SOIL ORGANIC CONTENT OF STANDS OF DIFFERENT AGES IN A SUBTROPICAL CHIR PINE (*PINUS ROXBURGHII*) FOREST OF PAKISTAN

AMIR, M.[1] – SAEED, S.[2] – LIU, X. D.[1*] – MANNAN, A.[3] – KHAN, A.[4] – LI, Z. Z.[2] – MUNEER, M. A.[2]

[1]*Beijing Key Laboratory for Forest Resources and Ecosystem Process, College of Forestry, Beijing Forestry University, 100083 Beijing, China*

[2]*School of Forestry, Beijing Forestry University, Beijing, China*

[3]*Punjab Forest Department Government of Punjab, Lahore 54000, Pakistan*

[4]*School of Soil and Water Conservation, Beijing Forestry University, Beijing 100083, China*

[*]*Corresponding author*
e-mail: xd_liu@bjfu.edu.cn; phone: +86-134-3921-2063

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Abstract. Soil organic carbon (SOC) storage in forest ecosystems plays a major role in the global carbon cycle. Soil carbon across an age sequence may vary of Chir pine forest. In this study, based on the silvicultural shelterwood management system, forest area was classified into stands of three different age groups, including young stand, mature stand, and over-mature stand. Soil carbon density was assessed in each stand at three depths (0-20, 20-40, and 40-60 cm) by Walkley Black method. The results showed that SOC was higher in different stand ages at a depth of 0-20 cm as compared to the 20-40, and 40-60 cm depth. Altogether, the average potential of soil carbon density at a depth of 0-60 cm in young, mature and over-mature stand ages of Chir Pine forest was 70.96 (Mg C ha^{-1}), 68.96 (Mg C ha^{-1}) and 61.02 (Mg C ha^{-1}), respectively. The result indicates that soil carbon stock decreases with increasing forest stand ages. The results confirm that-- current management operations such as cutting, thinning, tending, may affect the soil carbon. Moreover, we recommended the regular periodic survey of soil and permanent sample plot establishment is necessary for the accurate soil carbon measurement in different stand ages of Chir pine forest.
Keywords: *shelterwood system, management operation, soil bulk density, soil depths, Murree Hill*

Introduction

Soil carbon storage is one of the most essential and significant carbon sinks in the terrestrial ecosystems, which facilitates carbon sequestration. Monitoring and assessment of soil carbon in forests are critical for the mitigation of climate change. The soil is an important sink for carbon storage (Ahmad et al., 2018; Mannan et al., 2019; Saeed et al., 2019a). Among various forest carbon pools, the estimation of soil organic carbon (SOC) is essential, because the soil is the world's largest terrestrial carbon pools, which plays a vital role in the global carbon budget (Saeed et al., 2019b). Therefore, a slight change in the soil carbon storage can have large impacts on the global carbon cycle (Johnson et al., 2007). The increasing emission of carbon dioxide from the soil is reinforced by the rising of temperature and climate change (Yang et al., 2010). SOC stored in the world's soils is approximately 1100–1600 Pg (petagrams), more than twice the carbon stored in the living vegetation, which is 750 Pg (Sundquist, 1993). Soil releases carbon to the atmosphere through deforestation and other anthropogenic activities may significantly

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increase the greenhouse gases concentration (Watson et al., 2000). The potential capability and assessment of soil are essential for the mitigation of CO_2 emission, improvement of surface water quality, soil physical properties, enhancement of forest production and stability, as well as decreasing of soil erosion (Lal, 2004; Lal et al., 2007).

Stand age is an essential factor affecting storage of carbon in various forest components such as in upper-story vegetation, understory vegetation, deadwood, and soil (Martin et al., 2005; Zerva et al., 2005; Peichl and Arain, 2006). Moreover, stand age is a reliable predictor of the structure and function of a forest ecosystem which may affect the carbon density among carbon pools (Bradford and Kastendick, 2010). Furthermore, various carbon pools of a forest respond differently to stand age (Zhu et al., 2010). Therefore, it is essential to understand the relationship of stand age with soil carbon and sequestration rate (Cheng et al., 2014). Some studies underlined that the potential of soil carbon sequestration is age-independent (Song et al., 2018). Some studies showed that soil carbon increase with the increase in stand age (Cao et al., 2012; Zhao et al., 2014). The soil carbon inconsistency may be depending on many other factors such as forest types, climate, previous land use and soil properties (Taylor et al., 2007).

In Pakistan, the researchers focused on the forest carbon stock such as temperate forest (Ahmad et al., 2015, 2018; Ahmad and Nizami, 2015), subtropical forest (Nizami, 2012), and in the planted forest (Saeed et al., 2016). However, no up-to-date studies have been conducted on the soil carbon allocation concerning stand age. Therefore, the current study was carried out in subtropical Chir pine (*Pinus roxburghii*) forest. This species is widely distributed in the Murree, Swat, Dir, and Azad Kashmir. The height of *Pinus roxburghii* is 120 ft, and girth is 7 to 8 ft with the whole of the top canopy. The Chir pine canopy tends to be even-aged over a compact area. The heavy needle falls, and burning reduces the growth of shrubs. Only a few of the species of grasses and herbs are noticeable, the fire virtually immune the older trees from damage and seedling show resistant after the first year while rootstock persists and new shoots appears (Champion et al., 1965). A forest fire is a common behavior of Subtropical Chir pine forest. Regularly, the forest fire incidents took place from April to June during the summer season. The falling of dry needle of Chir pine acts as fuel for forest fire (Nafees and Asghar, 2009). This species is easily cultivated and naturally regenerated in the northern area of Punjab and Khyber Pakhtunkhwa province. *Pinus roxburghii* considered as relatively short-lived and fast-growing species. Because of its longevity and fast growth, seems a suitable species for wood production. *Pinus roxburghii* is a valuable species for forestation, afforestation, and reforestation of denuded areas of Pakistan (Sheikh, 1993; Amir et al., 2018).

Under the shelterwood management system, the Chir pine forest is managed. According to this system, the forest area is divided into various blocks on the base of age, following different management operations. Felling and cutting activities are concentrated on over-mature blocks. In the mature block, thinning operations are carried out. In the young stand, cleaning operations are carried out. These different management operations in each stand age block may influence the soil carbon. Therefore, the study was designed to assess soil carbon of *Pinus roxburghii* forest ecosystem in age-sequence and to outline, the effect of management operations practices on carbon stock in different stand age classes. Moreover, to provide necessary information on carbon storage potential for the reporting of the Kyoto protocol to provide future recommendations for suitable forest management and making policy decisions. Here, our objective of the study was to investigate SOC stocks in age-sequence along various soil depths.

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Materials and Methods

Study site

The present study was conducted in Murree Hill of Pakistan. The latitude and longitude of the study site were ranged from 33° 47'15" to 33° 54' 47" N and 73° 16' 54" to 73° 29' 18" E. The elevations of the Chir Pine forest from sea level are range from 939 to1873 m. The mean precipitation ranges from 500 to 1200 mm while the temperature of the studied forest varies from -5ºC in winter to 40ºC in summer. The sedimentary rocks are comprised of shales, limestone sandstones, and marls. The soil was loamy with a proportion of silt, clay, and sand (Sheikh, 1993). The study area is the natural zone of Chir Pine forest that is managed under a shelterwood system. The dominant tree species of the study area is Chir Pine. The major associated tree species are *Pinus wallichiana* (kail), *Pyrus pashia* (batangi), and *Quercus incana* (rhin). The associated understory flora consists of *Carissa spinarum* (granda), *Myrsine africana* (khukhal), *Dodonea viscosa* (sanatha), *Berberis lycium* spp. (sumblu), *Capparis decidua* (karir), *Cannabus sativa* (Bang) and *Adhatoda vasica* (Bahekar). The map shows the location of the study area (*Fig. 1*).

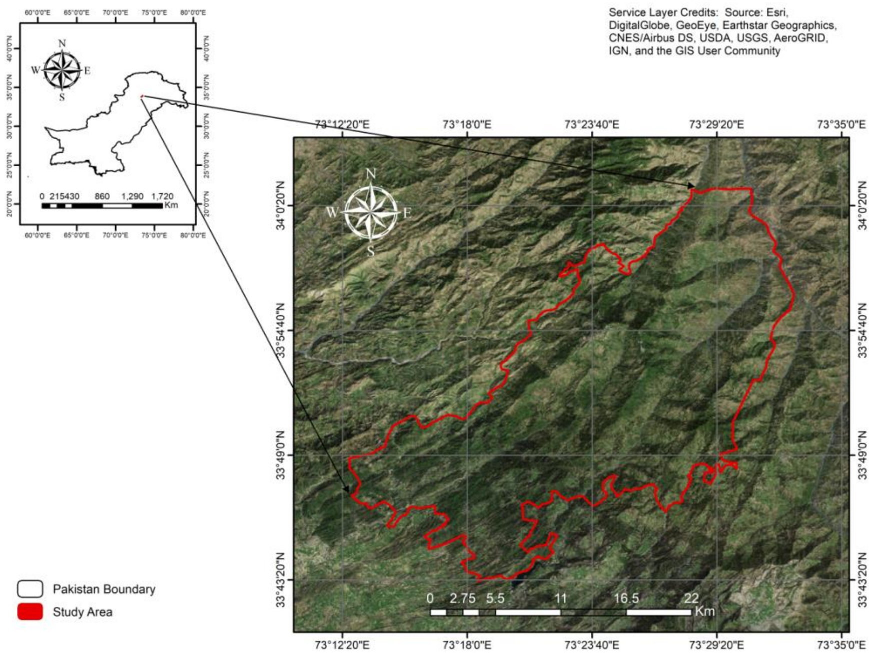

***Figure 1.** Range of study area, Murree Hill*

Research design

Pinus roxburghii forest was managed under a shelterwood system. Based on uniform shelterwood silvicultural management system, the Chir Pine forest consists of four periodic blocks (PBI, PBII, PBIII, PBIV) with rotation age (100 years) and regeneration period (25 years), which followed stands of four ages (1-25, 26-50, 51-75 and 76-100 years) (*Table 1*). According to the system, regeneration felling is carried out in PBIV, but some prescribed numbers of trees are retained for seed production. After the establishment of regeneration, then seed bearer is removed for the development of new trees. The duration between regeneration and final felling take 25 years. In PBIII, the thinning operations are carried out in PBIII for growth enhancement. The study area was classified into three age classes on the base of current forest structure and accurate measurement, PBI and PBII representing young age class (1-50 years), PBIII representing the mature stand (50-75 years) and PBIV representing over the mature stand (>75 years). Moreover, the comparison of SOC with growing stock and biomass of the *Pinus roxburghii* forest, information has mentioned in *Table 2* (Amir et al., 2018).

Table 1. *Sketch of the uniform shelterwood system*

Periodic Blocks	Age at the time of formation	Age at the end of the regeneration period
PBI	1-25	76-100
PBII	26-50	51-75
PBIII	51-75	26-50
PBIV	76-100	1-25

Table 2. *Characteristics of Chir Pine forest. Superscripts in each column show significant differences at α=0.1 and p= <0.0001)*

Age group	Mean Basal Area (m^2ha^{-1})	Mean Stand Density (trees ha^{-1})	Mean Stand Volume (m^3ha^{-1})	Mean total tree biomass (t ha^{-1})
Young	15.5±1.6 ^{B}	636.6±93.7 ^{A}	83.0±12.9 ^{C}	80.0±12.4 ^{C}
Mature	35.5±18.0 ^{A}	267±115.7 ^{B}	356.0±105.9 ^{B}	343.1±167.6^{B}
Over Mature	45.9±15.1 ^{A}	147.6±56.7 ^{C}	549.4±107.1 ^{A}	529.5±176.8 ^{A}
Mean	32.3	350.4	329.4	338.3

Soil samples collection and treatment

The area maps and topographic sheets were taken from the respective forest department. GPS determined coordinates and elevation (m) of each sample area. The stratified random sampling method was done in each block. The soil samples were collected from the subplot of (1×1 m^2) within quadrate at a depth of 0-20 cm, 20-40 cm, and 40-60 cm. In each stand, eight sample plots were taken with three replicate plots. The soil samples were collected by using the Soil Augur. The volume of the soil core was 198.24 cm^3 with 5.9 cm diameter and 7.25 cm height dimension. After weighing and packing of the labeled soil samples were brought into the laboratory to keep it in the oven for 48 hours at 72ºC for further analysis and the soil bulk density was determined from air-dried soil samples. The air-dried sample was ground with a pestle and passed through 0.5 mm sieve manually for SOC determination, as mentioned by Lu (1999).

Soil bulk density calculation

For the determination of total soil carbon, the soil bulk density of samples in each age group was determined from the weight of soil sample and known volume of soil core according to given *Equation (1)*.

$$SBD\,(g/cm^3) = \frac{WS}{VS} \qquad \text{(Eq.1)}$$

where SBD = soil bulk density (g/cm^3), WS = weight of soil sample (g) and VS = volume soil core (cm^3).

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Soil carbon measurement

Soil carbon density was determined by using the oxidizable organic carbon method (Walkley and Black, 1934; Rayment and Higginson, 1992; Anderson and Ingram, 1994; Ahmad and Nizami, 2015). In this method, the Potassium dichromate solution was prepared by drying in an oven at 105ºC for two hours and cooled it. Then distilled water was added into the 49.09 gm of dried potassium dichromate to dissolve and took one volume of solution from it. 196 gm of distilled water was added into the ferrous ammonium sulfate solution and to dissolve and prepared to one-liter volume. 5 ml sulfuric acid was taken and mixed with it and brought to one volume of solution. One gram of diphenylamine indicator was dissolve and added into the concentrated H_2SO_4. One gram of soil was taken in 500 ml beaker with 10 ml Potassium dichromate solution. 200 ml of concentrated H_2SO_4 was added, and the mixture was kept for 30 minutes, and then 10 ml of H_3PO_4 and 10 ml of water was added to the mixture, then the mixture was kept to cool for ten minutes, and 10-15 drops of Diphenylamine indicator was added to this mixture. When the mixture changes the color from violet-blue to green by the titration of 0.5 ferrous ammonium sulfate solution and then reading was noted. The total organic carbon was calculated by the given *Equation (2)*.

$$Oxidable\ organic\ carbon\ \% = \frac{blank\ volume - actual\ volume \times 0.3 \times M}{Weight\ of\ air-dry soil\ (gm)} \quad \text{(Eq.2)}$$

where M = Molarity of ferrous ammonium sulfate, $0.3{=}31{\times}0^{-3}{\times}100$, here 3 shows equivalent to the weight of carbon, Av = volume of Ferrous ammonium sulfate to titrate with sample and Bv = Ferrous ammonium sulfate solution volume to titrate it with the blank solution.

Soil carbon (Mg C ha^{-1}) calculation

Soil carbon (Mg C ha^{-1}) was calculated from the soil organic carbon (SOC %), soil bulk density (g/cm^3) and thickness of horizon (cm). Soil carbon (Mg C ha^{-1}) was calculated from the following equation (Pearson et al., 2007; Nizami, 2012; Ahmad et al., 2015).

$$Soil\ carbon\ (Mg\ C\ ha^{-1}) = SBD\ (g/cm^3) \times SOC\ (\%) \times SHT\ (cm) \times 100 \quad \text{(Eq.3)}$$

where SBD = Soil bulk density (g/cm^3), SOC = Soil organic content (%) and SHT = Soil horizon thickness (cm).

Statistical analysis

Sigmaplot (12.5 version) and Statistics (8.1 version) software were used for statistical analysis. Mean, standard deviation and standard error were calculated. Analysis of variance (One-way ANOVA) along with all pairwise comparison and least significance difference (LSD) test was performed to test the significant difference of the means values of the soil bulk density and soil carbon.

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Results

SOC was higher in young stand ages at a depth of 0-20 cm as compared to the 20-40, and 40-60 cm depth (*Fig. 2*). The average SOC in the young stand at a depth of 0-20, 20-40 and 40-60 cm was 36.49, 17.43 and 17.04 Mg C ha^{-1}, respectively. Moreover, the significant differences found between different stand ages at a depth of 0-20 cm, F (2, 14) = 71.70, p = 0.0000. The higher SOC was found at a depth of 0-20 cm in the mature stand age. The mean SOC in the mature stand at a depth of 0-20, 20-40 and 40-60 cm was 37.43, 17.22, and 13.31 Mg C ha^{-1}, respectively.

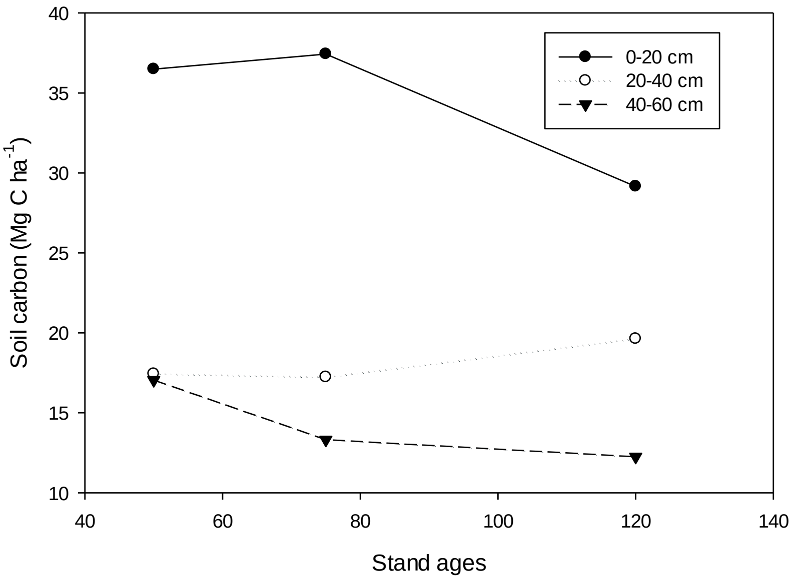

Figure 2. *Soil carbon stock of different stand ages at various soil depths*

The results showed significant differences at the top layer of the mature stand, F (2, 14) = 30.09, p = 0.0000. SOC was higher in over-mature stand ages at a depth of 0-20 cm as compared to 20-40, and 40-60cm depth. The mean SOC in over-mature stand at the depth of 0-20, 20-40 and 40-60 cm was 29.15, 19.62 and 12.25 Mg C ha^{-1}, respectively, with a significant difference, F (2, 14) = 10.14, p = 0.0019 (*Table 3 Table 4*).

Table 3. *SOC in young, mature and over-mature stand at various soil depths*

Soil depths	Young	Mature	Over-mature
0-20 cm	36.49 ± 3.98^{A}	37.43 ± 2.92^{A}	29.154 ± 3.3^{A}
20-40 cm	17.43 ± 4.23^{B}	17.22 ± 7.84^{B}	19.627 ± 6.81^{B}
40-60 cm	17.04 ± 4.06^{B}	13.31 ± 7.81^{B}	12.250 ± 8.11^{B}

Table 4. *Relationship type, equation and R^2 value of the Pinus roxburghii stands*

Soil depths	Parameters	Relationship type	Equation	R^2 Value
0-20 cm	SA & SC	Polynomial, linear	y = -0.1132x + 43.454	0.83
0-40 cm	SA & SC	Polynomial, linear	y = 0.0339x + 15.32	0.81
40-60 cm	SA & SC	Polynomial, linear	y = -0.0631x + 19.351	0.79

SA= Stand ages (<50, 50-75, >120 years old stands) and SC = Soil carbon (Mg C ha^{-1})

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SOC was higher in different stand ages at a depth of 0-20 cm as compared to the 20-40, and 40-60 cm depth. The average SOC in young, mature and over-mature stands were 36.493, 37.44 and 29. 15 (Mg C ha^{-1}), respectively. Moreover, the significant differences found between different stand ages at a depth of 0-20 cm, F (2, 21) = 14, $p = 0.0001$. While, the non-significant differences found at the depth of 20-40 cm (F (2, 21) = 0.34, $p = 0.72$), and 40-60 cm (F (2, 21) = 1.04, $p = 0.36$) for different stand ages. The average SOC for young, mature and over-mature stands were 17.43, 17.22, 19.62 Mg C ha^{-1} at a depth of 20-40 cm, while at a depth of 40-60 cm, there were 17.05, 13.31, 12.25 Mg C ha^{-1}, respectively (*Fig. 3*).

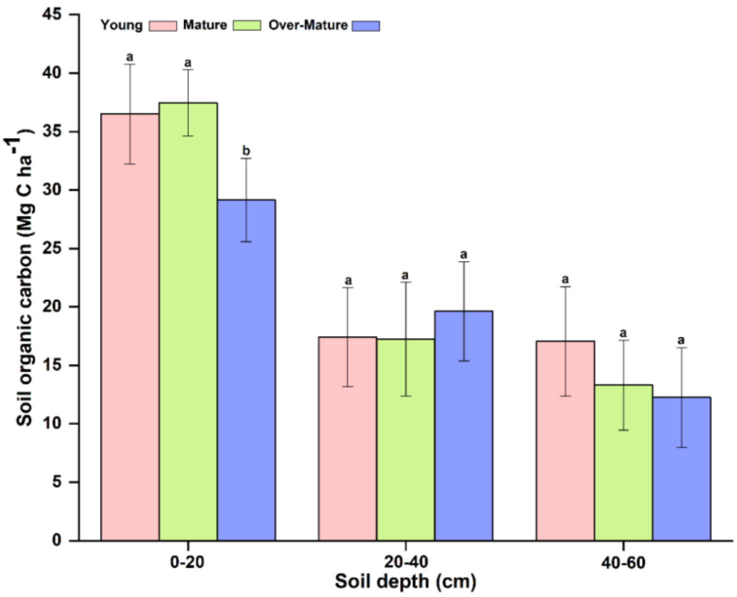

Figure 3. *Soil organic carbon contents. SOC in young, mature, and over-mature stand at various depths. The different letters on the bar are showing significant differences while the same letters are showing the non-significant different between different classes of stand ages. Alphabets on the top of each bar are showing the $LSD_{0.05}$ difference*

The soil organic matter (SOM) in the young stand at a depth of 0-20, 20-40 and 40-60 cm was 2.95, 1.68 and 1.38%, respectively. The SOM in the mature stand at a depth of 0-20, 20-40 and 40-40 cm was 2.88, 1.35 and 0.98%, respectively. SOM in the over-mature stand at a depth of 0-20, 20-40 and 40-60 cm was 2.76, 1.68 and 0.96%, respectively. The resulted that higher SOM was found at the 0-20 cm soil depth and following the decreasing trend from upper to lower soil depth in each stand. Soil Bulk density (SBD) results showed almost similar values in different stand ages at each depth. The mean SBD in young, mature and over-mature stands at a depth of 0-20 cm were 1.09, 1.04 and 0.93 (g/cm^3), respectively. Moreover, the significant differences found between different stand ages at a depth of 0-20 cm, F (2, 21) = 9.51, $p = 0.0011$. The average SBD in young, mature and over-mature stands at a depth of 20-40 cm were 1.13, 1.10 and 1.04 (g/cm^3), respectively. The significant difference observed at a depth of 20-40 cm in young stand ages, F (2, 21) = 4.34, $p = 0.0265$. The mean SBD in young, mature and over-mature stands at a depth of 40-60 cm were 1.15, 1.08 and 1.06 (g/cm^3),

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respectively. Meanwhile, the significant difference found in different stand ages $F (2, 21) = 3.64$, $p = 0.0440$ (*Fig. 4*).

Generally, the results of soil carbon analysis showed a decreasing trend with increasing stand age. Soil carbon showed decreased with increasing at different soil depth in each stand. This decreasing trend in soil may be attributed to the soil disturbance during the felling operations in the over-mature stand and decrease in the natural thinning with the stand age. Additionally, there was an inverse relationship between soil carbon and soil bulk density. Soil bulk density increased with a decrease in soil organic carbon at various depths. The higher degree of soil organic matter (SOM) showed a lower degree of bulk density. The SOM and SBD are inversely proportional to each other, which indicate higher infiltration and, proper aeration and granulation. The soil bulk densities in each stand age showed significant variation.

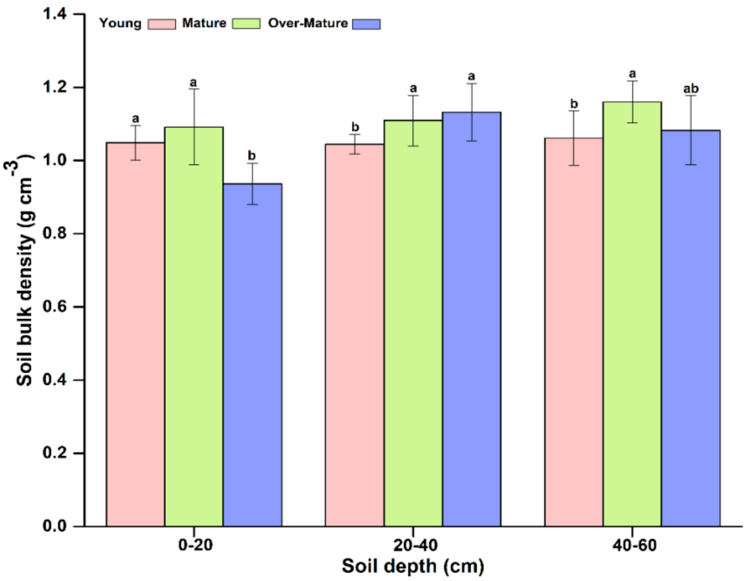

Figure 4. *Soil Bulk density. SBD in young, mature, and over-mature stand at various depths. The different letters on the bar are showing significant differences while the same letters are showing the non-significant different between different classes of stand ages. Alphabets on the top of each bar are showing the $LSD_{0.05}$ difference*

Discussion

It is quite difficult to compared SOC with forest biomass (Teklay and Chang, 2008; Laganiere et al., 2010), because SOC may be affected by various factors, such as stand age, tree species, forest type, climate, and soil chemical and physical properties (Richter et al., 1999; Guo and Gifford, 2002; Paul et al., 2002; Peichl and Arain, 2006; Mora et al., 2014; Wang et al., 2014). Soil organic carbon across stand age has been widely carried out with different results. In many studies, SOC stock increases with the forest stand age (Smal and Olszewska, 2008; Berthrong et al., 2009) while the opposite result was also found after afforestation (Teklay and Chang, 2008; Mao et al., 2010). Afforestation and reforestation have been considered to be essential to reduce atmospheric CO_2 emission by sequestering carbon in soils (Richter et al., 1999; Smal and Olszewska, 2008). Therefore, SOC in surface soil layer could be affected by the changes of the upperstory, understory, litter, shrub and herb biomass, and soil

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characteristics with stand age sequence (Six et al., 2002; Noh et al., 2010). Many studies found that the SOC content declines firstly and then increases with the increasing of stand age (Chen et al., 2005; Mao et al., 2010). The mean soil carbon showed decreased with respective stand age as well as with soil depth. The soil carbon was recorded higher in the topsoil of each stand as compared to deeper soil. It may be due to producing of SOC by the decomposition of the root system and litter, which entered into topsoil first near the ground as demonstrated by other studies (Peichl and Arain, 2006; Tian et al., 2010; Zhang et al., 2012). The current study showed carbon content of soil layer showed a decreasing trend with stand age while other study showed increasing of soil carbon with stand age due to the availability litter productivity in high older stands (Ming et al., 2014).

Soil carbon depends on the forest stand age, forest types, forest productivity, the chemical and physical properties, and litter decomposition rate of the soil (Gower, 2003; Kang et al., 2006; Jandl et al., 2007; He et al., 2013). Our study showed a decreasing trend with stand age. The proportion of soil carbon stock should need focus during any management activities for the enhancement of future soil carbon sequestration of *Pinus roxburghii* forest ecosystem.

The SOC exists different debates on whether or not SOC potential could change with respect to forest stand ages (Farley et al., 2004; Lemma et al., 2006). Some previous researches showed that soil carbon stock is not increasing with increasing stand ages significantly (Farley et al., 2004; Cheng et al., 2013), while some other researches showed that SOC increasing with increasing forest stand age (Hooker and Compton, 2003; Pregitzer and Euskirchen, 2004; Lemma et al., 2006). The difference may be due to various factors such as soil factor climatic factor and forest type (Peichl and Arain, 2006). However, generally, SOC increases with stand age in conifer forest, due to the accumulation of organic matter in older stages (Zerva et al., 2005; Li et al., 2013). However, in the case of the present study, the soil carbon decreases with increasing stand age, may be due to the result of management operations such as felling and thinning activates. During these operations, the top layer of the soil exposed to rainfall and wind, wash out the soil surface, which may lead to reducing of soil carbon.

Conclusion

The soil carbon density decreased with the increase of forest stand ages and decreased with increasing soil depths in different stand ages. However, the soil carbon showed gradually decreasing trend from young to over-mature stand. Furthermore, our results showed the effects of management operations on soil carbon. The decreasing of soil carbon value with respect to stand ages may be is the result of soil disturbances during management operations. Therefore, we suggest different protection measures such as reduced impact logging, soil conservation through mulching, maintaining understory vegetation and forest cover through regeneration, control grazing and effective management of forest fire should be considered. The appropriate management of forest could allow the importance of forest soil in the regional carbon budget.

Furthermore, the results highlighted that regeneration and afforestation of Chir Pine could promote the potential capability of SOC and provide necessary data for estimating the carbon stocks of Chir Pine forest ecosystem. The present results highlighted the importance of stand age in assessing forest soil carbon measurement for policymakers when modeling climate scenarios.

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