Moderate thinning increases soil nitrogen in a *Larix principis-rupprechtii* (Pinaceae) plantations

Junyong Ma [1] , **Hairong Han** [Corresp., 1] , **Wenwen Zhang** [1] , **Xiaoqin Cheng** [1]

[1] Beijing Forestry University, Key laboratory of ministry of Forest Cultivation and Conservation of Ministry of Education, Beijing, China

Corresponding Author: Hairong Han
Email address: hanhr6015@bjfu.edu.cn

Changes in the concentration of soil N or its components of the soil may directly affect forestry ecosystem functioning. Thinning of forest stands, a widely used forestry management practice, may transform soil nutrients directly by altering the soil environment, or indirectly by changing above- or belowground plant biomass. The study objectives were to determine how tree stem density affects the soil N pool and what mechanisms drive any potential changes. In this study, N and its active components were measured beneath a *Larix principis-rupprechtii* plantation across two entire growing season and under 12 25*25m plots: LT (low thinning forests, removal of 15% of the trees, three plot repetitions), MT (35% removal) and HT (50% removal) and contrast: CK (no thinning control). The environmental index like the light condition, soil reoperation, soil temperatures and prescription was measured in the plots. Results indicated that STN (soil total nitrogen) was affected by tree stem density adjustments in short-term, STN generally increased with decreasing tree stem density, reaching its highest concentration in the MT treatment before decreasing in HT; this pattern was echoed by DON/STN (DON, dissolve organic nitrogen), under MT, a lower DON/STN was measured across the seasons; and MBN (microbial biomass nitrogen) and the SOC/STN (SOC, soil organic carbon) ratios, density treatments had an influence on MBN concentration and inhibited SOC/STN (SOC, soil organic carbon). MT tended to accumulate more STN and produce lower DON/STN and generally higher microbial activity, which may be partly ascribed to the higher MBN value, MBN/STN ratio and lower DON/STN; and the water condition (water content, surface runoff and sediment loads) and light and soil temperatures may partly be responsible to the N pool dynamic in the different density treatments.

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Author information: Junyong Ma (Email: mjy172404707@me.com); Hairong Han* (Email: hanhr6015@bjfu.edu.cn). Wenwen Zhang (Email: 360486711@qq.com); Fengfeng Kang (Email: phoonkong@bjfu.edu.cn); Xiaoqin Cheng (Email: cxq_200074@163.com); All the authors from "Key laboratory of ministry of Forest Cultivation and Conservation of Ministry of Education, Beijing Forestry University, Beijing 100083, China". (*Corresponding author: Hairong Han)

Author Contributions: FFK, XQC and HHR conceived and designed the experiments. JYM WWZ and performed the experiments. JYM analyzed the data and wrote the manuscript.

Abstract

Changes in the concentration of soil N or its components of the soil may directly affect forestry ecosystem functioning. Thinning of forest stands, a widely used forestry management practice, may transform soil nutrients directly by altering the soil environment, or indirectly by changing above- or belowground plant biomass. The study objectives were to determine how tree stem density affects the soil N pool and what mechanisms drive any potential changes. In this study, N and its active components were measured beneath a *Larix principis-rupprechtii* plantation across two entire growing season and under 12 25*25m plots: LT (low thinning forests, removal of 15% of the trees, three plot repetitions), MT (35% removal) and HT (50% removal) and contrast: CK (no thinning control). The environmental index like the light condition, soil reoperation, soil temperatures and prescription was measured in the plots. Results indicated that STN (soil total nitrogen) was affected by tree stem density adjustments in short-term, STN generally increased with decreasing tree stem density, reaching its highest concentration in the MT treatment before decreasing in HT; this pattern was echoed by DON/STN (DON, dissolve organic nitrogen), under MT, a lower DON/STN was measured across the seasons; and MBN (microbial biomass nitrogen) and the SOC/STN (SOC, soil organic carbon) ratios, density treatments had an influence on MBN concentration and inhibited SOC/STN (SOC, soil organic carbon). MT tended to accumulate more STN and produce lower DON/STN and generally higher microbial activity, which may be partly ascribed to the higher MBN value, MBN/STN ratio and lower DON/STN; and the water condition (water content, surface runoff and sediment loads) and light and soil temperatures may partly be responsible to the N pool dynamic in the different density treatments.

Key words: forests thinning; soil total nitrogen; soil microbial environment; nitrogen solubility

1. Introduction

Forest ecosystems have often been proposed to play a part in the effective mitigation of climate change (Canadell and Raupach 2008; Miles and Kapos 2008). Forest soils play a major role in global nutrient cycles, providing regulating and supporting services, and hence soils are one of the most important components of forest ecosystems (Bravo-Oviedo et al. 2015). Previous studies have suggested that increasing levels of nitrogen (N) deposition could impact the sustainability of carbon (C) sinks in forest ecosystems (Townsend et al. 1996), as a result of interactions between the carbon and nitrogen cycles (Rastetter et al. 1997). However, due to the complexity of the interactions between both cycles, how these cycles are coupled remains poorly understood (Mcguire et al. 2003). Study (Aerts et al. 2012; Wieder et al. 2013) has shown that soil total nitrogen (STN), which has been widely studied in forest ecosystems (Hafner et al. 2005; Guan et al. 2015) and other land use conditions (Lehrsch et al. 2012; Zhao et al. 2017; Wang et al. 2017), responds to soil organic matter input; therefore, aboveground changes may potentially alter N pools in temperate forests.

Thinning treatments are frequently utilized in forest management to promote undergrowth renewal, increase biodiversity and improve soil fertility (Pariona et al. 2003). Management of stem density has been shown to be important for maintaining forest ecosystem services and long-term productivity, and is thus a focus of much scientific study (Jackson et al. 2002; Crow et al. 2002).

More than 80% of the N in soil exists in organic form (Schulten and Schnitzer, 1997). However, recent study in terrestrial ecosystems has been mainly focused on inorganic forms, such as ammonium (NH_4^+) and nitrate (NO_3^-) (Sigua and Coleman, 2006). STN is strongly correlated with the amount of available N in soil, and thus can influence soil microbial activity and humus formation (Bravo-Oviedo et al. 2015). Dissolved organic nitrogen (DON) availability may structure bacterial communities (Ren et al. 2016), responding rapidly to environmental change, DON dynamics can affect soil nutrient cycling, microbial activity and nutrient availability (Iqbal et al. 2010). Although total soil microbial biomass nitrogen (MBN) tends to be low in absolute value, its turnover represents a significant contribution to the global nitrogen cycle (Jenkinson et al. 1988). The MBN reflects the activity of microorganisms (Wardle, 1992; Jiang et al. 2010). Global stocks of soil organic carbon (SOC) had reached 2344 Pg (Stockman et al. 2013), as a large percentage of the global soil carbon pool is stored in forest soils (Houghton, 1995; Dixon et al. 1994). Due the close relationship between C and N in forest soils (Tateno et al. 1997; Cleveland et al. 2007) the SOC/STN ratio acts as an index of the degree of correlation between C and N availabilities (Ge et al. 2013), as well as a sensitive indicator of soil quality (Gravel et al. 2010). This SOC/STN ratio can also detect plant growth (Zhang et al. 2011; Wieder et al. 2013).

Tree stem density adjustment via thinning is a common management practice in forest plantations; this widely used approach can affect the growth of the forest stand (Duan et al. 2010), aboveground plant biomass (Jessica et al. 2007) and understory biological diversity (Karlsson et al. 2002; Lähde et al. 2002). Thinning regulates the distribution of open growing space so that standing trees may benefit from reduced competition, increasing growth and tree health (Smith et al. 1997; Jandl et al. 2007). Afforestation increases soil nitrogen accumulation and modifies nitrogen availability for micro-organismal growth (Deng et al. 2014), thereby potentially influencing elemental cycles in terrestrial ecosystems (Li et al. 2012; Li et al. 2014). Study (Aerts, et al. 2012; Wieder et al. 2013) has also shown that soil N responds to changes in soil organic matter inputs, which can then impact microbial processes. While many studies have focused on the soil carbon cycle in forest ecosystems (Lal et al. 2004; Zou et al. 2005; Ares et al. 2010), rather less attention has been paid to the relationship between C and N. Knowledge of how the active organic form of soil nitrogen varies with stand tree stem density and how SOC and STN are mechanistically linked is lacking.

In this study, within-growing-season variation in soil active nitrogen components was quantified for four different stand densities within a *Larix principis-rupprechtii* plantation located in a Northern Chinese montane secondary forest. Study hypotheses were first that adjustments in the tree stem density would affect STN and second that soil N-components would play an important role in N cycling. The specific objectives were to determine: (1) how STN varies with stand tree stem density; (2) the contributions of each soil nitrogen component to variation in the nitrogen pool overall under different stand densities and in different seasons; and (3) how the environmental factors changes related with the N pools.

2. Materials and methods

2.1 Study area and experimental design

The study was carried out in a plantation on Mt. Taiyue in Shanxi, North China (112°00′47″ E, 36°47′05″ N; 112°01′~112°15′E, 36°31′~36°43′N; elevation 2273–2359 m above sea level). This artificial forest is dominated by *Larix principis-rupprechtii* and has been protected since it was planted in the 1980s. The climate is the continental monsoon type with a humid, rainy summer and a cold, snowy winter. Mean annual air temperature is 8.7 ° C, with an average minimum temperature of - 10.4°C in January and an average maximum of 17.4 °C in July. The frost-free period lasts an average of 125 days, with the earliest frost generally in October and latest frost in April. Average annual rainfall ranges between 600 and 650 mm·yr^{-1}, with precipitation occurring mainly from July to September. The soil type in the study plantation are Haplic luvisols, ranging from 50–110 cm thick, according to the World Reference Base (WRB) soil classification system (IUSS Working Group WRB, 2006).

Sampling was performed in stands selected to reflect average altitude, grade, slope direction and soil conditions within the plantation, and measurements of these characteristics did not significantly vary among stands at the beginning of the experiment. After quantifying the initial characteristics of each quadrat, three 25 m × 25 m quadrats, or "sample areas", were designated within each treatment in July 2010. Field sampling was conducted in 12 study treatments, with initial stand densities averaging 2160 stems ha^{-1}.Three sample areas was designed for the 15% thinning (low thinning forests, LT) treatments randomly, with tree stem density adjusted to 1834 ± 12 stems ha^{-1} (mean of three replications); three 35% thinning (moderate thinning forests, MT) treatments, with tree stem density adjusted to 1418 ± 7 stems ha^{-1}; and three 50% thinning (heavy thinning forests, HT) treatments, with tree stem density adjusted to 1089 ± 3 stems ha^{-1}. Thinning treatments included three no thinning contrast (CK) with 2160 ± 12 stems ha^{-1}. The trees that were cut for thinning were removed from the plots and the understory plants remained. The dominant overstory vegetation in all stands was 35 years old *L. principis-rupprechtii*. Shrub species included *Elaeagnus umbellata* and *Rubus parvifolius*, and herbaceous species included *Carex rigescens* and *Dendranthema chanetii*. Detailed treatment characteristics are presented in Table 1 and Table 2.

2.2 Sampling and chemical analysis

Total soil carbon and nitrogen concentration were determined from soil samples collected from treatments at 0-10 cm, 10- 20cm, 20- 30cm, 30- 40cm, and 40- 50 cm depths using a cylindrical soil auger. Samples were collected at three time points throughout the growing season of 2015: spring, summer and autumn. Snow cover and freezing prevented collection of soil samples in the winter. Soil samples were collected from nine randomly chosen locations within each quadrat, and then combined according to depth to

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form one homogenous composite sample per depth. Visible stones and organic residues were removed and each sample was sieved through 2-mm mesh prior to chemical analyses. After sifting, each composite soil sample was divided into two subsamples. One subsample was stored in a 4°C incubator for later determination of DON and MBN concentration. The second was air-dried and passed through a 0.25-mm sieve before determination of soil organic carbon (SOC) concentration, STN concentration, through a 2-mm sieve for soil pH.

SOC and STN concentrations were determined by dry combustion using an elemental analyzer (Thermo Scientific FLASH 2000 CHNS/O, USA). The MBN concentration was measured using an HCl_4–fumigation extraction technique; 10.0 ± 0.5 g of fresh soil was fumigated with HCl_4, then extracted with 40 mL of 0.5mol·L^{-1} K_2SO_4, shaken for 1 h at 350 r min^{-1}, and filtered through a 0.45 μm membrane after centrifuging 5min at 3000 r min^{-1}. The filtrate concentration was quantified using a total organic carbon analyzer (Multi N/C 3000, Germany). The DON concentration was measured as the carbon concentration of non-fumigated soil samples (Boyer and Groffman 1996).

MBC was calculated as:

$$MBC=EC/k_{EC} \quad (2)$$

In (1) E_C = (organic C extracted from fumigated soils) - (organic C extracted from non-fumigated soils) and k_{EC} = 0.54

The soil texture was analyzed using the pipete method (Gee and Bauder, 1986). Air-dried soil samples that had been passed through a 1 mm sieve were used for soil pH determination; using a pH meter (Sartorius PB-10), pH was determined for a 1: 2.5 soil- water mixture. Gravimetric soil water concentration was measured as mass lost after drying for 24 h at 105 ºC. Meteorological data collected from a small fixed weather stations beside the sample area.

2.3 Environmental factors in the density adjustment plots

Soil respiration was measured with an LI-8100 soil CO_2 flux system (LI-COR Inc., NE., USA) in the middle and end of each moths during the three sampling seasons. The measurements were made on twelve PVC collars on each plot during 10:00–17:00 h over a one-day period. The PVC collars on each plot were systematically arranged. Soil temperature and volumetric soil water content at 5 cm depth were concurrently measured near each PVC collar. Soil temperature and volumetric soil water content at 5 cm depth were concurrently measured near each PVC collar. Each PVC collar is at 10 cm in diameter and 5 cm in height, with 3 cm insertion into soil.

Soil respiration for was the average of ever moth crossing the vegetation growing time (2 times each month), computation formula is as follows:

$$C_{RS} = \frac{R_S \cdot t \cdot C_{mol}}{10^6} \qquad (2)$$

C_{RS} (total carbon emission from soil respiration) $gC \cdot m^{-2}$; R_S (Soil respiration), $\mu mol \cdot m^{-2} \cdot s^{-1}$; t (time), s; C_{mol}, $12 g \cdot mol^{-1}$. C_{RS} in winter in this study accounted for 10% total carbon emission from soil respiration annual (Wang, et al., 2002).

The forests light environments were collected in July 2015 and 2016. The canopy analyzer (WIN SCANOPY 2010 a, Canada) was used to measured PPFD total over: photosynthetic photon flux density over the forest, PPFD total under: photosynthetic photon flux density under the forest. The plot was divided into three areas, which, in each region according to the left, middle and right is divided into three sub areas, a total of nine areas; in the center of each sub area the canopy analyzer was set up. Optical information collected and used the instrument software to analyze stand light environment (PPFD) back to the laboratory.

Surface runoff was measured by the runoff watershed method implementation, in each of the treatment, using asbestos shingle (set 5 m * 10 m) along the slope embedded in the dug trenches (depth of 0.25 m, 10 m long), guarantee not outflow runoff field runoff, a total of three sides runoff field (two 10m long side and one short side 5m up of the slope), runoff long downhill a short edge set a tilt in the horizontal plane of intercepting trough (5 m * 0.2 m * 0.2 m of PVC produced), intercept tank placed below level of end surface runoff collecting device (30 L plastic bucket), cover collection device with asbestos shingle, surface runoff data was collected directly by measuring the water in the bucket. Then shaken the water in the bucket and took 500 ml sediment content of water samples back to the laboratory and used the filtration experiment to calculate the sediment. The water was collected in crossing the vegetation in 2015.

2.3 Statistical Analysis

All data in the tables and figures are presented as means (n= 15, 3 plot repeats * 5 soil depths). Four-way analysis of variance was used to examine the impact of thinning treatment, season and soil depths, years and their interaction on STN, SOC, DON, MBN and soil pH values based on the post hoc Tukey-HSD test using the statistical package, IBM SPSS 20.0. One- way analysis of variance was used to examine the impact of thinning treatment in a season by T-test using SPSS. The least significant difference (LSD) test was used to compare treatment means, with significant effects having $p < 0.05$. Pearson correlation coefficients were calculated for pairs of carbon and nitrogen variables and two-tailed t-tests carried out using SPSS 20.

3. Results

3.1 General characteristics of the soil

Meteorological data, according to an automatic meteorological station indicated that precipitation was significantly higher in the summer than in the spring and autumn, the air temperature and 0- 10cm soil temperature also higher in summer, precipitation in 2016 was 1.3 times higher than 2015, Fig. 1.

No significant differences were found in the total phosphorus, bulk density, or mechanical composition of soil under different tree stem density treatments (Table 1). With tree stem density reduction, the forest understory became much brighter (from both direct, scattering and total radiation); the total photosynthetic photon flux density (PPFD) in the understory increased in the LT, MT and HT treatments, respectively, compared with the control ($p < 0.05$), Table 2. Soil respiration was higher in the MT plots while not significantly. A higher soil temperature was measured in MT, only significant in 2015. Soil moisture was significant higher in HT compared with CK, Table 2.

The total biomass gradually decreases with increasing intensity of density regulation, the variance analysis showed that only between CK and HT process has significant difference ($p < 0.05$) (Table 3).

Understory species composition was relatively simple in this *L. principis-rupprechtii* plantation, with the understory vegetation in the CK containing nine families, 13 genera and 14 herbaceous species. Dominant plants included species of *Compositae*, *Ranunculaceae* and *Rosaceae* families. In thinning treatments, understory plant species richness increased with decreasing tree stem density. Overall, the highest species richness was recorded in the MT treatment (Table S2). Soil nutrients decreased significantly with soil depth and generally accumulated to higher levels in summer (Table S2).

3.2 Soil total nitrogen

Tree stem density effects on STN were significant in five sampling seasons out of six, (Fig. 2-a). In 2015, spring ($p = 0.0027$), (g N Kg^{-1}): M (3.1 ± 0.21) > HT (2.9 ± 0.33) > CK (2.5 ± 0.05) > LT (2.3 ± 0.11 g). In summer ($p = 0.002$), (g N Kg-1): MT (3.6 ± 0.04) > HT (3.4 ± 0.21) > LT (2.9 ± 0.08) > CK (2.7 ±0.19). In autumn ($p = 0.110$), (g N Kg-1): HT (3.2 ± 0.42) > MT (3.1 ± 1.197) > CK (2.7 ± 0.29) > LT (2.5 ± 0.97). Thus, STN was highest in spring and summer in the MT treatment compared with other treatments. Mean STN concentrations were 25% higher in the MT (30% thinning) and HT (50% thinning) treatments than in the less severe thinning treatments (i.e. LT - 15% thinning, and CK - 0% thinning).

In 2016, the response of STN content to density adjustments was similar to 2015, but bigger differences between more and less severe thinning treatments. In 2016, the tree stem density effects on STN were significant in spring ($p= 0.003$), summer ($p= 0.026$) and autumn ($p= 0.003$). Across the three sampling seasons, (g N Kg^{-1}): MT (3.2 ± 0.44) > HT (2.8 ± 0.23) > CK (2.4 ± 0.24) > LT (2.3 ± 0.13).

Accumulation of STN content was greater for the more thinned treatments (MT, HT) than the less thinned treatments (CK, LT) in the two sampling years, resulting in 26.1%, 24.9%, and 22.5% increases between less thinned and more thinned treatments in spring, summer, autumn, respectively in 2015 (Fig. 3- a); resulting in 12.5%, 26.3%, and 48.9% increases between less thinned and more thinned treatments in spring, summer, autumn, respectively in 2016 (Fig. 3- b).

3.3 Tree density adjustment effects on soil organic nitrogen components

Tree stem density had little effect on dissolved organic nitrogen (DON) in the soil across the sampling seasons (Fig. 3a), only did in the summer of 2015 ($p = 0.034$) though DON concentration varied little among thinning treatments (standard deviation < 5.75 mg N kg^{-1}). However, DON varied with the seasons ($p < 0.001$), changing rapidly over the sampling period. The DON was 102.7% higher in summer than the other seasons.

The MBN, which reflects the microbial activity of forest soils, was highest in the MT treatment (compared with other treatments) across all seasons ($p = 0.012$ in spring; $p = 0.076$ in summer; $p = 0.035$ in autumn) in 2015. MBN generally increased with decreasing tree stem density up to the MT treatment and then decreased in the HT treatment (Fig. 4a) (mg N Kg^{-1}): in spring, CK < HT (7.7 ± 0.79) < LT (8.8 ± 1.16) < MT (10.8 ± 0.30); in summer, CK (29.9 ± 2.49)< LT (30.5 ± 1.32)) < HT (32.2 ± 2.97) < M (36.4 ± 0.93); and in autumn, LT (30.2 ± 0.80) < HT (33.0 ± 0.51) < CK (30.7 ± 3.37) < MT (35.8 ± 0.44). However, in 2016, MBN was not affected by density adjustment significantly, ($p = 0.165$ in spring; $p = 0.555$ in summer; p =

0.205 in autumn). In the summer of 2016, a significant higher MBN content was measured, which was 302.6% higher than the average MBN content across all the seasons and treatments.

3.4 Relationships among soil nitrogen components

The ratios of DON/STN and MBN/STN responded differently to both thinning treatments and seasonal changes (Fig. 3b and Fig. 4b). A one-way ANOVA revealed that both ratios differed among seasons ($p< 0.01$), being higher in autumn and summer versus spring.

As noted, different from DON content the ratio DON/STN varied with tree stem density significantly in four sampling times out of six. DON/STN generally decreased with decreasing tree stem density down to the MT treatment and then increased in the HT treatment (Fig. 3b) (%): in spring 2015 ($p= 0.027$), MT (1.42 ± 0.13) < CK (1.59 ± 0.05) < LT (1.73 ± 0.08) < HT (1.78 ± 0.04); in summer 2015 ($p= 0.003$), MT (6.13 ± 0.16) < CK (6.58 ± 0.20) < LT (7.79 ± 0.34) < CK (8.47 ± 0.76); in autumn 2015, not significant ($p= 0.10$); In spring 2016 ($p= 0.123$); in summer 2015 ($p= 0.047$), MT (2.70 ± 0.43) < HT (3.22 ± 0.91) < CK (4.18 ± 0.16) < LT 4.51 ± 0.55); in autumn 2015 ($p= 0.001$), MT (2.32 ± 0.15) < HT (3.18 ± 0.22)< CK (3.33 ± 0.23) < LT (3.45 ± 0.13). Within each season, DON/STN was minimized in the MT thinning treatment.

Strong, positive correlations were found between SOC and STN ($R = 0.894$, $p < 0.001$, n=360), DOC and DON ($R=0.926$, $p < 0.001$, n=360), and between the DON and both MBC ($R = 0.657$, $p < 0.001$, n=360) and DOC ($R = 0.926$, $p < 0.001$, n=360). In contrast, the SOC/STN ratio was negatively correlated with STN ($R = -0.427$, $p < 0.001$, n=360). (Fig. 5).

4. Discussion

4.1 Effects of thinning treatments on the soil N pool and forest ecosystem

The specific objectives of this study were to determine how STN varies with stand tree stem density in a *L. principis-rupprechtii* plantation, and how variation in each soil nitrogen component may drive patterns in STN. STN responded to density treatments, first increasing with decreasing density (up to the MT treatment) and then decreasing in HT (50% tree stem removal), indicating that thinning generally increased soil total nitrogen. However, this effect was limited to the growing season and was not seen in autumn.

The availability of soil N is widely regarded as a factor commonly restricting primary productivity (Sigurdsson, 2001) and the function of certain biochemical processes (Vitousek et al, 2010). Understory plant species were most abundant in the moderate thinning treatment of this study (MT, Table 2). Similarly, an experiment that followed mixed forests for 12 years after thinning showed that tree stem density reduction can improve the growth of woody species in stands significantly (Lei, 2005). Study in *Picea abies* (Heinrichs and Schmidt, 2009) and *Pseudotsuga menziesii* forests (Ares et al. 2010) also found that both forest species richness and the abundance of shrub and grass species increased with thinning intensity. Aboveground vegetation is one of the main source for soil N (nitrogen) pool (Achat et al. 2015), hence changes in species composition and biomass may affect STN. As we found here, understory plant species were most abundant in the moderate thinning treatment (MT, Table S1). A more biomass was found in this study under MT if the tree layers (thinned layers) was not included (Table 3), echoed to a higher STN and SOC in the 35% thinning plots.

The forest density adjustment conducted in the *L. principis-rupprechtii* plantation caused a change in the environmental factors both from the up ground plants and the below, soil respiration, temperature and moisture in the soil at different levels, which may contribute to the differences of soil N pool. Light and space availability in the understory can change with thinning (Richards and Hart, 2011; Roberts, 2004) (and here, Table 1). Here, thinning treatments altered the total PPFD under and had no impact on the total PPFD over the forest canopy (Table 2). Other crucial environmental factors like soil temperatures, soil respiration changed with density adjustments, higher soil temperatures and soil respiration were measured in the MT, while not significant generally. The soil moisture was enhanced by the density adjustment significantly, with the thinning increased cased a higher soil moisture content (Table 2).

According to the *intermediate disturbance hypothesis* (Fox, 1979; Roxburgh et al. 2004; Huston 2014), moderate rates of disturbance to plant communities can maintain high species diversity. This was observed in an experimental *Cupressus funebris* plantation, where moderate thinning enhanced the diversity indices of both understory shrub and herbaceous species (Gong et al. 2015). Combinations of various environmental factors, such as understory plant species composition and light and space availability, may alter the soil environment to different extents, thus affecting STN concentrations.

Close relationships were found between STN and other soil properties. Plotting all the data (across treatments and seasons), it can be seen that higher STN concentrations also correspond to higher concentrations of SOC, DON, DOC and MBN (Table 4). Bravo-Oviedo et al. (2015) and further analysis performed in the setting of this study revealed that density treatments affected various components of the soil N pool which are considered to be factors driving variation in total soil N.

4.2 Effects of thinning treatments on the SOC/STN ratio and soil N-components

This study tested the hypothesis that moderate thinning treatments should increase STN through changes to a) the environmental factors in the forests and b) soil N-components and the solubility of the N pool.

Changes in DON can lead to significant modifications in soil nutrient stoichiometry, thereby affecting microbial activity and STN concentration (Iqbal et al. 2010; Aerts et al. 2012). Even though there was no significant correlation found between tree stem density and DON, thinning treatments did alter soil nitrogen characteristics, with one unit of STN containing less DON in the more extreme thinning treatments (Fig. 3b). The amount of DON can affect STN dynamics, as a higher DON/STN means a greater possibility of nitrogen loss through leaching, which would affect nitrogen accumulation rates. The DON/STN ratio was smallest under the MT treatment ($p < 0.05$), the same treatment where the highest concentrations of STN were recorded in the spring and summer 2015, and summer, autumn 2016; this may partly explain STN dynamics across treatments, higher STN echo to a lower DON/STN.

Here, moderate tree stem density reduced nitrogen solubility, limiting nitrogen losses. An analysis of hydrological characteristics in the study area revealed abundant rainfall, which may cause fertilizer to wash away (Fig. 1 and Fig. 3). The effects of the moderate thinning treatment on the DON/STN ratio matched expectations, however DON alone did not.

Total soil DON and the DON/STN ratio varied with the season (Fig. 3). In summer, as the temperature gradually increased (Fig. 1), trees and grasses would have experienced abundant root growth, likely leading to an increase in root secretions or the amount of deciduous material around the root system (He et al. 2013); both soil STN and DON concentration rose continuously from spring to summer. Soil temperature was also higher and more precipitation occurred in the study plantation in the summer (Fig. 1). Higher temperatures can enhance microbial growth (Edwards et al. 2006), which can then be further facilitated by higher concentrations of DON providing more nitrogen for microbial growth (Iqbal et al. 2010), and in this study a close positive relation was measured between DON and MBC ($R = 0.657$, $p < 0.001$, n=360). Meanwhile, the higher precipitation which was reported to be an important factor which affected N pool (Yu et. al, 2017) and resultant continuous nitrogen losses (via higher DON) might explain the reduction in STN in autumn, a higher surface runoff was found in higher thinning treatments (Fig. 6a) and a more sediment content was measured in the HT compared with MT across the plant growing seasons (Fig. 6b). This confirms the hypothesis that moderate thinning reduced DON/STN; thus, enhanced the STN (Fig. 2a and Fig. 3b).

Tree stem density had a more complex effect on the microbial index like MBN, MBC and SOC/STN. 302% higher MBN content was measured in summer of 2016 compared with the average MBN content of the two years. The MBN concentration tended to increase with decreasing tree stem density, reaching its highest level in the MT treatment before decreasing in HT; this pattern was echoed by the STN concentration (Fig 2a and Fig 4b), while only significant in spring and summer of 2015. In the sampling year of 2016, when there was 130% more precipitation, MBN was not affected by density adjustments.

The MBN concentration and MBN/STN ratio were much higher in summer and autumn than in spring (Fig. 4), as also has been found in previous studies in temperate forest regions (Bohlen et al. 2008), indicating lower microbial activity at the beginning of the vegetative season. Adequate water availability and warmer temperatures for microbial growth likely produced the observed increase in MBN in summer (Fig. 1 and Fig 4a). The observed average MBN/STN ratio (2.5%) was similar to the other temperate forest soils (1–3%) (Zhong and Makeschin, 2006).

Previous study has indicated that a lower SOC/STN ratio indicates an increment of the rate of microbial decomposition and of the nitrogen mineralization nitrogen mineralization (Springob and Kirchmann, 2003), and here, the SOC/STN ratio was negatively correlated with STN (Table 4). The MT treatment likely provided a better environment for microorganism growth, thus enhancing the rate of microbial decomposition. Greater microbial biomass could then increase the concentration of MBN, as shown in the Pearson relation (Fig. 5), because MBN and MBC were strongly positively correlated. Soil microbial biomass (as MBC or MBN) can be sensitive to changing soil conditions, a slight variation in the composition of soil organic matter (Liu, 2010) or environmental (Yi et al., 2007) changes may have changed the content, and hence has been suggested as an index of both soil environmental change and nutrient supply capacity (Hargreaves et al. 2003). The highest MBN (Fig. 4a) and lowest SOC/STN (Fig. 4c) were observed in the MT treatment in some seasons, suggesting that microbes might have been more active under intermediate tree stem densities.

5. Conclusions

Clear effects of thinning treatments were found on STN in a *Larix principis-rupprechtii* plantation three years after thinning. The STN concentration was greatest in the MT treatment. Moderate thinning treatments may have enhanced the soil N pool by changing a) the environmental factors and b) the solubility of soil N pool. These influences of density adjustment on N pool are likely driven by density effects on the labile N pool, like DON or MBN, varied with seasons, with contents of these components peaking in the summer when the water and heat condition was better for the carbon cycle. A lower DON/STN under intense thinning responses to a higher STN content, indicating the solubility of soil N pool was changed by the density treatments. The lower solubility created by the MT treatments is the key factor caused the more STN accumulation in this treatments. Environmental factors: soil temperature, soil moisture and light of plots creating a moderate (via better) conditions for microorganism and the plants also contribute to the STN accumulation. We recommend moderate density adjustment (1404 trees per ha) to *L. principis-rupprechtii* plantations to promote N retention and agree with the *intermediate disturbance hypothesis*, but still long-term studies are required to validate these findings.

Acknowledgments

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Figure legends

Figure 1 Air temperature, 10cm soil temperature and average precipitation in the study treatments across the growing season.

Figure 2 Variation in the STN (a) and SOC (b) in different thinning treatments across the growing seasons in 2015 and 2016. CK, the no thinning, control treatments. LT, the low thinning treatments (15%

thinning). MT, the moderate thinning sample treatments (35% thinning). HT, the heavy thinning sample treatments (50% thinning). STN, soil total nitrogen; SOC, soil total organic carbon. Each bar represents an average value across three replicate samples (n =15), i.e. three plots repeats × five soil depths. Error bars represent standard errors around the three plot repeats. Different lowercase letters demarcate a significant difference among different density adjustments within the same sampling season ($p < 0.05$). The same for Figure 3, 4 and 5.

Figure 3 Variation in the DON (a) and DON/STN (b) in different thinning treatments across the growing seasons. CK, the no thinning, control treatments. LT, the low thinning treatments (15% thinning). MT, the moderate thinning sample treatments (35% thinning). HT, the heavy thinning sample treatments (50% thinning). DON, dissolved organic nitrogen; STN, soil total nitrogen. Each bar represents an average value across three replicate samples (n =15), i.e. three plots repeats × five soil depths. Error bars represent standard errors around the three plot repeats. Different lowercase letters demarcate a significant difference among different density adjustments within the same sampling season ($p < 0.05$).

Figure 4 Variation in the MBN (a), MBN/STN (b) and SOC/STN (c) in different thinning treatments across the growing seasons. CK, the no thinning, control treatments. LT, the low thinning treatments (15% thinning). MT, the moderate thinning sample treatments (35% thinning). HT, the heavy thinning sample treatments (50% thinning). MBN, microbe biomass nitrogen; STN, soil total nitrogen; SOC, soil organic carbon. Each bar represents an average value across three replicate samples (n =15), i.e. three plots repeats ×

five soil depths. Error bars represent standard errors around the three plot repeats. Different lowercase letters demarcate a significant difference among different density adjustments within the same sampling season ($p < 0.05$).

Figure 5 Pearson relationship of different soil properties across thinning treatments, seasons and soil depths. n= 360, i.e. four density treatments * three seasons * three repeats * five soil depths * two years.

Figure 6 The surface runoff (a) and sediment (b) concentration under different thinning treatments across seasons. The data was collected in 2015.

Figure 1

Figure 1 Air temperature , 10cm soil temperature and average precipitation in the study treatments across the growing season.

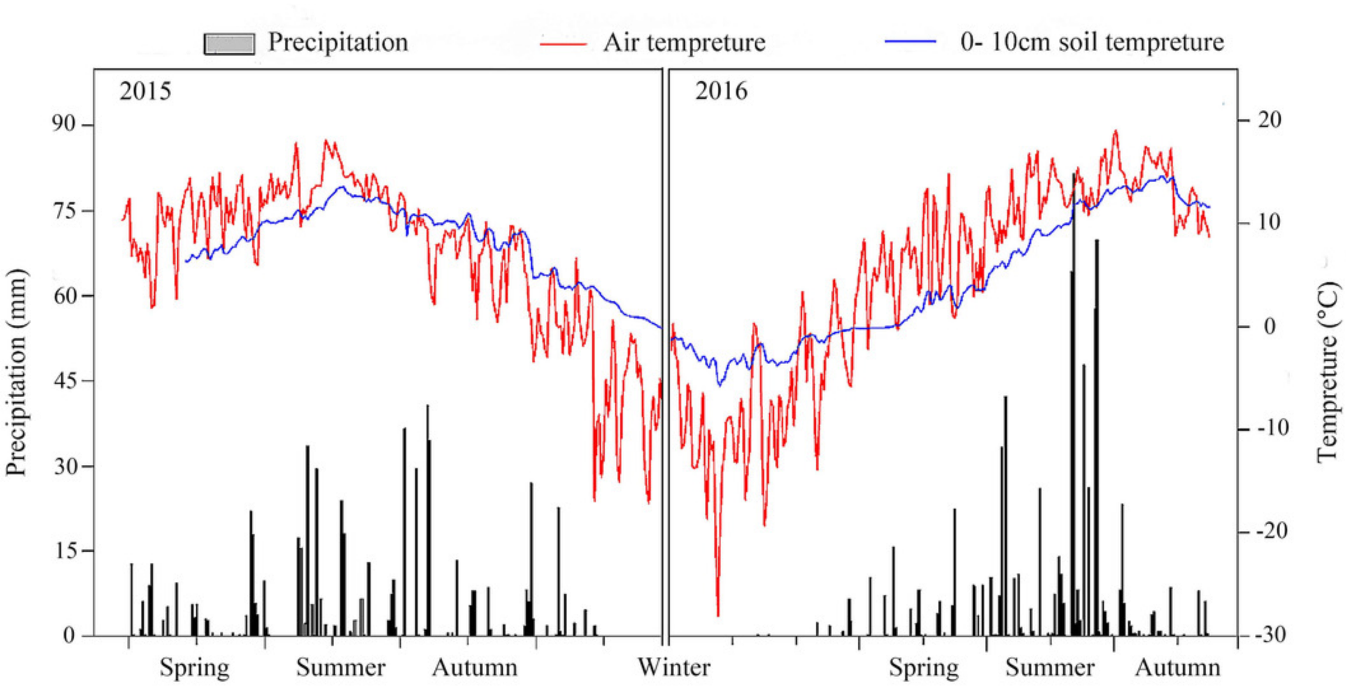

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Figure 2

Figure 2 Variation in the STN (a) and SOC (b) in different thinning treatments across the growing seasons in 2015 and 2016.

CK, the no thinning, control treatments. LT, the low thinning treatments (15% thinning). MT, the moderate thinning sample treatments (35% thinning). HT, the heavy thinning sample treatments (50% thinning). STN, soil total nitrogen; SOC, soil total organic carbon. Each bar represents an average value across three replicate samples (n =15), i.e. three plots repeats × five soil depths. Error bars represent standard errors around the three plot repeats. Different lowercase letters demarcate a significant difference among different density adjustments within the same sampling season ($p < 0.05$). The same for Figure 3, 4 and 5.

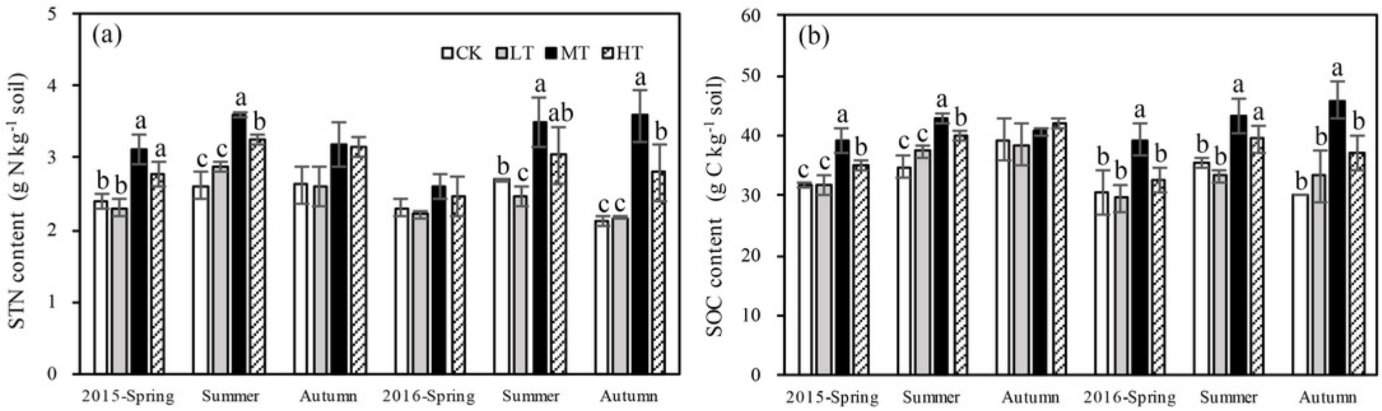

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Figure 3

Figure 3 Variation in the DON (a) and DON/STN (b) in different thinning treatments across the growing seasons.

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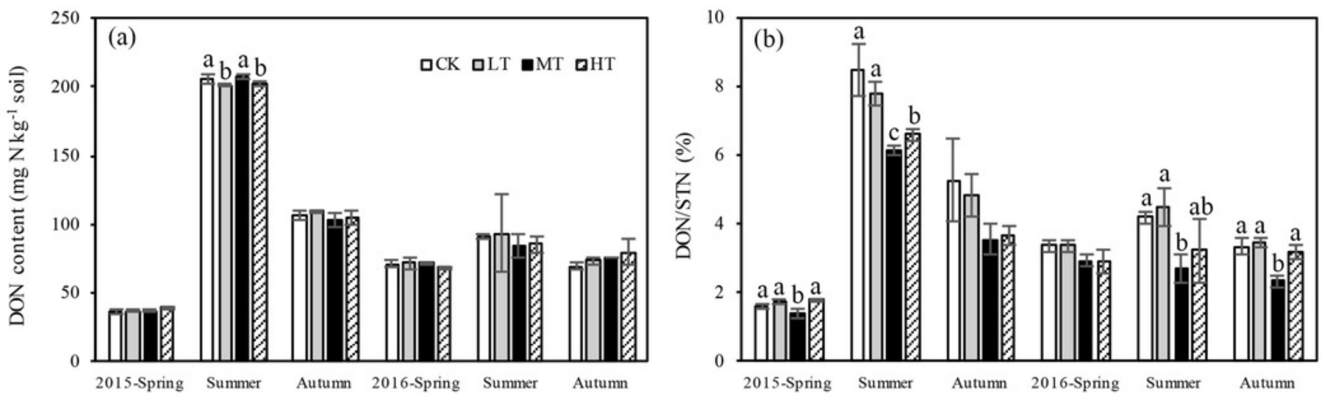

Figure 4

Figure 4 Variation in the MBN (a), MBN/STN (b) and SOC/STN (c) in different thinning treatments across the growing seasons.

CK, the no thinning, control treatments. LT, the low thinning treatments (15% thinning). MT, the moderate thinning sample treatments (35% thinning). HT, the heavy thinning sample treatments (50% thinning). MBN, microbe biomass nitrogen; STN, soil total nitrogen; SOC, soil organic carbon. Each bar represents an average value across three replicate samples (n =15), i.e. three plots repeats × five soil depths. Error bars represent standard errors around the three plot repeats. Different lowercase letters demarcate a significant difference among different density adjustments within the same sampling season ($p < 0.05$).

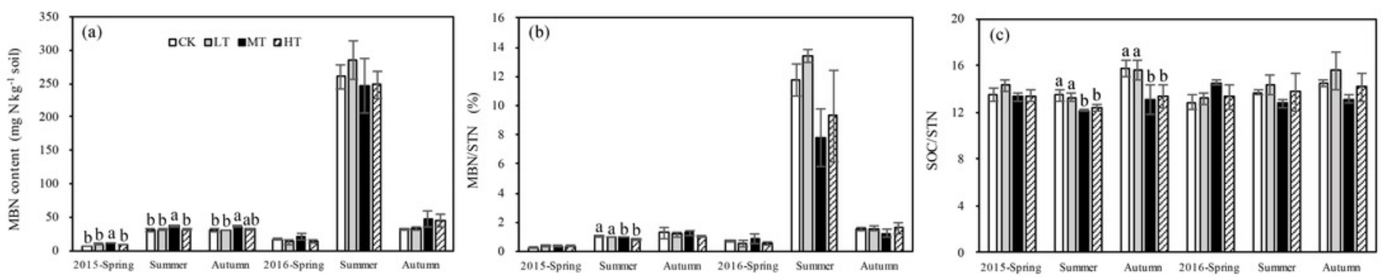

Figure 5

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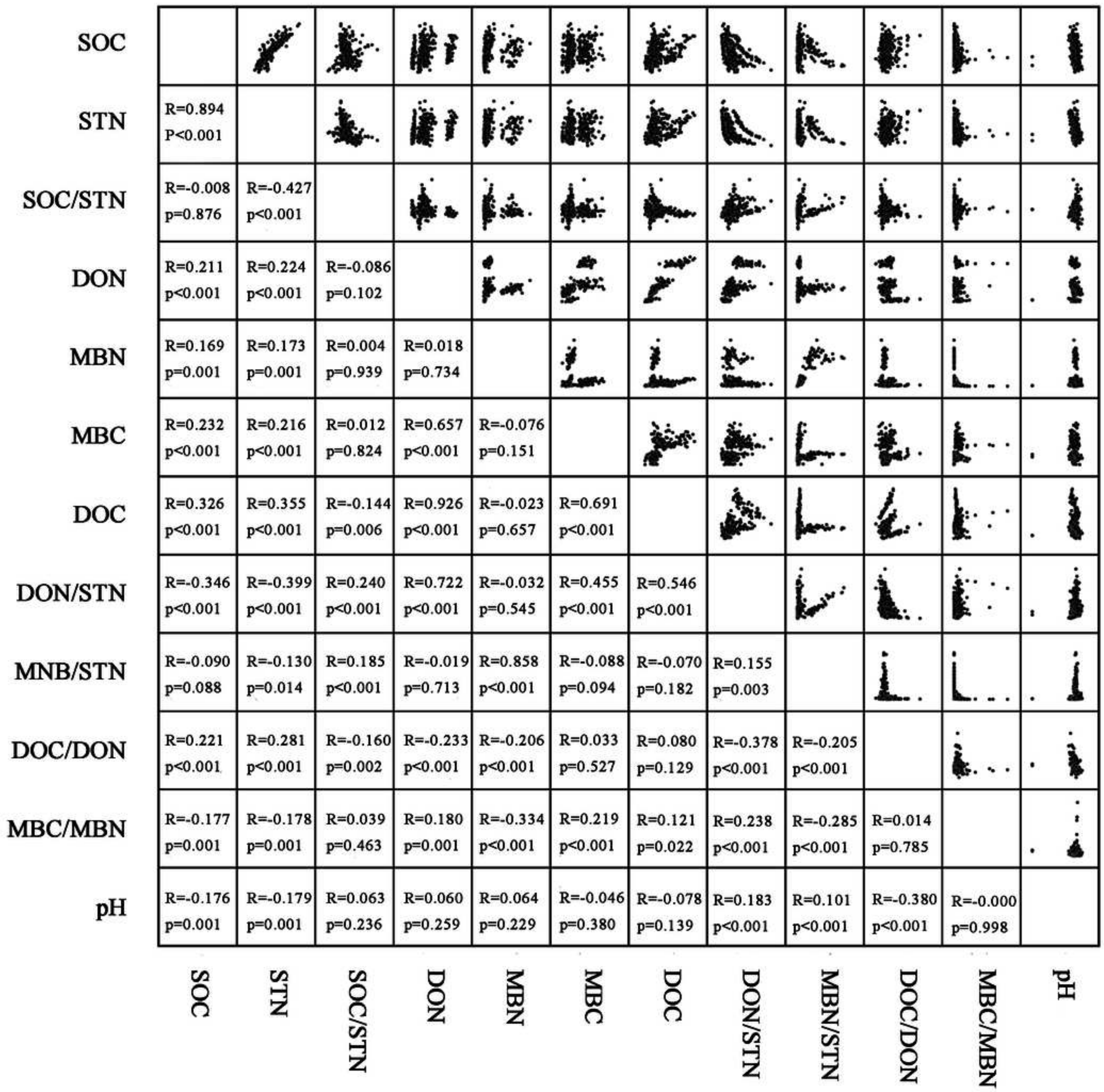

Figure 6

Figure 6 The surface runoff (a) and sediment (b) concentration under different thinning treatments across seasons.

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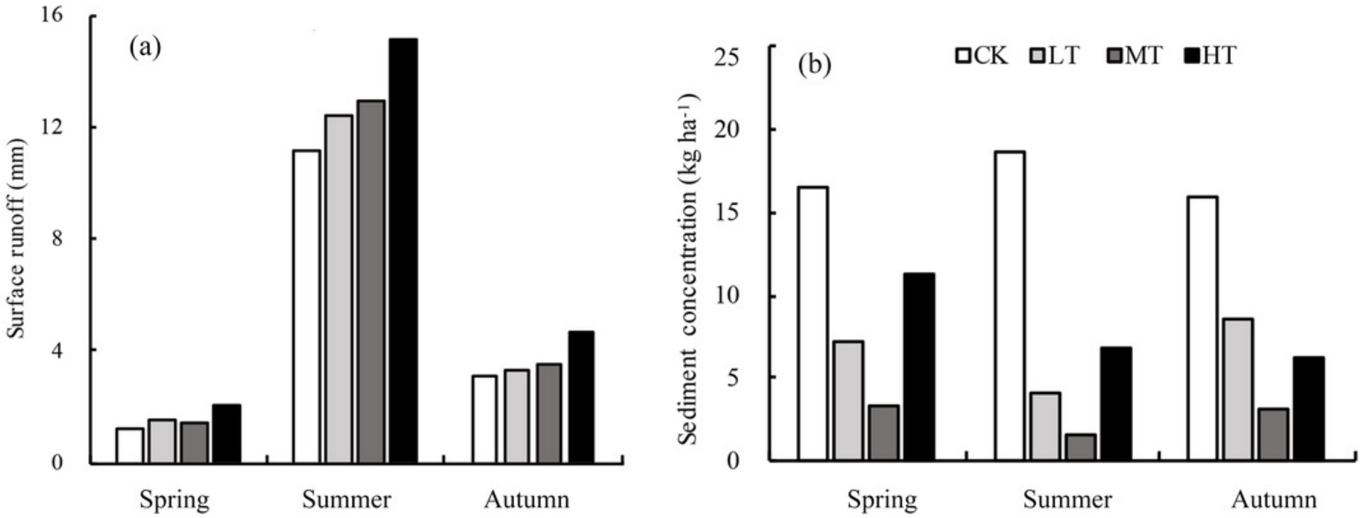

Table 1(on next page)

Table 1 Average characteristic measurements of experimental stands for density adjustment treatments in a 35-year-old *Larix principis-rupprechtii* plantation.

Standard errors of the mean are presented within parenthesis. Treatments: NFC: no thinning, control forest, LTF: low thinning forest, M: moderate thinning forest, H: heavy thinning forest. Density adjustments and measurements of characteristics were performed in July, 2012. Total phosphorus, bulk density, mechanical composition or bulk density of soil was measured in July of 2015 (means ± SD, n = 3)

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July of 2015 (means ± SD, n = 3)

Treatment	Stems (ha^{-1})	Thinning (%)	Slope Gradient(°)	Mean Height (m)	Mean DBH (cm)	Total soil phosphorus ($g\ kg^{-1}$)	Soil bulk density $g\ cm^{-3}$	Mechanical con < 0.002mm	0.002~0.0 m
CK	2173	0	25	14.5	13.3	0.50	0.91	20.83	30
	(±12)		(± 3.6)	(± 1.21)	(± 1.29)	(± 0.032)	(± 0.070)	(± 4.263)	(± 0.
LT	1834	15	25	19.3	14.9	0.51	0.87	22.07	29
	(±12)		(± 3.6)	(± 1.21)	(± 1.29)	(± 0.032)	(± 0.070)	(± 4.263)	(± 0.
MT	1418	30	23	16.6	16.3	0.60	0.95	18.27	31
	(± 7)		(± 0.5)	(± 0.21)	(± 0.02)	(± 0.071)	(± 0.024)	(± 2.117)	(± 2.
HT	1089	50	24	16.9	17	0.57	0.86	17.43	33
	(± 3)		(± 2.0)	(± 0.31)	(± 0.65)	(± 0.034)	(± 0.010)	(± 1.156)	(± 2.

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Table 2(on next page)

Table 2 The environmental factors of *L. principis-rupprechtii* plantation with different thinning treatments.

Soil respiration: carbon flux of soil respiration; PPFD total over: photosynthetic photon flux density over the forest, PPFD total under: photosynthetic photon flux density under the forest. The Soil respiration, soil temperature, soil moisture was measured in the vegetation growing seasons and the values were the means of 7 months from April to October. PPFD was measured in the summer seasons, July each year. Different superscripts indicate significant difference at $p< 0.05$ in thinning treatments, $n= 3$.

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Environmental factors	Year	CK	LT	MT	HT
Soil respiration (g C m^{-2})	2015	297.6 ± 22.1 a	280.43±31.97 a	391.1 ± 40.6 a	356.7 ± 33.6 a
	2016	421.6 ± 47.3 a	391.08±70.42 a	507.5 ± 55.4 a	438.8 ± 45.3 a
PPFD total under ($MJ·m^{-2}·d^{-1}$)	2015	4.6 ± 0.5c	5.9 ± 0.47b	6.5 ± 0.5 b	9.7 ± 0.5 a
	2016	4.8 ± 0.3 c	5.86 ± 0.21b	6.4 ± 1.0 b	8.1 ± 0.4 a
PPFD total over ($MJ·m^{-2}·d^{-1}$)	2015	27.9 ± 1.2	28.3 ± 1.23	28.4 ± 0.3	28.5 ± 0.6
	2016	28.87 ± 1.07a	29.25 ± 0.14a	29.5 ± 1.1 a	30.4 ± 1.2 a
Soil temperature（℃）	2015	6.1 ±0.1 b	6.3 ±0.3 b	7.0 ± 0.2 a	6.5 ± 0.2 ab
	2016	7.7 ± 0.4 a	7.7 ±1.2 a	8.5 ± 0.8 a	7.8 ± 0.6 a
Soil moisture（%）	2015	22.1 ± 0.8 b	24.2 ±3.3 ab	27.1 ± 1.9 ab	28.7 ± 2.1 a
	2016	22.7 ± 1.4 b	24.1 ± 2.9 ab	25.0 ± 2.2 ab	28.2 ± 1.2 a

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Table 3 (on next page)

Table 3 Biomass (t ha-1) of *L. principis-rupprechtii* plantation with different thinning treatments.

Values mean ± SD; different superscripts indicate significant difference at $p< 0.05$ in thinning treatments in thinning treatments; the biomass data of tree were surveyed in July 2014

Table 3 Biomass (t ha^{-1}) of *L. principis-rupprechtii* plantation with different thinning treatments. Values mean ± SD; different superscripts indicate significant difference at $p < 0.05$ in thinning treatments in thinning treatments; the biomass data of tree were surveyed in July 2014

Components	Treatments				
	CK	LT	MT	HT	Mean
Tree layer	189.58 ± 2.06 a	159.17 ± 7.59 b	144.98 ± 5.58 bc	135.55 ± 3.44 c	157.32 ± 23.60
Understory layer	2.24 ± 0.25 a	2.83 ± 0.42 a	5.56 ± 1.14 a	6.95 ± 1.57 a	4.40 ± 2.23
Litter layer	61.88 ± 10.53 a	57.71 ± 14.55 a	62.35 ± 14.49 a	60.84 ± 19.38 a	60.70 ± 2.09
Total	253.70 ± 8.72 a	219.70 ± 22.48 ab	212.90 ± 17.33 ab	203.33 ± 18.67 b	222.41 ± 21.92

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Table 4 (on next page)

Table 4. Four-way ANOVA analysis of soil carbon-containing components to years, soil depth, seasons and density (or thinning treatment).

TN (Soil total nitrogen), MBC (microbial biomass carbon), DOC (dissolved organic carbon), SOC (soil organic carbon), EOC (KMnO4 oxidizable carbon). * means a significant difference under $p < 0.05$, ** $p < 0.05$; ns means not static significant. For each components data was pooled from 360 independent samples e.g. Two sampling years*three seasons* four density treatments*five soil depths* three repetition.

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Table 4. Four-way ANOVA analysis of soil carbon-containing components to years, soil depth, seasons and density (or thinning treatment). TN (Soil total nitrogen), MBC (microbial biomass carbon), DOC (dissolved organic carbon), SOC (soil organic carbon), EOC (KMnO4 oxidizable carbon). * means a significant difference under $p < 0.05$, ** $p < 0.05$; ns means not static significant. For each components data was pooled from 360 independent samples e.g. Two sampling years*three seasons* four density treatments*five soil depths* three repetition.

	SOC	TN	LOC	C/N	MBC	DOC	PH	LOC/SOC	MBC/SOC	DOC/SOC
Year	ns	ns	**	ns	**	**	**	**	*	**
Season	**	**	**	**	**	**	**	**	**	**
Treatment	**	**	**	**	**	*	ns	**	**	**
Depth	**	**	**	ns	**	**	**	ns	**	**
Year * Treatment	ns	ns	**	ns	ns	**	ns	**	*	**
Year * Depth	ns	**	ns	**	ns	**	ns	ns	ns	**
Season * Treatment	ns	ns	ns	ns	**	**	ns	ns	**	**
Season * Depth	*	ns	**	ns	**	**	ns	**	**	*
Treatment * Depth	ns	ns	**	ns	**	**	ns	ns	*	ns
Year * Treatment * Depth	*	ns	ns	ns	ns	ns	**	ns	ns	ns
Season * Treatment * Depth	*	ns	ns	ns	**	*	**	ns	**	*