elementar Vario EL, Hanau, Germany); § analyzed potentiometrically in 1 M KCl [1:2.5 (m/v)]; ¶ cation exchange capacity determined with the $BaCl_2$ compulsive exchange method (Gillman and Sumpter1986); ** base saturation. Values are mean and standard deviation (n = 3). Different letters in each column indicate significant differences ($P < 0.05$).

In the same experimental plots, Zeleke Asaye (2011) identified significantly higher annual fine root biomass production in the thinned plot (554-603 g m^{-2}) as compared to the unthinned plot (327-364 g m^{-2}). Above and belowground fine litter are a major component of biomass (Clark *et al*., 2001); larger input of the former materials under the experimental plots in the thinned part was also expected and this could probably contribute to larger carbon input. Two years after thinning treatment, Hwang and Son (2006) found out mean SOC concentrations (g kg^{-1}) for pitch pine and Japanese larch plantations of 39.6 and 58.8 for the control and 44.0 and 63.4 for the thinned plot. In a forest ecosystem, inorganic matter assimilation is performed by fine roots (Yunusa *et al*., 2012) and they also function as a major source of organic matter in the soil (Du and Fang, 2014).

Soil microbial biomass

The relative abundance of individual fatty acid profiles extracted from soil samples under the experimental plot is shown in Figure 2. The normal saturates 16:0 and monounsaturated 18:1ω7c, methyl branched 10:Me16b and branched chain saturated i15 were the most abundant PLFAs, accounting for 54% of the total concentration. Monounsaturated 16:1ω7, 18:1ω9, and Cyclopropyl fatty acid cy19:0 were formed the second most predominant group (24%). Branched-chain saturates (a15 and i16), monounsaturated 16:1ω5 and methyl branched 10:Me16a were 19% of the total concentration. 18:2ω6 and 16:1ω9 were 3% of the total PLFA concentration. There are no major differences in mole percentage of individual PLFAs between the two plots, which indicates the lack of noticeable effects of soil origin and land use history.
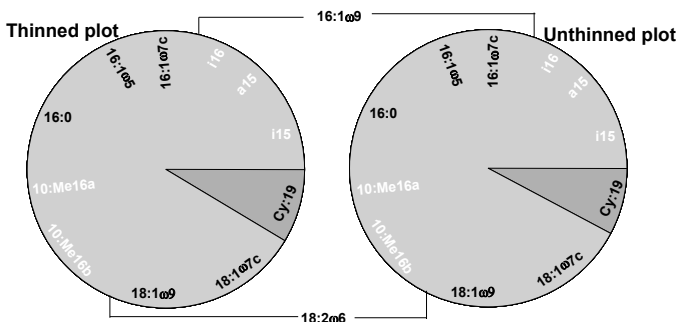

Figure 2. Proportion of individual fatty acids under the two-forest plots

With the exception of lipid marker a15, i16, 16:1ω9, 16:0, 10:Me16b, 18:2ω6, and Cy19, the mol fraction of individual PLFAs decreasing in proportional abundance with soil depth. However, in terms of PLFA concentration, each lipid marker followed the patterns of soil organic C and TN content, and decreased from 0-10 to 10-25 cm soil depth (Table 2). At 0-10 cm soil depth, total PLFA concentration tended to be larger in soil under managed plot (271.5 ± 39.1 nmol PLFA g^{-1} dry soil). The soil under unmanaged plot exhibited less concentration (166.2 ± 57.1 nmol PLFA g^{-1} dry soil). From the depth 10-25 cm, PLFAs concentration under the managed plots (136.1 ± 9.3 nmol PLFA g^{-1} dry soil) had contained larger concentration than the unmanaged ones (76.5 ± 6.4 nmol PLFA g^{-1} dry soil).

Table 2. Proportion of PLFA profiles, expressed as mol% in soil from 0-10 cm and 10-25 cm soil depth thinned and unthinned plot

PLFA	Thinned plot		Unthinned plot	
	0-10 cm	10-25 cm	0-10 cm	10-25 cm
i15	10.22 (0.90)	10.15 (1.30)	9.32 (1.06)	8.12 (0.72)
a15	5.01 (1.14)	5.09 (1.16)	4.63 (0.47)	4.36 (0.53)
i16	4.97 (0.35)	5.61 (0.37)	5.05 (0.37)	4.73 (0.30)
16:1ω9	1.41 (0.13)	1.53 (0.09)	1.12 (0.39)	1.02 (0.44)
16:1ω7c	7.47 (0.49)	6.47 (1.49)	7.66 (0.99)	6.69 (0.72)
16:1ω5	4.70 (0.61)	4.31 (0.84)	4.85 (0.54)	4.43 (0.67)
16:0	16.95 (1.87)	17.23 (1.11)	18.45 (1.76)	19.00 (2.54)
10:Me16a	3.89 (0.37)	3.42 (0.76)	3.95 (0.21	3.74 (0.07)
10:Me16b	12.81 (1.19)	14.09 (1.82)	11.99 (2.89)	12.34 (1.36)
18:2ω6	1.82 (0.61)	2.24 (0.56)	2.34 (0.64)	2.86 (0.38)
18:1ω9	8.38 (1.14)	8.12 (1.31)	8.89 (0.04)	9.95 (0.52)
18:1ω7c	13.79 (0.77)	12.65 (0.67)	14.00 (0.32)	14.30 (0.66)
Cy19	8.58 (0.26)	9.09 (1.45)	7.77 (1.99)	8.47 (2.43)
Total PLFAs*	271.5 (39.1)	136.1 (9.3)	166.2 (57.1)	76.5 (6.4)

Values are Averaged mol% of individual fatty acids (SD in parenthesis, n = 3);
* stands for Total amounts of PLFAs in nmol g-1 dry weight of soil

At a depth of 0-10 cm soil layer, the biomass of fungal marker indicator estimated as the sum of PLFA concentration 18:2ω6 and 18:1ω9 (Zelles, 1997) tended to be larger in soil under managed plot (27.7 nmol fungal PLFA g-1 dry soil). The corresponding value for unmanaged plot was found to be 18.7 nmol fungal PLFA g-1 dry soil). At the same soil depth, biomass of bacterial marker indicator estimated from PLFA concentration

i15:0, a15:0, i16:0, 18:1ω7c and cy19:0 (Zelles, 1999) were found to be larger in soil under the thinned plot (115.6 nmol bacterial PLFA g^{-1} dry soil) and 67.7 nmol bacterial PLFA g^{-1} dry soil (unthinned plot). From the depth of 10-25 cm soil layer, biomass of fungal and bacterial marker indicators PLFAs concentration was found 14.1 and 58 nmol PLFA g^{-1} dry soil vs. 9.8 and 30.6 nmol PLFA g^{-1} dry soil, for managed vs. unmanaged plot respectively (Table 2). At both soil depths, the thinned plot contained larger concentration of NLFA16:1ω5 (arbuscular mycorrhizal fungi biomarker) with values of 3.6 and 1.9 NLFA g^{-1} dry soil vs. 2.1 and 1.1 NLFA g^{-1} dry for thinned vs. unthinned plot respectively.

The reduction of stand densities and opening canopy encourage the establishment of understoryy vegetation and functional diversity in gaps (Lindh and Muir, 2004; Ares *et al*., 2010). Although I didn't attempt to enumerate the understory vegetation in a scientific manner, the area of the managed plot had clear influence on the abundance of the understory vegetation but the opposite was observed under the control plot (personal observation). Understory plant can have important and diverse effects on quality of substrate inputs into the soil through changing litter inputs and altering the soil microclimate (Zhao *et al*., 2018). As a consequence of these changes, the composition of the soil microbial community may be altered or microbial activities may increase (Mikola *et al*., 2000). In the present study forest stand, Zeleke Asaye (2011) found out higher production and turnover rate of fine roots in thinned plot than control plot. Fine root biomass production and its relationship to aboveground biomass have been studied by various authors (see Leppälammi-Kujansuu *et al*., 2014 and reference cited there). This could be a potential explanation for the differences between the two experimental plots in microbial biomass. Carbon input through aboveground litter and belowground input through fine root production and turnover and associated microbial activity are well-documented processes in the carbon and nutrient cycling of forest ecosystems (Majdi and Kangas, 1997). Based on the present finding, one could expect that belowground and/or aboveground litter input might increase soil microbial activity and induce changes of the microbial biomass between the thinned and control plot. Positive relations between fine roots and microbial biomass parameters have been frequently reported (e.g. by Wardle, 1992; Lee and Jose, 2003). Similarly, Hwang and Son (2006) found larger fine root input to the soil after thinning and argued that this was enough organic matter input to offset potential losses of soil OC by soil respiration. On the other hand, the declining PLFAs concentration

with soil depth is the result of decline in resource availability, predominately a function of decline in organic matter and is a common pattern across many ecosystems (Feng et al., 2003).

CONCLUSION

Forest management practices such as thinning operations are necessary parts of forestry. The present study has showed that soil chemical and biological properties are changed by forest management intervention. At both soil sampling depth, the thinned plot had higher C, N, CEC and microbial concentration than the control plot that could be resulted from larger above ground and belowground biomass accumulated by individual potential crop trees. The opening of forest stands due to thinning also favor the flourishing of understory biomass in the managed plot than the control plot. In addition, the stump roots left at thinned plot together with understory vegetation could have contributed the differences in the chemical and biological properties of the soil. This increased fertility of the soil could provide a valuable nutrient supply to the succeeding forest stand, particularly if it is given proper protection. From these results, it can be concluded that thinning operation has a positive effect on soil quality in Cupressus plantation in the Munessa forest, Ethiopia. However, information from the present study will aid in understanding the general and sites specific responses of soil chemical and microbial biomass properties to thinning treatment. The issue involved in understanding the effect of thinning on soil quality should be more holistic, and it needs further investigation using a longer-term study.

ACKNOWLEDGEMENTS

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