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Effects of stand density on soil organic carbon storage in the top and deep soil layers of *Fraxinus mandshurica* plantations

Auswirkungen der Bestandesdichte auf die Kohlenstoffspeicherung in oberen und tiefen Bodenschichten von *Fraxinus mandshurica* Aufforstungen

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Keywords: *Manchurian Ash; Afforestation; Litter input; Carbon sequestration; Refractory; Respiration; Dissolved organic carbon; Fine root biomass*

Schlüsselbegriffe: *Mandschurische Esche; Streufall; Kohlenstoff Sequestrierung; Atmung, Gelöster organischer Kohlenstoff; Feinwurzelbiomasse*

Abstract

Forests stand density has been reported to influence soil organic carbon (SOC) storage, yet this effect is often inconsistent. Especially for SOC in deep soil layers few studies examined its changes with stand density. In this study we investigated the effects of stand density on SOC storage by collecting soil samples from a *Fraxinus manshurica* plantation at three different stand densities. We took samples at two soil depths from 0-10 cm (top layer) and 40-60 cm (deep layer), fractionated the soil material with a 0.4 mm mesh to remove the labile fraction and then used the sieved material for laboratory incubation. Soil properties in different stand densities were examined before the incubation. After the incubation, soil respiration and the final

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carbon balance were determined. Our results indicate that the SOC storage increased with increasing stand density in both top and deep layers. The fractionation lowered the carbon concentration in both layers with the reduction in the top layer being highest at the low stand density site, while in deep layer it was highest at middle stand density. At the end of the incubation, total respiration in the top layer decreased with increasing stand density, whereas it remained invariable in the deep layer. The specific respiration decreased with increasing stand density in both layers. Addition of leaf litter after incubation resulted in an increase of the carbon content in top soil samples with the scale of accumulation increasing with increasing stand density. We concluded that the increasing SOC storage with stand density is due to its resistance versus microorganisms in top soil layer and not related to deep soil layer.

Zusammenfassung

Die Bestandsdichte eines Waldes soll einen entscheidenden Einfluss auf die Speicherung von organischen Kohlenstoff im Boden haben, allerdings ist dieser nicht immer eindeutig. Insbesondere für den organischen Kohlenstoff in tieferen Bodenschichten gibt es nur wenige Studien, die den Effekt der Bestandesdichte untersucht haben. Diese Studie erforscht die Auswirkungen der Bestandsdichte auf die Lagerung des organischen Kohlenstoffs mittels Bodenproben aus drei verschiedenen Bestandsdichten und aus zwei Bodenschichten in 0-10 cm (oberste Schicht) und 40-60 cm (tiefe Schicht) für eine *Fraxinus manshurica* Aufforstung. Die Proben wurden mit einem 0.4 mm Sieb getrennt, die labilen Komponenten entfernt und dann für die Inkubation im Labor verwendet. Vor der Inkubation wurden die Bodeneigenschaften in verschiedenen Bestandsdichten ermittelt. Nach der Inkubation wurde die Bodenatmung und die gesamte Kohlenstoffbilanz gemessen. Unsere Ergebnisse zeigten, dass die organische Kohlenstoffspeicherung mit zunehmender Bestandsdichte sowohl in oberen wie auch in unteren Bodenschichten zunahm. Die Fraktionierung reduzierte die Kohlenstoffkonzentration in beiden Schichten, wobei die Abnahme in den oberen Bodenschichten am größten bei niedriger Bestandsdichte war, während die Abnahme in den tieferen Bodenschichten am höchsten bei mittlerer Dichte war. Am Ende der Inkubation nahm die Gesamtrespiration im Oberboden mit zunehmender Bestandsdichte ab, während sie in in den tieferen Bodenschichten unverändert blieb. Die spezifische Respiration nahm in beiden Schichten mit zunehmender Bestandsdichte ab. Nach der Inkubation führte die Zugabe von Laubstreu zu einer Erhöhung des Kohlenstoffgehalts in oberen Bodenproben, wobei das Ausmaß der Akkumulation mit zunehmender Bestandsdichte zunahm. Wir schlussfolgeren, dass die zunehmende organische Kohlenstoffspeicherung mit der Bestandsdichte eher auf die Widerstandsfähigkeit gegen Mikroorganismen in der obersten Bodenschicht als auf die tiefen Bodenschichten zurückzuführen ist.

1. Introduction

Soil is the largest carbon pool in terrestrial ecosystems and exceeds the amount of carbon in atmosphere and biomass (Post et al. 1982; Jobbágy 2000). Thus even a small change of soil organic carbon (SOC) sinks affects not only soil properties and microorganism activity (Bonner et al. 2018), but may have a substantial effect on the carbon balance, potentially leading to global climate change (Schlesinger and Andrews 1990; Davidson and Janssens 2006).

Studies suggested that the forest soil plays an important role in carbon sink (Brown et al. 1993; Peng et al. 2008). A number of variables have been reported to affect SOC storage in forests, such as tree species composition (Langenbruch et al.2012; Vesterdal et al. 2013), management practices (Frouz et al. 2009; Klumpp et al. 2009; Shahzad et al. 2012), climate conditions (Razanamalala et al. 2017), etc. As one of the management practices, stand density of afforestation has been reported to influence SOC storage (Jandl et al. 2007; González et al. 2012; Zhou et al. 2013), yet its effects are somewhat inconsistent, as SOC storage both increased (Fernández-Núñez et al. 2010; Sitters et al. 2013) and decreased (Noh et al. 2013) with increasing stand density. The variation of stand density will likely lead to the differences in the carbon input and output of soils (Litton et al. 2003; Fang et al. 2007; Noh et al. 2010), which may cause the change of recalcitrance of SOC, which in turn affects SOC storage. However, the effect of variation of stand density on SOC recalcitrance is poorly explored, which may explain our ambiguous understanding discrepant of stand density effects on SOC storage. We conclude that the evaluation of the size and recalcitrance of soil carbon sink by stand density is an important task.

Kirkby et al. (2014) suggest that SOC fractions can be separated into coarse and fine fractions according to the size and density of the particles. The coarse fraction, which is mainly composed of the residuals of plants and animals, has a high microbial activity, while the fine fraction, which is mainly composed by refractory humus, has a slow turnover time (Falloon and Smith 2000). The fine fraction in soil usually represent a larger share of the entire SOC pool compared to the coarse fraction (Kirkby et al. 2011).

Many studies have examined the properties of SOC (Cui et al. 2014; Liang et al. 2017). The surface soils received more attention than the subsoils, although the latter also store large amounts of carbon as well (Batjes 1996; Jobbágy 2000; Fontaine et al. 2007). The surface SOC sink is mainly regulated by the input of fresh organic carbon, unlike in subsoils the carbon sink is affected by physical disturbance (Salomé et al. 2010). Therefore, surface soils and subsoils should be both considered when studying SOC storage, which could lead to a better understanding of the mechanism of SOC storage change.

Manchurian ash (*Fraxinus mandshurica*) is a commonly used species for afforestation

in northeast China, which helps to upscale the findings of this study on SOC in ash forests for understanding the regional soil carbon sink and provide advice for future afforestation. We investigate the effects of variation of stand density on SOC recalcitrance and subsequently on SOC storage in different layers by sampling both top and deep soil layers from 18-year-old ash plantations at three different stand densities. The objective of this study is to reveal the mechanisms of how stand density regulates SOC storage. We hypothesized that the SOC storage in different stand density sites is affected by the recalcitrance of SOC.

2. Materials and methods

2.1 Study area

This study area is located in Maoershan Experimental Station of Northeast Forestry University, Heilongjiang Province, China (127°36′47″ E, 45°18′13″ N) with an average altitude of 300 m. The climate type of this region is temperate continental monsoon climate with a mean annual temperature of 2.8 °C and mean annual precipitation of 700 mm (Zhou 1994). The forests in this region are mainly composed of *Fraxinus mandshurica*, *Larix gmelini*, *Betula platylhylla*, *Acer mono*, *Phellodendron amurense* and *Populus davidiana*.

The studied ash plantations were planted in 1998 with 2-year-old seedlings with three different afforestation densities (2200, 4400 and 10000 trees per hectare). The plantations were planted on a north-facing slope with an inclination of less than 10° and the soil type is an Alfisol. Those plantations were not artificially thinned. Due to natural thinning the stand density was lower than when the stands were planted. The dead trees were removed in every winter, as local residents collected fuel wood usually in October and November. The stand structure of the plantations are shown in Table 1.

Table 1: Stand structure of ash plantations with different original stand density, current stand density, average tree height and average diameter at breast height are presented as means ± standard error (n = 3).

Tabelle 1: Merkmale von Eschenaufforstungen mit unterschiedlicher Bestandsdichte. Für aktuelle Bestandesdichte, durchschnittliche Höhe und durchschnittlichen Durchmessers bei Brusthöhe zeigen wir Mittelwert ± Standardfehler (n = 3).

	Original density (trees·ha^{-1})	Current density (trees·ha^{-1})	Average height (m)	Average diameter at breast height (cm)
Low density	2200	1407 ± 87	10.3 ± 0.5	9.0 ± 0.3
Middle density	4400	2943 ± 137	9.7 ± 0.7	7.5 ± 0.5
High density	10000	4011 ± 182	9.4 ± 0.7	6.4 ± 0.3

2.2 Methods

2.2.1 Sampling

Three sampling sites with a size of 20 m by 20 m were established under each stand density (9 sampling sites in total) in early September 2015. The density plantations were separated by a 20 m buffer, and the three replicate sites were also separated by a 20 m buffer. For sampling roots, six plots from each site were randomly chosen located at 50 to 70 cm distance from trees. A soil core with inner diameter of 60 mm was used for 0-10 cm layer (top soil) and for 40-60 cm layer (deep soil) to measure the fine root biomass with a diameter of less than 2 mm (Eissenstat et al. 2000). For sampling soils three profiles were dug in each site from 0-10 cm and 40-60 cm. Soil samples from the three profiles of each site were homogeneously and equally mixed, and passed through a 2 mm mesh to remove stones and plants debris, before the soil properties were measured. The properties of soil samples were described in Table 2. As suggested by Kirkby et al. (2011), a fractionation was used to obtain the refractory SOC fraction, which was subsequently used for incubation by using a 0.4 mm mesh. Besides, we collected the annual fresh leaf litter of ash trees from the forest floor to use as a substrate subsequently in the incubation. The leaf litter was grounded into pieces with 1 to 2 mm size and dried before application.

2.2.2 Incubation setup

A weight of 60 g air dried soil sample from each density site of top layer and deep layer was put into a 500 ml jar with the water content of 40 % water holding capacity (WHC). All the samples were put into an incubator in the dark at 25 °C for 8 days of pre-incubation. To simulate field conditions, all the topsoils were amended with annual litter as the substrate. The deep soil samples did not get additional litter input, as the deep soil layer receive little litter carbon input. After the pre-incubation, 6.44 mg C g^{-1} grounded air-dried annual ash litter (carbon concentration 454.97 mg g^{-1}; nitrogen concentration 20.04 mg g^{-1}; phosphorus concentration 0.88 mg g^{-1}) was added into the topsoils from all the density sites, while deepsoil samples did not receive additional substrate. Subsequently, the water content was adjusted to 65 % WHC with the soil and added litter getting mixed. A 25 ml beaker filled with 10 ml 1 mol L^{-1} NaOH solution was suspended up inside the jar for trapping CO_2 released from soil (Aye et al., 2018). All the jars were sealed airtight at 25 °C for the incubation of 121 days. There were three replicate sites for each stand density with three top layer and deep layer replicate soil samples for each site, which was 54 jars to be incubated (3 densities * 3 sites * 2 layers * 3 replicates). At the day 2, 4, 7, 11, 18, 29, 36, 45, 61, 79, 100 and 121, CO_2 trapped in NaOH solution was measured with titration. When the incubation ended, samples from topsoils were fractionated to separate the refractory fraction and not fully decomposed litter by using "dry sieving and winnowing" method mentioned by Kirkby et al. (2011). Using this method, soil particles that were not able to pass through the 2 mm mesh, were taken as the light fraction and

considered as the not fully decomposed litter in this experiment. The particles that passed through 0.4 mm mesh were taken as the heavy fraction, known as the refractory fraction of SOC. The particles that passed 2 mm mesh but not passed the 0.4 mm mesh compounded both fractions, which were separated with blowing wind as only the light fraction rather than the heavy fraction can be blown away. The weight and carbon content of the refractory fraction and the not fully decomposed litter before and after incubation were measured to determine the carbon balance.

2.2.3 Analysis

The carbon and nitrogen concentration was measured with Vario MACRO Elementor Co., Germany. The mineral nitrogen concentration was the sum of ammonium concentration and nitrate concentration, which was extracted with 2 mol L^{-1} KCl and measured ammonium and nitrate respectively by Auto Analyzer 3. Microbial biomass was determined with the chloroform fumigation method (Vance et al. 1987). Carbon was extracted with 0.5 mol L^{-1} K_2SO_4 and subsequently measured by Liqui TOCII. Microbial biomass carbon was estimated as the difference between the fumigated soil sample and unfumigated sample. DOC was extracted with deionized water and measured by Liqui TOCII.

2.2.4 Statistic

All the data were presented as the average value of the three replicate sites of each stand density. Means were compared with the ANOVA method using the LSD criterion. The SOC storage and fine root biomass in top layer were calculated as the total amount per unit area within the 10 cm while in deep layer were calculated within the 20 cm. Correlations between indexes were calculated as Pearson Correlation Coefficient. The total respiration is presented as the amount of accumulative CO_2 released during the incubation period normalized to the sample weight, while the specific respiration is presented as the amount of accumulative CO_2 released during incubation period normalized to the SOC content of the sample. All the data were statistically analyzed with SPSS 19.0.

3. Results

3.1 SOC storage and other soil properties in top and deep soil in different stand density

In field condition, the SOC storage was found higher in high stand density site in both top layer and deep layer, with 15.52 % and 10.90 % higher in topsoils and 31.81 % and 9.99 % higher in deepsoils compared to the low stand density site and middle stand density site, respectively. The microbial biomass carbon and dissolved organic carbon were both found higher in low stand density site and lowest in high stand

Table 2: Soil properties and fine root biomass in top layer and deep layer at three different stand densities. We present mean ± standard error (n = 3). The different capital letters represented the significant difference of samples among stand densities in the same layer, and the different lowercase letters represented the significant difference of samples between two layers in the same stand density (P<0.05).

Tabelle 2: Bodeneigenschaften und Feinwurzelbiomasse in der oberen und tiefen Bodenschicht unter drei unterschiedlichen Bestandesdichte. Wir zeigen Mittelwert ± Standardfehler (n = 3). Die unterschiedlichen Großbuchstaben repräsentierten signifikante Unterschiede hinsichtlich Bestandesdichte in derselben Schicht und die unterschiedlichen Kleinbuchstaben repräsentierten signifikante Unterschiede zwischen den zwei Schichten in derselben Bestandesdichte (P<0.05).

Parameters	Top layer (0-10 cm)			Deep layer (40-60 cm)		
	Low density	Middle density	High density	Low density	Middle density	High density
SOC storage ($t \cdot ha^{-1}$)	36.21±1.99 Ba	37.72±3.94)Ba	41.83±2.78 Aa	15.78±1.00 Bb	18.91±0.71 Ab	20.80±1.49 Ab
Microbial biomass carbon ($mg \cdot kg^{-1}$)	1453.80±116.18 Aa	1288.51±106.32 Ba	1244.15±115.59 Ba	339.46±45.79 Bb	326.78±42.88 Bb	534.16±45.55 Ab
Dissolved organic carbon ($mg \cdot kg^{-1}$)	35.82±1.94 Aa	34.81±2.24 Aa	29.71±2.05 Ba	34.50±2.25 Aa	36.31±2.59 Aa	28.68±1.46 Ba
Available nitrogen ($mg \cdot kg^{-1}$)	15.83±1.36 Aa	16.60±1.59 Aa	17.07±1.98 Aa	1.41±0.35 Bb	1.28±0.16 Bb	1.87±0.10 Ab
pH (1M KCl, 1:2.5)	5.03±0.04 Aa	5.04±0.08 Aa	5.08±0.03 Aa	4.09±0.11 Ab	4.15±0.03 Ab	4.02±0.06 Ab
Clay (%)	42.63±3.42 Aa	44.18±4.12 Aa	43.77±2.88 Aa	38.52±4.12 Ba	36.82±2.39 B a	38.71±2.79 Ba
Silt (%)	33.63±2.61 Ba	31.59±3.04 Ba	32.69±3.70 Ba	39.66±4.50 Aa	41.06±2.58 Aa	41.62±5.26 Aa
Sand (%)	23.74±3.16 Aa	24.23±3.86 Aa	23.54±3.09 Aa	21.82±3.96 Ba	22.12±2.68 Ba	19.67±4.45 Ba
Fine root biomass ($g \cdot m^{-2}$)	408.63±26.32 Aa	341.78±29.91 Ba	241.16±14.90 Ca	13.12±2.89 Cb	34.19±5.62 Bb	47.16±6.30 Ab

density site in topsoils. In deepsoils, however, the microbial biomass carbon was found the lowest in high stand density site, which is opposite to the trend of dissolved organic carbon. The pH was 5.03 to 5.08 in topsoils and 4.02 to 4.15 in deepsoils, which remained unchanged with stand density. There was no difference of particle distribution with stand density in both topsoils and deepsoils. For the fine root biomass, it was found increasing with stand density in topsoils but decreasing with stand density in deepsoils (Tab. 2).

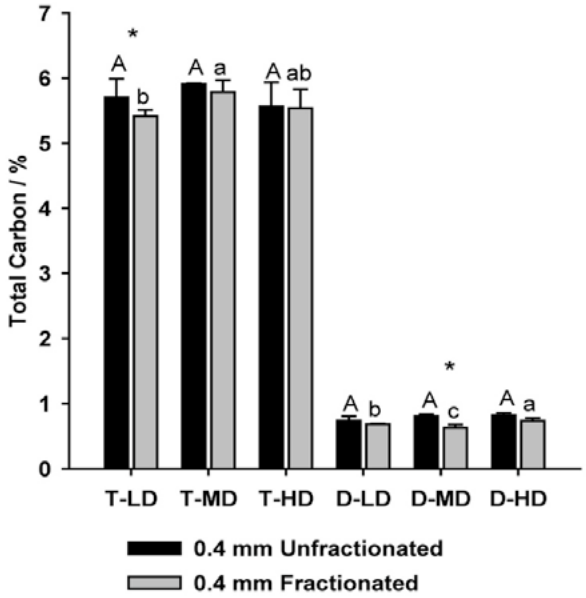

Figure 1: The total carbon concentration of soil samples in top layer (T) and deep layer (D) from low density (LD), middle density (MD) and high density (HD) with and without fractionation. The different capital letters represented the significant difference of unfractionated samples among stand densities in the same layer, and the different lowercase letters represented the significant difference of fractionated samples among stand densities in the same layer. The asterisks represented the significant effects of fractionation on carbon concentration (n = 3; P<0.05).

Abbildung 1: Die Kohlenstoffkonzentration der Bodenproben in der oberen (T) und der tieferen Bodenschicht (D) mit niedriger (LD), mittlerer (MD) und hoher Bestandesdichte (HD) mit und ohne Fraktionierung. Die verschiedenen Großbuchstaben zeigen signifikanten Unterschiede der unfraktionierten Proben zwischen den Bestandesdichten in derselben Schicht, und die unterschiedlichen Kleinbuchstaben repräsentierten den signifikanten Unterschied der fraktionierten Proben zwischen den Bestandesdichten in derselben Schicht. Die Sterne repräsentieren die signifikanten Auswirkungen der Fraktionierungauf die Kohlenstoffkonzentration (n = 3; P<0.05).

3.2 Carbon concentration in top and deep soil in different stand density changed by fractionation

There was not any significant variation of soil carbon concentration with changing stand density before fractionation. After fractionation, the soil carbon concentration in topsoils was found highest in middle density site with 4.42 % and 6.79 % higher than in high density site and low density site, respectively. In deepsoils, it was found highest in high density site with 16.73 % and 7.48 % higher than in middle density site and low density site, respectively. Furthermore, the carbon concentration of each soil sample decreased with the application of fractionation. In topsoils, the decre-

ments are getting slighter with increasing stand density while in deepsoils the scale of decrements presents the highest in middle density site and lowest in low density site (Fig.1).

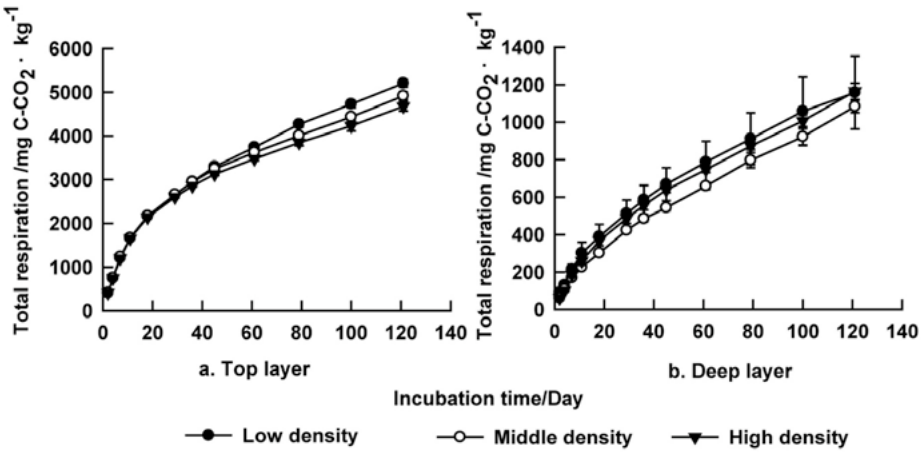

Figure 2: The accumulated total respiration of samples in top layer (a, left) and deep layer (b, right) for low, middle and stand density with simulating litter accessibility expected in the field.

Abbildung 2: Die kumulierte Gesamtrespiration der Proben in der oberen Bodenschicht (a, links) und tiefen Bodenschicht (b, rechts) für niedrige, mittlere und hohe Bestandesdichte, unter simulierten Streuinput.

3.3 Respiration of topsoils and deepsoils from different density sites

Total respiration in topsoils is significantly higher than in deepsoils, but its change patterns with different stand density are distinct in top layer and deep layer. Total respiration was found significantly higher in topsoils than in deepsoils and decreased in topsoils with stand density while it remained unchanged in deepsoils (Fig.2). The specific respiration in deepsoils was higher than in topsoils, which in both layers presenting the highest in low density site (Fig. 3).

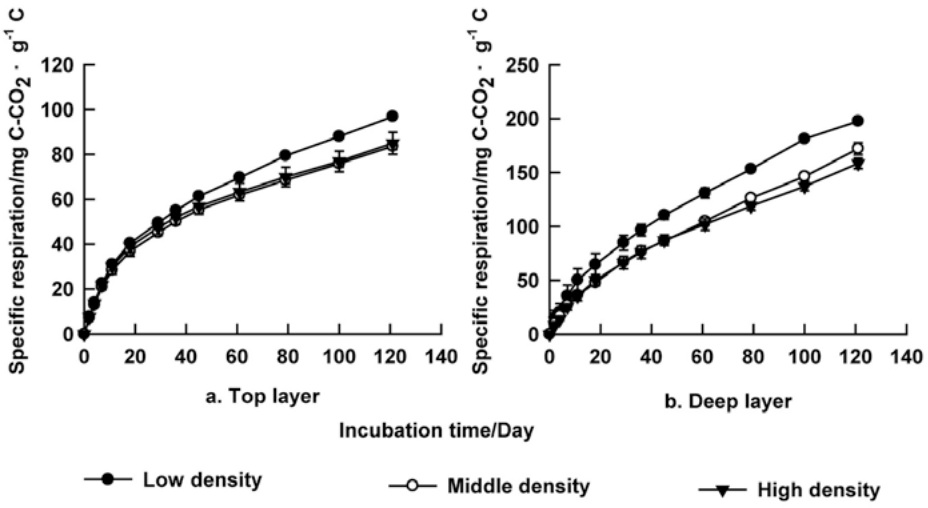

Figure 3: The accumulated specific respiration of samples in top layer (a, left) and deep layer (b, right) at low, middle and stand density while simulating litter accessibility expected in the field.

Abbildung 3: Die kumulierte spezifische Respiration von Proben in der oberen Bodenschicht (a, links) und tiefe Bodenschicht (b, rechts) für niedrige, mittlere und hohe Bestandesdichte, unter simulierten Streuinput.

3.4 Carbon balance in topsoils from different density sites

During the incubation, the content of refractory fraction of organic carbon increased by 4.64 % to 9.08 % compared to the samples before incubation. The scale of increment increased significantly with stand density. After the incubation, only 29.66 % to 31.68 % of added litter remained. The amount of litter decomposition in the duration of incubation did not vary with stand density (Tab. 3).

Table 3: Changes of refractory SOC content and substrate carbon content of the 121 days of incubation in top layer at low, middle and high stand density (g · kg^{-1}). We present means ± standard error (n = 3). The different lowercase letters represented the significant difference among stand density (P<0.05).

Tabelle 3: Veränderungen des organischen Kohlenstoffgehalts und des Kohlenstoffgehalts des Substrats während der 121-tägigen Inkubation in der obersten Bodenschicht bei niedriger, mittlerer und hoher Bestandesdichte (g · kg^{-1}). Wir zeigen Mittelwert ± Standardfehler (n = 3). Die unterschiedlichen Kleinbuchstaben repräsentieren signifikante Unterschiede zwischen der Bestandesdichte (P<0.05).

	Refractory SOC fraction			Carbon from substrate		
	Before incubation	After incubation	Increment	Before incubation	After incubation	Decrement
Low density	(54.15±0.93) b	(56.66±3.28) a	(2.51±0.45) c	6.44	(2.04±0.32) a	(4.40±0.32) a
Middle density	(57.82±1.86) a	(62.43±0.82) a	(4.60±0.79) b	6.44	(1.99±0.59) a	(4.45±0.59) a
High density	(55.37±2.91) ab	(60.41±4.30) a	(5.03±0.53) a	6.44	(1.91±0.20) a	(4.53±0.20) a

3.5 Correlations of SOC indicators with changing stand density in top and deep soil.

In top soil layer, the SOC storage was found positively correlated to SOC increment and negatively correlated to available nitrogen. The dissolved organic carbon was found negatively correlated to both substrate carbon decrement and SOC increment, and positively correlated to fine root biomass. There was also a negative correlation between SOC increment and fine root biomass (Tab. 4).

In deep soil layer, the SOC storage was found positively correlated to available nitrogen and negatively correlated to specific respiration. The available nitrogen was found negatively correlated to both total respiration and specific respiration. Obviously, the positive correlation was found between total respiration and specific respiration (Tab. 5).

Table 4: The Pearson correlations among SOC storage, Litter decomposition amount, SOC increment, Dissolved organic carbon, Available nitrogen and Fine root biomass in top soil layers (n=9). The asterisk represented the significant correlation between the two indexes.

Tabelle 4: Die Pearson-Korrelationen zwischen der organischen Kohlenstoffspeicherung, Streudekomposition, organischem Kohlenstofzuwachs, gelöstem organischen Kohlenstoff, verfügbarem Stickstoff und Feinwurzelbiomasse im Oberboden (n = 9). Der Stern zeigt die signifikante Korrelation zwischen den beiden Indices.

	SOC storage	Litter decomposition amount	SOC increment	Dissolved organic carbon	Available nitrogen	Fine root biomass
SOC storage	1					
Litter decomposition	0.60	1				
SOC increment	0.81 *	0.42	1			
Dissolved organic	-0.06	-0.68 *	-0.74 *	1		
Available nitrogen	-0.66 *	-0.35	0.13	0.16	1	
Fine root biomass	-0.60	-0.14	-0.82 *	0.72 *	-0.06	1

Table 5: The Pearson correlations among SOC storage, Total respiration, Specific respiration, Dissolved organic carbon, Available nitrogen and Fine root biomass in deep soil layers (n=9). The asterisk represented the significant correlation between the two indexes.

Tabelle 5: Die Pearson-Korrelationen zwischen der organischen Kohlenstoffspeicherung, Gesamtrespiration, spezifischer Respiration, gelöster organischer Kohlenstoff, verfügbarem Stickstoff und Feinwurzelbiomasse in tieferen Bodenschichten (n = 9). Der Stern repräsentiert signifikante Korrelation zwischen den beiden Indices.

	SOC storage	Total respiration	Specific respiration	Dissolved organic carbon	Available nitrogen	Fine root biomass
SOC storage	1					
Total respiration	-0.30	1				
Specific respiration	-0.69 *	0.88 *	1			
Dissolved organic	-0.22	-0.16	-0.10	1		
Available nitrogen	0.61	-0.68 *	-0.66 *	-0.39	1	
Fine root biomass	0.63 *	-0.06	-0.33	-0.40	0.46	1

4. Discussion

4.1 The effects of stand density on carbon storage in topsoils

In the ash plantations, the SOC storage in topsoils increased with the magnitude of stand density (Tab.2), possibly due to the increase of the refractory SOC pool as we hypothesized. After fractionation, the light fraction, known to be microbial active with a high carbon concentration, has been removed by the 0.4 mm sieving. The retained heavy fraction is known to be refractory with relatively low carbon concentration. That could be the explanations of the generally lower carbon concentration after sieving (Magid and Kjaergaard 2001; Kirkby et al. 2011; Fig. 1). Only for the low stand density site we discovered a significant decrease in carbon concentration after sieving (Fig. 1), indicating the larger amount of easily degradable carbon contained in topsoil at a low stand density. Thus, at the high stand density site, higher percentage of refractory fraction in topsoils may lead to potentially higher recalcitrance of SOC. We considered the recalcitrance as one possible reason for the higher SOC storage in high stand density site compared to low stand density site.

After incubation, the carbon content of all the topsoils increased compared to those before incubation (Tab. 3), indicating that under natural conditions (with litter input) the refractory fraction of SOC will be continuously accumulated. This was consisting with the results of studies of SOC storage in chronosequence forests (Sharma et al. 2009; Uri et al. 2014). Meanwhile, after incubation, the accumulation amount increased with increasing stand density (Tab.3), indicating higher stand density of ash will not only increase the percentage of refractory fraction of SOC in topsoils, but also benefit for its accumulation. This is also confirmed by the positive correlation between SOC storage and the increment after incubation (Tab. 4).

With the incubation of 121 days, no differences of soil samples receiving carbon from substrate were found in different stand density sites, but the increment of carbon content of the topsoils increased significantly when stand density gets higher (Tab. 3), indicating less SOC derived carbon emission stimulated by litter input with increasing stand density. As the addition of fresh carbon will stimulate the mineralization of SOC, which is known as the priming effect (Kuzyakov et al. 2000; Fontaine et al. 2004; Blagodatskaya et al. 2011), we inferred that the priming effect of the refractory fraction of SOC will be restricted with increasing stand density in topsoils (Fig. 2). Creamer et al. (2015) reported that the chemical similarity of SOC and added substrates would alter the intensity of priming effect due to the "co-metabolism". The more chemically similar they are, the stronger potential priming effect of SOC will be, as the promoted microorganism by the addition of substrate better equipped to decompose SOC. In our experiment, all the substrates are annual litter. As Pascault et al. (2013) mentioned the positive relationship between priming effect intensity and substrate degradability, we considered that with increasing stand density, it is the SOC microbial availability decreases. Also, our results of decreased microbial biomass

with increasing stand density supported this point (Tab. 2). Thus, the decreasing microbial availability of refractory fraction of SOC with increasing stand density is also considered to be an explanation of higher SOC storage in higher density site.

4.2 The effects of stand density on carbon storage in deepsoils

Similar to the topsoils, SOC storage in deepsoils also increases with increasing stand density (Tab. 2). The scale of decrement of soil carbon concentration with fractionation presented the highest in middle density site (Fig. 1), indicating the largest percentage of easily decomposed SOC in the deep layer from middle density site. This result, however, is not considered as the contribution to the change of SOC storage in deepsoils as Salomé et al. (2010) pointed out that the recalcitrance of SOC in deepsoils from microbe is mainly caused by the incapability of the microbes to contact the substrate it can use, which is known as the spatial isolation. The high amount of easily decomposed SOC in deepsoil of middle density site might be potentially not available to the microorganism because of the isolation, subsequently affects SOC storage barely.

It has been reported that the intensity of specific respiration could express the recalcitrance of SOC (Lv et al. 2005). Therefore, the negative correlation between specific respiration and SOC storage in our results (Tab. 5) probably indicated that the increasing SOC storage with increasing stand density is due to the higher recalcitrance of SOC. As some studies pointed out that DOC is one kind of labile carbon for microorganism in soil which is also allowed to move freely in soil solution (Neff and Asner 2001; Qiu et al. 2016), we expected DOC could somehow explain the variation of SOC recalcitrance, in turns the SOC storage with stand density changing. However, no significant correlations were found between DOC and specific respiration or SOC storage (Tab. 5). Thus, the recalcitrance of SOC in deepsoils might not be considered as the reasons for SOC storage variation with stand density. Moreover, the specific respiration is a key path for soil carbon pool output (Guenet et al. 2012; Heitkötter et al. 2017). The weaker output of carbon with increasing stand density could partly explain the accumulated SOC storage with increasing stand density.

Guo et al. (2005) reported the crucial effects of fine root on SOC storage. In our results, a positive correlation was found between fine root biomass and SOC storage in deepsoils (Tab. 5), implying the impact of fine root biomass on SOC storage. Normally, soil in deep layer is not able to contact the forest floor to receive the carbon feedback, which makes the fine root in deep layer one of the few paths for carbon input (Hu et al. 2016). Thus, we speculated the SOC storage increasing with increasing stand density could also be explained by the higher input level, as more root exudates and litter will be when more fine root biomass exists (Iversen et al. 2008).

5. Conclusion

In conclusion, the SOC storage of ash plantations increased with increasing stand density in both top soil layer and deep soil layer. In topsoils, as we hypothesized, the increasing SOC storage is due to the recalcitrance of SOC that increased with increasing stand density. With stand density increasing, higher percentage of refractory fraction exists, with this fraction being also more recalcitrant for microorganism. In deep soils, however, the recalcitrance of SOC might not be taken as the reason for SOC variation with stand density as we hypothesized. It is the higher input and lower output of carbon with increasing stand density that resulted in the higher SOC storage.

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