Higher stand densities can promote soil carbon storage after conversion of temperate mixed natural forests to larch plantations

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Received: 6 October 2020 / Revised: 16 November 2020 / Accepted: 15 December 2020 / Published online: 23 December 2020
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Abstract
Soil carbon (C) reservoirs held in forests play a significant role in the global C cycle. However, harvesting natural forests tend to lead to soil C loss, which can be countered by the establishment of plantations after clear cutting. Therefore, there is a need to determine how forest management can affect soil C sequestration. The management of stand density could provide an effective tool to control soil C sequestration, yet how stand density influences soil C remains an open question. To address this question, we investigated soil C storage in 8-year pure hybrid larch (Larix spp.) plantations with three densities (2000 trees ha−1, 3300 trees ha−1 and 4400 trees ha−1), established following the harvesting of secondary mixed natural forest. We found that soil C storage increased with higher tree density, which mainly correlated with increases of dissolved organic C as well as litter and root C input. In addition, soil respiration decreased with higher tree density during the most productive periods of warm and moist conditions. The reduced SOM decomposition suggested by lowered respiration was also corroborated with reduced levels of plant litter decomposition. The stimulated inputs and reduced exports of C from the forest floor resulted in a 40% higher soil C stock in high- compared to low-density forests within 8 years after plantation, providing effective advice for forest management to promote soil C sequestration in ecosystems.

Keyword Soil carbon · Stand density · Larch forest · Harvesting natural forest

Introduction
Forests play a key role in governing global carbon (C) cycling. There will be about 60 GtC exchanged between terrestrial ecosystems and the atmosphere every year, of which forests account for 80% (Schimel 1995; Kurbanov and Post 2002). Forest soils are important reservoirs of C, equivalent to double that existing in the atmosphere (Lal 2004). Thus, increases of soil C can reduce carbon dioxide (CO2) in the atmosphere and mitigate global warming (Li et al. 2013). Assuming negligible levels of intersystem exports and imports via, e.g., dissolved organic C (Sanderman and Amundson 2008; Kindler et al. 2011), the soil C pool is determined by the balance between C input by plants via litter and root deposits and the release of C during decomposition of soil organic matter (SOM) (Ajami et al. 2016). However, anthropogenic forest management activities can shift this balance (Laganière et al. 2010; Fernández-Romero et al. 2014; Ajami et al. 2016), which could result in changing soil C storage.

Stand density is one important aspect of forest management (Eldegard et al. 2019). Strategies have been forwarded to get larger individual growth and higher yield per unit area (Sun et al. 2018) in young forest plantations, but recent studies have found that different stand densities can also affect soil C storage (Noh et al. 2013; Hernández et al. 2016; Na et al. 2017; Truax et al. 2018 Sun et al. 2018). To date, studies have primarily focused on how stand density influences soil C in tree plantations on agricultural lands (Fernández-Núñez et al. 2010; Scott et al. 2006; Sitters et al. 2013; Hernández et al. 2016). For example, Sitters et al. (2013)
found that soil C storage increased with higher tree density in *Acacia zanzibarica* forest established on agricultural land, Hernández et al. (2016) found that increased density reduced soil C in *Eucalyptus grandis* plantation established on grassland, while Scott et al. (2006) found that stand density had little effect on soil C sequestration in *Pinus radiata* forest established on pasture. However, few studies about how stand density affects the soil C pool in forest plantations based on natural forests have been conducted. In recent decades, it has been noted that clear-cut harvesting of natural forest has resulted in soil C losses because of reduced C inputs, accelerated decomposition and erosion (Jandl et al. 2007; Mayer et al. 2017). In order to investigate the influence of tree density on the preservation and promotion of soil C stocks following the harvesting of natural forests, tree plantations at different densities are being established (Truax et al. 2018; Mayer et al. 2020). This land-use change from natural forests to plantations is likely to influence the soil C pool by shifting litter quality and quantity as well as soil nutrients under managed ecosystems with different vegetation composition and community structure. Nonetheless, it remains unresolved whether and how different stand densities influence the balance between soil C input and output by changing microclimate conditions of forest during afforestation.

There are few attempts to distinguish between the mechanisms that determine the effect of stand density on the soil C pool of forests. Several studies have shown that increased stand density can enhance forest productivity and increase the amount of above- and belowground litter. These increased soil C inputs may stimulate the decomposition of pre-existing SOM (Fontaine et al. 2007; Chen et al. 2014) and also be offset or even exceeded by C loss via SOM decomposition, leading to an uncertain net soil C balance (Fernández-Núñez et al. 2010; González González et al. 2012). Besides, some findings have suggested that soil C stocks decrease with stand density probably by facilitating microbial activity and respiration rates (Litton et al. 2004; Nilsen and Strand 2008), while other studies have found that increased stand density can decrease soil temperatures and thus reduce SOM decomposition (Li et al. 2006). However, Mayer et al. (2020) found that management of stand density had a small effect on soil C pool. Taken together, it is unclear how tree densities affect soil C sequestration.

Here, we investigated how stand density influenced the soil C pool of a larch (*Larix* spp.) plantation established after harvesting secondary natural forest. We aimed to determine the above- and belowground ecological factors that altered soil C contents of tree stands with different densities and to test the hypothesis that higher stand densities would increase soil C storage in plantations converted from natural forests.

### Materials and methods

#### Characteristics of the study site

The experiment was conducted at Maoer Mountain of Heilongjiang province in northeastern China (45°21′~45°25′ N, 127°30′~127°34′ E). The study area is situated at 300 m above sea level. The existing vegetation is dominated by secondary forest and also includes some plantations. Secondary forest of this area mainly consists of *Quercus mongolica*, *Betula platyphylla*, *Populus davidiana*, *Tilia* spp., *Fraxinus mandshurica* and *Juglans mandshurica*. The major tree species composition of tree plantations includes *Larix* spp., *Pinus sylvestris*, *Pinus koraiensis*, *Fraxinus mandshurica* and *Juglans mandshurica*. The zonal soil is classified as Alfisol (FAO, 1998). This area had a temperate monsoon climate, with dry and mild springs, short, warm and wet summers, windy but dry falls, and cold and dry winters. The mean annual air temperature is 2.8 °C, and accumulated air temperature (≥ 10 °C) is 2638 °C. The mean annual precipitation is 723 mm, and the mean annual evaporation is 1094 mm. The frost-free season is between 120 and 140 days.

#### Experimental site properties

The study was carried out at pure hybrid larch forests which were established with three densities including 2000 trees ha⁻¹ (LD), 3300 trees ha⁻¹ (MD), and 4400 trees ha⁻¹ (HD), following harvest of secondary natural forest in the spring of 2007. Larch plantations with different densities are located downhill, which has a flat slope side on a gradient of about 10%. Sites for the different tree plantations were chosen to be similar in microclimate and soil properties. The species composition in these larch plantations was the same, which mainly contains *Acanthopanax senticosus*, *Evonymus sacrosancta*, *Paris verticillata*, *Urtica angustifolia* and *Potentilla chinensis*. The understory vegetation of these forests was cut and removed from sites; therefore, the contribution of understory vegetation to the aboveground litterfall is negligible in this study. The experiment was carried out in 20 × 20 m plots in larch plantations with three densities, and each treatment had three replicates. The basic characteristics of the larch plantations were determined in May 2015 (Table 1).

#### Soil sampling

A soil profile (60 cm) was dug using a spade in the center of the plot in August 2015, and soil was taken at 0–10 cm, 10–20 cm, 20–40 cm and 40–60 cm layer for estimating soil C storage. In addition, five soil cores were randomly collected per plot using a cylindrical auger of 10 cm diameter,
and each core was split into 0–10-cm- and 10–20-cm-depth increments for measurements of soil dissolved organic C (DOC), microbial biomass C (MBC) and microbial biomass N (MBN) as well as mineral N concentrations in May, July and September of the years 2016 and 2017, thus resolving the growing seasons. All the soil samples were stored at 4 °C for less than one week before subsequent analyses.

Root biomass

Coarse roots were sampled by an excavator in August 2015. Fine roots (< 2 mm) at 0–20-cm-depth soil were collected using an auger (5.3 cm diameter). Samples were then sieved with a 2-mm mesh screen and pressure-washed with water. All the roots were oven-dried at 80 °C to constant weight. C storage of coarse root was estimated by coarse root biomass, C concentration, area occupied by coarse roots and tree density. The calculation of C storage of coarse roots was as follows:

\[ A = D \times \left( w \times c \right) / \left( \pi \times (d/2)^2 \right) \]

where \( A \) is the C storage of coarse roots (g C m\(^{-2}\)), \( D \) is the number of trees at each plot, \( w \) is the coarse root dry weight (g), \( c \) is the C concentration (g kg\(^{-1}\)) and \( d \) is the estimated diameter of area occupied of coarse roots from one sample tree.

Forest floor litter

Samples from forest floor layer (L, F and H layers) were composited from five random points on the soil surface in each plot using the 30 × 30 cm sampling template without living plants in larch plantations in August 2015. Forest floor layer was around 3–4 cm from the top soil, and mineral soil was beneath forest floor. The samples were oven-dried at 80 °C to constant weight. The calculation of forest floor C storage was as follows:

\[ B = S \times M \times C / s. \]

\( B \) is the C storage of forest floor (g C m\(^{-2}\)), \( S \) is the area of plot (20 × 20 m), \( C \) is the C concentration of forest floor (g kg\(^{-1}\)), and \( s \) is the sampling area (30 × 30 cm).

Annual litterfall

Eight circular litter traps (0.5 m\(^2\)) were located in each plot, and the bottom of basket was about 30 cm above the ground. Litter from each trap was collected and separated into needles and branches from May 2015 to May 2017. The collected samples were oven-dried at 80 °C until constant weight to estimate litter biomass.

Litter decomposition

Litter decomposition was assessed using the litterbag method to estimate weight loss rate of litter (Li et al. 2009). We collected litter from larch forests with different densities in this study and filled 10 g air-dried litter in each nylon litterbag (10 × 10 cm) with 0.5-mm mesh size. Ten litterbags were randomly set in plots of larch plantations where larch litter came from, clung to the soil surface and covered with fresh litter in May 2016. Litterbags placed in the fields were retrieved in November 2016 and dried at 65 °C to constant weight.

Measurements of soil respiration rate and soil temperature and water content

Six PVC soil collars with 10.6 cm diameter and 10 cm long were inserted 3–4 cm into the soil in each plot to estimate soil respiration rate in the end of April 2016 and left in the field throughout the course of the experiment. Respiration rates were measured at the middle and end of each month with a portable photosynthesis system (LI-COR 6400, Lincoln, NE, USA) coupled to a soil respiration chamber (LI-COR 6400–09, Lincoln, NE, USA) from the end of May 2016 to the end of October 2017, thus covering the growing seasons at the site. Measurements were carried out in the time when field conditions were suitable to avoid raining or puddles of water on the soil surface after raining, and were made between 9:00 and 11:00 h to minimize the effect of

### Table 1 Basic characteristics of larch plantations with different stand densities

| Stand density | Planting density trees ha\(^{-1}\) | Existing density trees ha\(^{-1}\) | Mean DBH cm | Mean height m | Stem basal area cm\(^{-2}\) | Canopy closure |
|---------------|------------------------------------|----------------------------------|-------------|---------------|-----------------|---------------|
| LD            | 2000                               | 1283 ± 74                        | 9.8 ± 0.3   | 12.4 ± 0.8    | 75.0 ± 5.2      | 0.7           |
| MD            | 3300                               | 2525 ± 66                        | 8.9 ± 0.3   | 14.1 ± 2.0    | 62.4 ± 7.5      | 0.9           |
| HD            | 4400                               | 3308 ± 196                       | 8.4 ± 0.1   | 12.4 ± 2.7    | 55.4 ± 2.4      | 0.9           |

Values are given as mean ± SE (n = 3). LD, MD and HD are low density, medium density and high density, respectively. DBH is tree diameter at breast height.
daily fluctuations on soil respiration. The ground vegetation within PVC collars was cut by scissor before each measurement to avoid the disturbance. During measurements of soil respiration, soil temperature (°C) and water content (%) were estimated at 10 cm depth with a digital probe (DeltaTRAK, Pleasanton, USA) and a water sensing probe (TDR300, Spectrum, California, USA), respectively. Soil temperature was fit to the following exponential model:

$$R_S = \alpha \times e^{\beta T}$$

where $R_S$ is the measured soil respiration rate (μmol CO₂ m⁻² s⁻¹), $T$ is the measured soil temperature (°C) and $\alpha$ and $\beta$ are the constants fitted with nonlinear regression equation. $Q_{10}$ values were calculated as:

$$Q_{10} = e^{10\beta}$$

where the $Q_{10}$ value is the increment of $R_S$ for every 10 °C increase in $T$.

Soil water content was fit to the following model:

$$R_S = a \times x^b$$

where $x$ is the measured soil water content (%) and $a$ and $b$ are constants fitted with nonlinear regression equation.

**Soil analysis**

Bulk density of soil was determined on a separate set of samples at 0–10-cm, 10–20-cm, 20–40-cm and 40–60-cm layer. A 100-cm³ cutting ring was pressed vertically into soil profile at different layers until cutting ring was filled with soil samples. Three samples were collected at each plot. The cutting ring was covered by top and bottom lid, weighed and then dried for 48 h at 105 °C to constant weight. The soil pH was measured by a soil and distilled water rate of 1:5, and the value of pH was determined by a model PHS-3C pH meter (INESA, China).

**Measurements of total C and N**

Total C and N concentrations were measured by using an elemental analyzer (Vario Macro, Germany). The calculation of soil C storage was as follows:

$$S = \sum_{i=1}^{l} D_i C_i H_i / 10$$

where $S$ is the soil C storage per unit area (kg C m⁻²), $i$ is the number of soil depth, $D_i$ is the bulk density at $i$ layer (g cm⁻³), $C_i$ is the soil C content at $i$ layer and $H_i$ is the soil depth at $i$ layer (cm).

**Measurements of soil DOC**

A sample of 30 g of fresh soil sieved through a 2-mm mesh was extracted with 60 ml deionized water, shaken for 30 min and then filtered with filter paper. The filtrate was centrifuged for 10 min (10,000 r min⁻¹) and then filtered through a 0.45-μm membrane filter, and DOC was determined using a TOC analyzer (Multi N/C 2100s, Analytik Jena, Germany).

**Measurements of soil MBC and MBN**

Soil MBC and MBN were estimated with the chloroform fumigation–extraction method (Vance et al. 1987). Twenty-five grams of fresh soil sieved through a 2-mm mesh was fumigated with ethanol-free chloroform in a sealed desiccator in the dark for 24 h at 25 °C, after which fumigated and non-fumigated soil samples were extracted with 50 ml 0.5 M K₂SO₄ solution and shaken for 30 min. Extracts were filtered with filter paper and analyzed using a TOC analyzer (Multi N/C 2100s, Analytik Jena, Germany). MBC and MBN were determined as:

$$MC = EC / k_{EC} \text{ and } MN = EN / k_{EN}$$

where $MC$ is the soil MBC concentration (mg kg⁻¹) and $MN$ is soil MBN concentration (mg kg⁻¹). $EC$ is the difference in extractable C extracted between fumigated and non-fumigated samples, and $EN$ is the difference in extractable N extracted between fumigated and non-fumigated samples. $k_{EC}$ is an extraction efficiency coefficient with a value of 0.45 (Wu et al. 1990), and $k_{EN}$ is an extraction efficiency coefficient of 0.54 (Brookes et al. 1985).

**Measurements of mineral N of soil**

A sample of 10 g of fresh soil sieved through a 2-mm mesh was extracted with 60 ml 2 M KCl solution, shaken for 1 h (200 r min⁻¹) and then filtered with filter paper. Ammonium and nitrate were analyzed on an AutoAnalyzer 3 (SEAL, Germany).

**Statistical analysis**

All statistical analyses were performed in SPSS 18 (IBM, Chicago, USA). Soil C storage, biomass and C storage of the litter and root were tested with a one-way ANOVA. DOC, soil respiration, temperature and moisture, soil MBC and MBC/MBN as well as soil mineral N were tested with a repeated-measures ANOVA. Pair-wise comparisons were determined using Tukey’s test. The relationship between soil respiration rate and temperature, water content, MBC and mineral N, respectively, was examined using correlation and regression analysis. Figures and tables were made.
using Sigmaplot 12.5 (Systat, California, USA) and Microsoft Excel 2013.

**Results**

**Soil properties**

Soil properties were not affected by different densities ($P > 0.05$; Table 2).

**Soil C storage in larch plantations and natural forest**

Total soil C storage at 0–60 cm and C storage at different depths tended to increase with stand density in larch plantations. The tree density had a significant effect on total soil C storage ($P = 0.03$), and C storage for HD was significantly higher than LD ($P = 0.03$). In addition, soil C storage in different plantations decreased with depth, and C storage at 0–20 cm layer accounted for more than 70% of total soil C storage (Table 3).

**Changes in DOC**

DOC concentration tended to increase with stand density in May but not in other time points (Fig. 1). Stand density and the interaction between stand density and time had no impact on DOC ($P > 0.05$; Fig. 1). However, time had a significant effect on DOC at 10–20 cm of soil depth ($P = 0.049$; Fig. 1).

**Biomass and C storage of forest floor and root**

Biomass and C storage of forest floor and root increased with higher stand densities. There was a distinguishable difference between root biomass in LD and in HD ($P = 0.05$), showing root biomass in HD was significantly higher than in LD ($P < 0.047$). In addition, stand density also has a significant effect on C storage of forest floor ($P = 0.049$; Table 4).

**Annual litterfall and weight loss rate of litterfall**

Annual litterfall in larch plantations tended to increase with higher stand density although differences were not statistically distinguishable between different densities ($P = 0.2$). Weight loss rate of litterfall showed a tendency for a slight decline with stand density ($P = 0.1$; Table 5).

**Soil respiration and temperature and moisture**

Overall soil respiration rate was affected significantly by time ($P < 0.0001$) and the interaction between stand density and time ($P = 0.008$), and tended to be affected slightly by density ($P = 0.09$; Fig. 2a). There was also a significant difference between LD and HD on June 15 of the first year ($P = 0.05$). Mean annual soil respiration rate for HD was marginally higher than the respiration for LD during the first year ($P = 0.08$) (Fig. 2a). The cumulative soil respiration for the whole year tended to decrease with higher tree density ($P = 0.1$; Table 3). The cumulative soil respiration was

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**Table 2** Soil properties in larch plantations with different densities

| Depth (cm) | Density | Temperature (°C) | Water content (%) | pH          | Bulk density (g cm$^{-3}$) | Total C (g/kg) | Total N (g/kg) |
|------------|---------|------------------|-------------------|-------------|---------------------------|----------------|----------------|
| 0–10       | LD      | 12.7 ± 1.1       | 20.5 ± 1.4        | 5.2 ± 0.0   | 0.7 ± 0.2                 | 66 ± 3.5       | 4.4 ± 0.3      |
|            | MD      | 12.3 ± 1.0       | 20.7 ± 1.4        | 5.4 ± 0.1   | 0.8 ± 0.1                 | 57 ± 4.5       | 5.5 ± 0.4      |
|            | HD      | 12.3 ± 1.1       | 19.9 ± 1.5        | 5.4 ± 0.1   | 1.0 ± 0.1                 | 63 ± 7.0       | 4.9 ± 0.5      |
| 10–20      | LD      | 5.0 ± 0.1        | 1.0 ± 0.1         | 21 ± 2.7    | 2.8 ± 0.3                 |               |               |
|            | MD      | 5.2 ± 0.1        | 1.0 ± 0.1         | 16 ± 1.4    | 2.7 ± 0.2                 |               |               |
|            | HD      | 5.0 ± 0.1        | 1.1 ± 0.2         | 25 ± 1.5    | 2.9 ± 0.1                 |               |               |

Values are given as mean ± SE ($n = 3$). LD, MD and HD are low stand density, medium stand density and high stand density, respectively. There are no significant differences between different tree densities at the same soil depth ($P > 0.05$) based on Tukey’s test.

**Table 3** Soil carbon storage at different layers in larch plantations with different densities (kg C m$^{-2}$)

| Density | 0–10 cm       | 10–20 cm      | 20–40 cm      | 40–60 cm      | Total       |
|---------|---------------|---------------|---------------|---------------|-------------|
| LD      | 4.6 ± 0.8a (53%) | 2.1 ± 0.3ab (23%) | 1.2 ± 0.2a (13%) | 0.9 ± 0.1a (11%) | 8.8 ± 0.5a (100%) |
| MD      | 5.8 ± 1.6a (59%) | 1.6 ± 0.1a (17%) | 1.3 ± 0.4a (13%) | 1.1 ± 0.0a (11%) | 9.8 ± 1.6ab (100%) |
| HD      | 6.3 ± 1.5a (51%) | 2.7 ± 0.4b (22%) | 1.7 ± 0.9a (14%) | 1.6 ± 0.6a (13%) | 12.3 ± 1.3b (100%) |

Values are given as mean ± SE ($n = 3$), and figures in brackets are percentages of soil carbon storage. Different lowercase letters indicate significant differences by density ($P < 0.05$) based on Tukey’s test. LD, MD and HD are low density, medium density and high density, respectively.
affected significantly by stand density in the summer time of 2016 ($P=0.04$; Fig. 3), showing a significant decline with density, although this effect was not observed in the summer time of 2017 ($P=0.4$; Fig. 3).

The mean annual soil temperature tended to decrease with higher stand density, but the difference was not significant ($P=0.17$; Fig. 2b), although soil temperature for LD was significantly higher than MD on September 15 of the first year and also higher than MD and HD on June 15 of the second year ($P<0.05$; Fig. 2b). The soil temperature varied with time ($P<0.0001$), but there were no significant effects of stand density or interaction between density and time ($P>0.05$; Fig. 2b). Furthermore, the pattern of soil respiration rate followed the temporal pattern of soil temperature (see Sect. 3.6).

The mean annual soil moisture had no obvious change with stand density though soil moisture for LD tended to be slightly lower than moisture for MD and HD ($P=0.06$; Fig. 2c). Soil moisture was higher in 2016 and varied over time during 2016 and 2017 ($P<0.0001$), but without any influence by stand density or interaction between density and time ($P>0.05$; Fig. 2c).
Microbial biomass C and N

MBC at 0–10-cm depth was not affected by stand density \((P = 0.58; \text{Fig. 4})\), but was significantly affected by time \((P < 0.0001; \text{Fig. 4})\) and the interaction between time and density \((P = 0.001; \text{Fig. 4})\). Time resulted in a significant effect on MBC at 10–20-cm depth \((P = 0.02; \text{Fig. 4})\), but stand density and density combined with time had no effect on MBC at 10–20-cm depth \((P > 0.05; \text{Fig. 4})\).

Overall MBC/MBN was affected by stand density \((P = 0.01)\) and showed an increase for MD (Fig. 5). Besides, time \((P = 0.007)\) and the interaction between time and density \((P = 0.003)\) resulted in a significant effect on MBC at 0–10-cm depth (Fig. 5). MBC/MBN at 10–20-cm depth was also affected by time \((P = 0.009)\) and the interaction between time and stand density \((P < 0.001; \text{Fig. 5})\).

Soil mineral N concentration

The density had no significant effect on \(\text{NH}_4^+\) concentration \((P > 0.05, \text{Fig. 6})\), but \(\text{NH}_4^+\) concentration was affected significantly by time \((P < 0.001)\), by being higher in May and September but lower in July, but was not affected by the interaction between time and density (Fig. 6). Stand density and the interaction between time and density had no effect on \(\text{NO}_3^-\) concentration either \((P > 0.05)\), but time had a significant effect on \(\text{NO}_3^-\) concentration \((P < 0.001; \text{Fig. 7})\).

Temperature and moisture dependences of soil respiration

The soil respiration rate increased exponentially with higher soil temperature and as a power function with water content (both \(P < 0.001; \text{Fig. 8}\)). The temperature sensitivity \(Q_{10}\) was undistinguishable between the tree density treatments \((P = 0.27)\) and consistently about 3.4 (Fig. 8). While temperature could explain 85%–89% of the variability of soil respiration for each stand (Fig. 8), the relationship between soil respiration and water content accounted for 21%–33% of the variability (Fig. 9).

There were links between MBC and soil respiration rate in early growing season \((R = 0.61–0.82, P < 0.01)\), and then, this correlation had a slight decline but was still significant \((R = 0.55, P < 0.05; \text{Table 6})\). Soil respiration in the whole growing season had a strong negative correlation with \(\text{NH}_4^+\) concentration \((P < 0.01; \text{Table 6})\).
Effect of stand density on soil C input

Higher stand density increased soil C storage by about 40%, from about 8.8 kg C m\(^{-2}\) in low-density to about 12.3 kg C m\(^{-2}\) in high-density plantations (Table 3), within 8 years of planting. This result is consistent with that of previous studies in deciduous and coniferous trees (González González et al. 2012; Sitters et al. 2013; Na et al. 2017; Truax et al. 2018). As candidate ecological drivers for this change, we found that aboveground litter and belowground deposits increased with stand density (Table 4) apparently reducing...

Fig. 4 Microbial biomass carbon (MBC) concentration at different depths in larch plantations with different densities in 2016 and 2017. Values are given as mean ± SE (n = 3). Given are P values for the factors density, time and interaction between density and time obtained from a repeated-measures ANOVA. LD, MD and HD are low density, medium density and high density, respectively. Note that the shown estimates include the growing seasons (from May to September) of the two years 2016 and 2017.

Fig. 5 Microbial biomass carbon (MBC) and nitrogen (MBN) ratio (mass) at different depths in larch plantations with different densities in 2016 and 2017. Values are given as mean ± SE (n = 3). Given are P values for the factors density, time and interaction between density and time obtained from a repeated-measures ANOVA. LD, MD and HD are low density, medium density and high density, respectively. Note that the shown estimates include the growing seasons (from May to September) of the two years 2016 and 2017.

Discussion
the C loss following clear cutting of natural forest. In addition, DOC was also an important component of the soil C pool (Ziegler et al. 2013), which previously has been found to be a sensitive indicator of physical movement (see discussion below) or chemical transformation of SOC (Chen and Xu 2008; Ma et al. 2018). Consistent with this finding, Kalbitz and Kalbitz (2012) found that dissolved organic matter (DOM) produced during litter decomposition could promptly associate with silt and clay fractions in the top soil mineral layer, promoting aggregation, which contributed to the stability of SOM. A recent study also proposed that SOM formation through the DOM–microbial pathway can stabilize by organo-mineral interactions, leading to protection from microbial decomposition (Cotrufo et al. 2015). In this study, soil DOC tended to increase with higher stand density, time and interaction between density and time obtained from a repeated-measures ANOVA. LD, MD and HD are low density, medium density and high density, respectively. Note that the shown estimates include the growing seasons (from May to September) of the two years 2016 and 2017.
density (Fig. 1), which is likely to have contributed to the increased soil C storage.

**Effect of stand density on soil C output**

Soil C output is also an important factor influencing the soil C pool. Although soil dissolved organic C leaching also can lead to soil C losses, it normally accounts for a small proportion (Kindler et al. 2011). For example, Sanderman and Amundson (2008) found that dissolved organic C leachate from O horizon was 31.7 g C m$^{-2}$ year$^{-1}$ in redwood–Douglas fir forest, while soil CO$_2$ flux was 571 g C m$^{-2}$ year$^{-1}$, thus accounting for only about 5% of output. Therefore, soil respiration dynamics is likely to be the dominant soil C output. Although others have found soil respiration decreased with tree densities (Litton et al. 2004; Nilsen and Strand 2008), our study showed that soil respiration rate tended to decrease with higher stand density (Fig. 2a), leading to transient periods of reduced soil C loss via respiration (Fig. 3). This finding might be due to several factors. First, it is well known that soil temperature is a dominant controller of soil respiration (Birgander et al. 2013; Davidson and Janssens 2006; Kirschbaum 1995). This was also reflected in the significant positive dependence of soil respiration rate on temperature. To examine the relationship, we determined the temperature sensitivity, indexed as Q$_{10}$, in different stands, finding similarly high Q$_{10}$ values at around 3.4 in all tree densities (Fig. 8). A secondary controller of soil microbial activity and decomposition is soil moisture (Chomel et al. 2019; Moyano et al. 2013; Schimel 2018). As such, we also found a significant correlation between soil respiration rate and water content, which accounted for 21–33% of the soil respiration variability (Fig. 9). The dependence of soil respiration on water content tended to increase in strength at higher tree densities (Fig. 9), indicating that soil moisture grew into a stronger controller of soil respiration at higher tree densities. This might be because that there is a more intense competition for water among tree roots at higher densities (Adler et al. 2010; Miriti et al. 2001; Schenk and Mahall 2002), thus depleting the resource also for microbial use. However, during the summer 2016 when distinguishable differences in soil respiration between tree densities could be shown (Figs. 2a and 3), while soil temperature and moisture were both similar between different stand densities (Fig. 2b and c) excluding these factors as drivers for the effect (Li et al. 2006). This indicated that there were factors in addition to soil temperature and moisture that could reduce respiration at higher stand density. One such factor could be that higher tree densities led to higher root biomass (Table 4). Higher root densities could have enhanced a rhizosphere effect where the level of root exudation decreased when exposed to drought (Williams and de Vries 2020), driving a decline in soil respiration, where the summer of 2016 was both warm and moist (Fig. 2b and c), increasing

Table 6 Pearson product-moment correlation coefficients between soil respiration rate and microbial biomass carbon and available nitrogen

| Respiration rate (μmol CO$_2$ m$^{-2}$ s$^{-1}$) | MBC (mg/kg) | NH$_4^+$ (mg/kg) | NO$_3^-$ (mg/kg) |
|---------------------------------------------|-------------|-----------------|-------------------|
| $R_s$(5/15–5/30) | 0.61** | 0.02 | −0.12 |
| $R_s$(7/15–7/30) | 0.82** | −0.08 | 0.14 |
| $R_s$(9/15–9/30) | 0.55* | −0.24 | 0.04 |
| $R_s$ growing season | 0.22 | −0.663** | −0.104 |

$Rs$ means the soil respiration rate (μmol CO$_2$ m$^{-2}$ s$^{-1}$) in different growing seasons.

*Indicates a significant correlation (α = 0.05), **indicates a highly significant correlation (α = 0.01)
the effect size of the tree density factor on soil respiration. Corroborating the results from soil respiration, we found that litter decomposition rates during the same study period also decreased with higher tree densities (Table 5).

It is often assumed that the size of the soil microbial community plays a crucial role in regulating SOM decomposition (Allison et al. 2010; Schimel and Weintraub 2003). We found that there was a significant positive relationship between soil MBC and soil respiration rate (Table 6). While this result was consistent with the microbial biomass being rate-limiting for the decomposition of SOM (Allison et al. 2010; Schimel and Weintraub 2003), an alternative explanation is that, under stable-state conditions, both were linked to the availability of OM to microbes (Hobbie and Hobbie 2013; Rousk 2016). On the other hand, Ehtesham and Bengtson (2017) found that there was less SOC priming after adding glucose into forest soil with higher density, which suggested that increased stand density might decrease soil C release. This result was interpreted to have been due to higher abundance of fungi in stands with higher density (Ehtesham and Bengtson 2017). Additionally, fungal biomass is thought to contribute more than bacterial biomass to forming stable SOM (Clemmensen et al. 2013; Liang et al. 2017) and thus higher fungal dominance in soil can lead to a higher soil C accumulation (Soares and Rousk 2019; Waring et al. 2013). While we did not resolve between fungal and bacterial biomass in this study, it has been suggested that the stoichiometry of microbial biomass can be used to infer its fungal/bacterial composition (Fanin et al. 2013; Waring et al. 2013) due to the systematic difference in elemental composition of the groups (Kirkby et al. 2011; Strickland and Rousk 2010). In this study, the MBC/MBN ratio was affected by stand density, but not in a systematic way. The MBC/MBN ratio for medium density was higher than lower density (Fig. 5), implying that fungi/bacteria were higher in that treatment (Qiu et al. 2016; Waring et al. 2013). However, it is not obvious why the intermediate level treatment (medium tree density) should lead to the highest fungal/bacterial ratio, warranting further research.

**Effect of tree species and age on soil C**

It is possible that tree density effects are species dependent and also depend on the age of the stand. Tree species and stand age are key factors that influence soil C storage (Compton and Boone 2000; Paul et al. 2002; Vesterdal et al. 2013). Guo and Gifford (2002) found that tree species had different C allocation strategies that could result in distinct qualities and quantities of soil C input. For instance, soil C increased with higher tree density in *Eucalyptus grandis* forests, while it decreased with density in *Pinus taeda* forests (Hernández et al. 2016). The reason for these differences could be because that roots of larch might have secreted some phenolic acids inhibiting microbial activity (Bertrand et al. 2006), thus reducing SOM decomposition in stands with high density. Furthermore, total standing biomass in forests is expected to be independent of the initial densities according to the law of constant final yield (Weiner and Freckleton 2010). This could imply that differences in C input from forest litterfall resulted from different stand densities could decrease or even disappear with older stand age (Fernández-Núñez et al. 2010). This is because there would be a higher competition for light, water and nutrients among trees at higher density, providing a negative feedback to tree growth, concomitant with a weaker negative feedback due to lower competition in lower density stands. For example, Noh et al. (2013) found that there was no difference in C storage of tree litter between low density (983 trees ha⁻¹) and high density (1517 trees ha⁻¹) in a mature *Pinus densiflora* forest, but that there was a significant difference in soil respiration. This might mean that C input would play a progressively smaller role in soil C storage as forests approach canopy closure. However, the stand in our study was a young forest, so C input still increased with density, and was also a key factor for soil C sequestration at different stand densities. Therefore, we still need further study about the causal mechanism where stand density affects soil C, particularly in forest plantations used for productivity, which remains young ecosystems.

**Conclusions**

Our findings suggest that higher tree density can increase soil C storage in larch plantations established on harvested natural forest. These changes in the C-budget depend on a combination of both increased input and reduced output of soil C from the forest floor. In the present study, above- and belowground litter biomass and C storage increased with higher tree densities, which promoted soil C accumulation. Furthermore, we observed transient periods of reduced soil respiration and litter decomposition with higher tree densities, especially during warm and moist conditions of high tree productivity, indicating that higher density could also reduce soil C release. Overall, our findings suggest that the establishment of tree plantations with a higher stand density after natural forest harvesting can enhance the capacity for soil C sequestration, with up to a 40% increase in soil C within 8 years of plantation, providing effective advice for forest management that can promote soil C sequestration in forest ecosystems.

**Acknowledgements** The study was carried out under the research project “Mechanisms of logging residue removal effects in reforested larch (Larix olgensis Henry) forests,” financially supported by the National Natural Science Foundation of China (Grant number 31770670). Meng Na is supported by Chinese Scholarship Council.
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