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Different responses in leaf-level physiology to competition and facilitation under different soil types and N fertilization

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Abstract Knowledge of how competition and facilitation affect photosynthetic traits and nitrogen metabolism contributes to understanding of plant-plant interaction mechanisms. We transplanted two larch species, *Larix kaempferi* and *L. olgensis*, to establish intra- and interspecific interaction experiments under different types of soil. Experiment 1: Two different soil types were selected, one from a *c.* twenty years old *L. kaempferi* plantation (named larch soil) and another from a secondary natural forest (named mixed forest soil). The experiment included three types of plant interactions (*L kaempferi* + *L. kaempferi*, *L. olgensis* + *L. olgensis*, and *L. kaempferi* + *L. olgensis*) and two soil types. Experiment 2: N fertilization was applied to larch soil. The experiment included the same three types of plant interactions as in Experiment 1 and two N treatments. The growth of *L kaempferi* was negatively affected by larch soil and accelerated by N fertilization, particularly under interspecific interaction. The effects of soil type combined with plant-plant interactions or N fertilization influenced the chlorophyll pigment content, net photosynthetic rate (*Pn*), photosynthetic N use efficiency (PNUE) and total non-structural carbohydrates of leaves (TNC). *Chl a*/*Chl b* (ratio of chlorophyll a to chlorophyll b) was higher when the growth of *L. kaempferi* was facilitated by the presence of *L. olgensis* in mixed forest soil. However, the ratio significantly declined when *L. kaempferi* confronted strong competition from *L. olgensis* in larch soil without N fertilization. Under N fertilization in larch soil, *Chl a*/*Chl b* of *L. olgensis* significantly increased by the presence of *L. kaempferi*. Plant-plant interactions and soil types affected the number of chloroplasts, especially in *L. kaempferi*, which had a greater number of chloroplasts under interspecific

interactions than in monoculture when growing in mixed forest soil. *L. olgensis* enhanced its ability to absorb N-NO_3^- under interspecific interactions in larch N- soil, while *L. kaempferi* enhanced its ability to absorb N-NH_4^+ under interspecific competition in mixed forest soil. Competition or facilitation modified the photosynthetic traits and nitrogen metabolism depending on the type of soil. Differences in these physiological processes contribute to divergent performance among individuals growing under interspecific or intraspecific competition, or in isolation.

Keywords: C and N coordination, forest plantation, plant-plant interactions, plant-soil feedback.

1. Introduction

Competition and facilitation are plant-plant interactions that are strongly influenced by resources. Facilitation indicates the positive impact of one plant on another through improving its recruitment, growth and survival (Sthultz et al., 2007). The magnitude or direction of net plant-plant interactions are largely determined by the absorption or exploration of soil resources (Boyden et al., 2005; Trinder et al., 2012; García-Cervigón et al., 2013; Loranger et al., 2017; Song et al., 2017), which are heterogeneously distributed in space. Soil properties (physical, chemical and microbial) also vary greatly among different environments. Changes or shifts between competition and facilitation among plants have been previously examined across abiotic stress gradients, but some of the results are contradictory (Bertness and Callaway, 1994; Boyden et al., 2005; Maestre et al., 2005; Sthultz et al., 2007; Biswas and Wagner, 2014; Guo et al., 2017). Most studies have been based on survival, growth rate, biomass production or nutrient absorption. It is still largely unknown, how competition or facilitation affects physiological characteristics related to photosynthesis and nitrogen metabolism in different soil conditions.

Niche complementarity has been proposed as an explanation for species coexistence and interactions. Interspecific differences in plant traits related to physiology (e.g. leaf nitrogen metabolism and photosynthesis) or morphology (e.g. root architecture) are often the basis to select species to reduce competition intensity or to sufficiently make

use of soil resources when constructing mixed-species plantations (reviewed by Richards et al., 2010). Cai et al. (2009) have revealed that in certain types of forests lianas fix more carbon and are more efficient in using water and nitrogen than trees, thus being better competitors (Schnitzer, 2005). However, few studies have examined whether tree species with a higher photosynthetic ability (carbon fixation) and nutrient efficiency (such as nitrogen) are expected to have superior competitiveness, especially when variation or shift between competition and facilitation are affected by differences in soil conditions.

Mixed-species plantations are known to have higher productivity and C sequestration than monocultures (Forrester et al., 2006; Kelty, 2006; Richards et al., 2010). Monoculture forest plantations always confront decreasing productivity during successive rotations, mainly due to a decline in soil qualities, including soil compaction, nutrient deficiencies or intraspecific allelopathy (reviewed by, e.g., Fox, 2000; O'Hehir and Nambiar, 2010, Xia et al., 2016). Partitioning in the uptake of different nutrition forms is one factor regulating the results of interactions (Ahmad-Ramli et al., 2013; Chen et al., 2014). Chen et al. (2014) have found that *Populus cathayana* females have a strong ability to absorb NH_4^+ and NO_3^- leading to a higher photosynthetic capacity and superior competitiveness compared to *Populus* males during competition under well-watered conditions. Absorbed NO_3^- can be converted into NH_4^+ by series of enzymes, including nitrate reductase (NR). The converted NH_4^+ and the absorbed NH_4^+ can be further converted into glutamine (Gln)

and glutamate (Glu) by glutamine synthetase (GS), glutamate synthase and glutamate dehydrogenase (GDH). Gln and Glu provide precursors for the biosynthesis of N-containing compounds, such as most amino acids (Forde and Lea, 2007) and photosynthetic-related compounds, particularly Rubisco and light-harvesting complexes (Zhu et al., 2008).

Larches are deciduous trees within the genus *Larix* with high economic and ecological value and they have a wide range of distribution across the Northern Hemisphere, including Siberia, northeastern China and Japan (Agathokleous et al., 2017). *Larix kaempferi* and *L. olgensis* are two important larch plantation species. Li et al. (2016) found that *L. kaempferi* has higher growth rates, net photosynthetic rates and total leaf non-structural carbohydrate contents, but a lower leaf NO_3^- concentration compared to *L. olgensis* in the absence of plant-plant interactions. We conducted two experiments to investigate the effects of intra- and interspecific interactions between *L. kaempferi* and *L. olgensis* under different soil conditions. Our previous results have suggested that the productivity of the two *Larix* species grown in mixture is greatly promoted under N fertilization (Guo et al., 2017). Most importantly, competition or facilitation of the two species responding to soil type and N fertilization showed great changes during the experimental period. Although significant increases in the contents of N and non-structural carbohydrates (NSC) were found under facilitation, there is still a need to further explore how competition or facilitation affect photosynthetic traits and nitrogen metabolism in different soil conditions. Interspecies differences in

physiology that result from plant-plant interactions compared to monoculture may be specific to soil conditions. Our main hypothesis was that growth rates, photosynthetic traits and nitrogen metabolism of one species are facilitated or inhibited by another depending on the soil conditions.

2. Methods and materials

Two experiments were conducted from late October 2013 to early September 2015. In Experiment 1, soils from a *L. kaempferi* plantation and from a natural secondary forest were selected. In this experiment, we focused on the performance of *L. kaempferi*: is the interspecific relationship of *L. kaempferi* with *L. olgensis* different when using *L. kaempferi* soil or soil from a secondary natural forest? In Experiment 2, N fertilization was applied to the larch soil. In this experiment, we studied the effects of inter- and intraspecific interactions on growth and ecophysiological responses under N fertilization in *L. kaempferi* soil.

2.1. Study region and experimental design

The study was conducted at the Qingyuan Experimental Station of the Forest Ecology, Institute of Applied Ecology, Chinese Academy of Sciences, Liaoning Province, Northeast China (41º51'N, 124º54'E). The mean annual rainfall is 811 mm (80% in summer). The mean annual air temperature varies between 3.9 ºC and 5.4 ºC (the coldest month of January averaging -12.1 ºC and the warmest month of July averaging 21.0 ºC) (Lu et al., 2018). The two selected types of soil were from a *c.* twenty years old *L. kaempferi* plantation and from a natural secondary forest, where *Quercus mongolica* and *Juglans mandshurica* are the dominant tree species. Hereafter, we call the two soils larch soil and mixed forest soil, respectively. The properties of the larch

soil were as follows: pH 5.65, C 18.61 g kg^{-1}, N 1.82 g kg^{-1}, while those of the mixed forest soil were as follows: pH 6.24, C 42.78 g kg^{-1}, N 3.89 g kg^{-1}. One-year old seedlings of the two species with approximately the same crown size and height were selected from a local nursery garden to be used as experimental materials.

2.2. Experiment 1: soil type

The intra- and interspecific plant-plant interactions were designed as follows: monoculture *L. kaempferi* + *L. kaempferi* and *L. olgensis* + *L. olgensis* representing intraspecific interactions, and mixed cultures *L. kaempferi* + *L. olgensis* representing interspecific interactions. In late October, 2013, two seedlings were planted in each plastic pot (external diameter and height 56 cm and 33 cm, respectively). The layout of Experiment 1 was two (larch soil and mixed forest soil) × three (*L kaempferi* + *L. kaempferi*, *L. olgensis* + *L. olgensis*, and *L. kaempferi* + *L. olgensis*).

2.3. Experiment 2: N fertilization

The Experiment 2 was performed only in the larch soil at the same time. Its layout was two (with and without N fertilization) × three (*L kaempferi* + *L. kaempferi*, *L. olgensis* + *L. olgensis*, and *L. kaempferi* + *L. olgensis*). Urea (46.3% N) was applied as N fertilization and added twice a year, in May and June (5.1 g each time) during years 2014 and 2015. More detailed information is available in Guo et al. (2017).

In addition, three individuals of each species were planted in isolation in each type of soil and in the fertilized soil at the same time. Based on our pervious results, *L. kaempferi* confronts strong competition from *L. olgensis* in the larch soil without N fertilization (in contrary, *L. olgensis* is facilitated by *L. kaempferi*), whereas it is facilitated significantly by *L. olgensis* in the mixed forest soil (in contrary, *L. olgensis* confronts strong competition from *L. kaempferi*. Guo et al. 2017).

2.4. Sampling and measurements

The final height and stem diameter of five replicates from each treatment were measured to calculate the growth rates in late August, 2015. The relative height and stem diameter growth rates were calculated as follows: relative growth rate = (Ln (final) - Ln (initial))/(t_2 - t_1), where the denominator is the time elapsed between the initial and final measurements.

The net photosynthetic rates (*Pn*) were measured by using a portable photosynthesis system (LI-6400; Li-Cor Inc., Lincoln, NE, USA) at the end of July and beginning of September, 2015. Healthy leaves were selected. From each treatment, four replicates were chosen randomly. The measurements were performed between 08:30 and 11:30 h under the following conditions: leaf temperature 25 °C, relative air humidity from 69% to 71%, photosynthetic photon flux density 1500 μmol $m^{-2}s^{-1}$ and ambient CO_2

concentration 350 ± 5 μmol mol^{-1}. After that, some leaves were immediately sampled and dried at 70 °C for 72 hours. The dried leaves were ground into powder, and leaf N concentration was determined by the semi-micro Kjeldahl method (Mitchell, 1998). Nitrogen concentration variation from July to September was calculated using the following formula: N concentration variation = [N $concentration_{Sep}$ - N $concentration_{Jul}$]×100%/ N $concentration_{Jul}$, where N $concentration_{Jul}$ and N $concentration_{Sep}$ represent leaf N concentration in late July and early September, respectively. Higher concentration variation indicates greater N absorption during this period.

Fresh leaves were sampled after *Pn* measurements in early September, 2015. For the measurement of chlorophyll pigments conducted according to the method of Lichtenthaler (1987), four replicates from each treatment were chosen. The total chlorophyll content (*Tchl*) was the sum of chlorophyll *a* and *b*. Three or four replicates of fresh leaves from each treatment were sampled to determine the activities of GS, GDH and NR by using ELISA kits (Shanghai Enzyme-linked Biotechnology Co., Ltd.).

Healthy leaves (1-2 mm in length) were used for transmission electron microscopy (TEM) observations by H-600IV TEM (Hitachi, Tokyo, Japan) in early September, 2015. The specific procedures followed the descriptions of Chen et al. (2014).

$NH_4{}^{15}NO_3$ and $^{15}NH_4NO_3$ were used to reveal nitrogen uptake in different types of soil. Three randomly chosen cuttings per treatment were supplied either with 60 mg $NH_4{}^{15}NO_3$ or 60 mg $^{15}NH_4NO_3$ per pot. Then, 72 h later, leaves from the upper parts of individuals were sampled from each treatment and dried at 70 °C for 72 hours. The ground powder was used for the analysis of the ^{15}N isotope composition ($\delta^{15}N$) by Isotope Ratio Mass Spectrometer (DELTA V Advantage, Thermo Fisher Scientific, Inc., Waltham, Massachusetts, USA). In addition, fine powder of leaf samples (six replicates from each treatment) was used to determine the carbon isotope composition ($\delta^{13}C$). The $^{13}C/^{12}C$ ratio was determined by Isotope Ratio Mass Spectrometer (DELTA V Advantage, Thermo Fisher Scientific, Inc., Waltham, Massachusetts, USA).

After all abovementioned measurements, individuals were harvested on the 4th of September, 2015. All harvested materials were dried at 70 °C for 72 hours. The dried leaves were ground into powder to determine non-structural carbohydrates (starch and soluble sugar) and hydrolysable amino acids of leaves. Total non-structural carbohydrates (TNC) were defined as the sum of starch and soluble sugars. The methods used to determine non-structural carbohydrates and amino acids followed those of Guo et al. (2017) and Chen et al. (2015), respectively.

2.5. Data analysis

Data were checked for normality and homogeneity of variances, and values were log-transformed when needed. Three-way analyses of variance (ANOVA) to determine the effects of factors in Experiment 1 and Experiment 2, respectively. After a significant interactive effect of factors was found, Tukey's *b* tests were conducted as post hoc tests to discover differences among treatments. All data were analyzed with the software Statistical Package for the Social Science (SPSS) version 20.0.

3. Results

3.1. Growth rates

The soil type, N fertilization and interactions significantly affected the growth rates of both species (Fig. 1). The larch soil showed a negative effect on the growth of *L. kaempferi*, particularly on the relative growth of stem diameter, compared to the effect of the mixed forest soil. N fertilization removed the negative effect and promoted the growth of *L. kaempferi* as well as that of *L. olgensis*. *L. olgensis* facilitated the stem growth of *L. kaempferi* in both mixed forest soil and in larch soil with N fertilization (larch soil N+; Fig. 1a).

3.2. Photosynthetic traits

The soil type (So) alone had no or only a marginally significant effect on *Chl a*, *Chl b*, *Caro*, *Thcl* and *Chl a*/*Chl b*. However, significant mutual effects between soil type and interactions (Int) were observed (Table 1). When the trees were grown in mixture, the *Chl a*/*Chl b* ratio of *L. kaempferi* was significantly higher in mixed forest soil than in larch soil without N fertilization (larch N- soil). N fertilization promoted the concentrations of *Chl a*, *Chl b* and *Thcl* in both species. In *L. kaempferi*, the *Chl a*/*Chl b* ratio was lower when grown in mixture compared to monoculture in larch soil without fertilization. In *L. olgensis*, the *Chl a*/*Chl b* ratio was significantly higher when grown in mixture compared to monoculture under N fertilization in larch soil. The soil type affected *Pn* and PNUE in late July and early September, 2015 (Fig.

2a-d). Under interspecific interactions in mixed forest soil, *L. kaempferi* exhibited a stronger net photosynthetic rate (*Pn*), while *Pn* and PNUE of *L. olgensis* declined (Fig. 2a, b).

The factor Int showed a significant effect on the non-structural carbohydrate concentration of leaves (Fig. 3). In both species, there was a tendency that leaf starch and TNC were higher under interspecific interactions than in monoculture in both larch N- soil and in mixed forest soil (Fig. 3a, c). Leaf TNC of *L. olgensis* was significantly higher under interspecific interactions than when grown in monoculture in the larch N+ soil (Fig. 3c).

Observations on the mesophyll cells showed that the two species possessed differences in chloroplasts (Fig. 4). The granum sizes of *L. kaempferi* chloroplasts were bigger and the granum numbers were greater than in *L. olgensis* in the same treatment. Plant-plant interactions and soil type affected the number of chloroplasts, especially in *L. kaempferi*. Its chloroplast numbers tended to be lower in mixture than in monoculture in larch N- soil (Fig. 4a, b). However, in mixed forest soil, the chloroplast number of *L. kaempferi* was greater in a mixture than in monoculture (Fig. 4i, j). The $\delta^{13}C$ value was affected only by the mutual effect between the plant-plant interaction and soil type (Fig. 5a).

3.3. N metabolism

NO_3^- was the main N form absorbed by the two species. In larch N- soil, *L. olgensis* enhanced its ability to absorb N-NO_3^- in mixture (Fig. 5b), while in mixed forest soil, *L. kaempferi* enhanced its ability to absorb N-NH_4^+ in mixture (Fig. 5c). Nitrogen fertilization influenced the leaf N concentration in both July and early September (Fig. 6a, b). Plant-plant interactions affected the leaf N concentration only early September when the N concentration tended to be higher in mixture than in monoculture in both species (Fig. 6b). Generally, the N concentration variation from late July to early September tended to be higher when the two *Larix* were grown in mixture (Fig. 6c). The activities of GDH and GS were not significantly affected by either factor (Fig. 7a, b). The activity of NR was significantly affected only by the mutual effect between species and plant-plant interactions (Fig. 7c). Our results indicated that none of the hydrolysable amino acids showed significant differences (Table 2). However, according to the results of the three-way ANOVA, the mutual effect between the soil type and plant-plant interactions marginally affected the concentrations of some amino acids, such as aspartate, glycine and cysteine (see supplementary Table S1). Concentrations of most amino acids were a little higher under interspecific interactions in both species compared with those under monoculture in the larch N-soil, whereas this tendency was opposite in mixed forest soil (Table 2).

4. Discussion

In our study, we found that photosynthetic traits and nitrogen metabolism characters were affected by competition or facilitation in different soil conditions.

4.1. Negative effects of larch soil

A given plant species can alter soil environment in a way that decreases its own growth rate relative to that of others, resulting in a negative feedback (Harrison and Bardgett, 2010; Xia et al., 2016). Plants release a wide range of molecules that play important roles in interactions between roots and soil organisms, such as amino acids, sugars and proteins (Haichar et al., 2014; Zhang et al., 2015). Our results indicated that the growth rates of *L. kaempferi* were negatively influenced by *L. kaempferi* soil without N fertilization (larch N- soil). A negative plant-soil feedback caused by changes in nutrient availability and microbial communities (Harrison and Bardgett, 2010) leads to changes in interspecific competition (Hendriks et al., 2015). Differences in chemical properties are one reason causing differences on the leaf level between the two types of soil (Miatto et al., 2016). In this study, the soil type was an important factor to drive differences in photosynthesis-related traits and in some nitrogen metabolism processes. For example, the effects of soil type on *Pn* and PNUE were remarkable in late July and early September. We found that the soil type influenced the uptake of NO_3^--N and NH_4^+-N, especially under the mutual effect with plant-plant interactions. The use of N fertilization is suggested to be an efficient forest management approach to solve the problem of declining productivity along successive

rotations of plantations (Fox, 2000; O'Hehir and Nambiar, 2010). In our study, nitrogen fertilization removed the negative effect, accelerated growth in *L. kaempferi* and promoted biomass production (Guo et al., 2017), by not only increasing soil N availability but also by altering microbial communities (Zechmeister-Boltenstern et al., 2011). We have found that nitrogen fertilization altered alpha diversity and composition of bacteria and fungi in the rhizosphere soil of the two species (data unpublished).

4.2. Effects of competition or facilitation

Previously, isolated *L. kaempferi* was thought to have superior physiological leaf nutrition traits, because it displayed higher *Pn*, PNUE and photosynthetic N use efficiency compared to isolated *L. olgensis* under N and P fertilization (Li et al., 2016). However, our study did not show such clear species differences, mainly due to the effects of intra- or interspecific interactions. Plants have to optimize performance in morphology and physiological activity when they share resources with their conspecific or heterospecific neighbors (Kozovits et al., 2005; Cai et al., 2009; Duan et al., 2014). Our results indicated that *Pn* of *L. kaempferi* was facilitated while that of *L. olgensis* was inhibited by interspecific interaction in late July in mixed forest soil. The effects of competition and facilitation on *Pn*, and PNUE are evidently complex dynamic processes. It has been shown that higher *Pn* (mass-based), PNUE and $\delta^{13}C$ values (higher $\delta^{13}C$ indicating higher integrated water-use efficiency) in lianas than in

trees throughout the year (especially during the drought season) confers a competitive advantage to lianas during the dry season and suggests that lianas capture resources more efficiently than trees (Cai et al., 2009).

The mass-based photosynthetic rate reflects the influence of the leaf structure on CO_2 uptake (Lichtenthaler et al., 2007). The proportion of cell wall constituents range between 18-70% of leaf dry mass and is typically associated with the fraction of leaf N invested in photosynthetic proteins (Onoda et al., 2017). Cell walls (CW) of *L. kaempferi* were thicker under N fertilization and affected by competition or facilitation, which may lead to differences in leaf morphology (e.g. increasing leaf weight).

Leaf chlorophyll pigments that are directly related to photosynthesis provide valuable information about the physiological status of plants (Lichtenthaler et al., 1981, 1982, 2007). The organization of the chlorophyll pigments and the relative levels of *Chl b* and *Tchl* as well as the ratio of *Chl a* to *Chl b* showed considerable differences among treatments. *Chl a/b* variation may be positively related with the number of chloroplasts in the mesophyll structures of leaves. For example, *Chl a/b* of *L. kaempferi* under interspecific interactions was significantly lower than that under monoculture in the larch N- soil. Leaves with higher *Chl a/b* seemed to have more chloroplasts. Significantly higher values of *Chl a/b* were found in sun leaves and in the sunlit leaves from the upper canopy level, characterized by a higher

light-saturation of photosynthesis and higher chloroplast numbers (see Lichtenthaler et al. 1981) compared to shade leaves and leaves from the lower canopy level (Lichtenthaler et al., 1981, 2007; Hölscher, 2004). In addition, higher *Chl a/b* indicated the presence of fewer light-harvesting chlorophyll proteins (LHCII) and probably a larger number of reaction center pigment proteins (Lichtenthaler et al., 1981, 1982).

Non-structural carbohydrates play a key role in physiological processes and are thought to reflect a balance between carbon supply (photosynthesis) and demand (such as growth and respiration) (Koch, 2004; Sala et al., 2012). Liu et al. (2004) have demonstrated that the total carbohydrate content (sugars and starch) of European beech (*Fagus sylvatica*) declined when it confronted strong competition with Norway spruce (*Picea abies*) when exposed to elevated CO_2. Species was the primary factor to affect leaf starch concentrations, being higher in *L. kaempferi* than in *L. olgensis*. The result was consistent with the leaf mesophyll structure: the number and size of starch grains were greater in *L. kaempferi* compared to *L. olgensis*. It implied that the two larch species may differ in starch synthesis within a chloroplast or in the transport of triose phosphate from a chloroplast to cytoplasm. Interspecific interactions greatly affected the non-structural carbohydrates of leaves, particularly in *L. olgensis*, which had higher leaf TNC when grown in mixture. However, the mechanism of how competition or facilitation modulates leaf TNC remains unresolved, because we did not accurately measure how much carbon was fixed and exported for growth,

respiration, defense and secretion.

The partitioning in nutrient uptake regulates competitive relationships (Ahmad-Ramli et al., 2013; Chen et al., 2014). We provided evidence that the two larch species grown together show slight differences in N-source absorption partitioning. Most NH_4^+ can be assimilated locally, while the rest is transported to leaves or other parts, but most absorbed NO_3^- is transported to leaves, where it is assimilated by a series of enzymes after uptake into roots (Black et al., 2002; Li et al., 2012; Xu et al., 2012). Luo et al. (2013) found that the activities of leaf NR and GS show no differences between fast and slowly growing species in response to different N availabilities. However, the uptake of NH_4^+ and NO_3^-, and transcript levels of most ammonium (AMTs) and nitrate (NRTs) transporter genes in leaves showed considerable changes. The isolated *L. kaempferi* has a higher growth rate than isolated *L. olgensis* (Yin et al., 2008; Li et al., 2016), while the growth rates of the two species also show differences caused by plant-plant interactions, as discovered in the experiments of the present study. However, the activities of leaf GDH, GS and NR showed no or only slight variation between different soil conditions.

Amino acids are used for protein biosynthesis and N storage (e.g. arginine and arginine-rich proteins). In our study, the concentrations of N and amino acids showed no significant changes among treatments. The probable reason was the dilution effect of biomass. Competitive responses are dynamic processes that change along with

nitrogen capture and biomass production (Trinder et al., 2012). Variation in leaf N concentrations tended to be higher when the two species were grown together compared to monoculture, which reflects their differences in the preferred N form or ability to absorb or store N resources when interacting with heterospecific and conspecific plants.

4.3. Carbon and nitrogen interactions

Plants must integrate external and internal signals to modify their growth. It has been reported that starch integrates the metabolic status and the total protein content, thus suggesting that the regulatory network that determines starch and protein contents contributes to the regulation of biomass production (Matt et al., 2001; Sulpice et al., 2009). Diverse conclusions have been made in studies involving plants grown in monoculture or mixture, for example, by Liu et al. (2004) and Kozovits et al. (2005). Knowledge about the performance of plants acquired from plants growing in monoculture (or in isolation) may not be transferred to plants grown under interspecific interactions (Kozovits et al., 2005). Our results on the growth and physiological traits of the two studied species growing under interaction conditions differed from those by Li et al. (2016), where experimental individuals were planted in isolation. This implies that the coordination or balance between carbon and nitrogen was influenced by competition or facilitation, which enabled interacting plants to optimize their competitive performance.

5. Conclusions

Plants integrate external and internal signals to regulate the balance of carbon and nitrogen, and to optimize their performance in different conditions. Our findings indicated that physiological processes are affected by competition or facilitation under different types of soil. Divergent responses and performance under intra- and interspecific interactions in varied conditions reflect plant adaptation.

Author Contribution Statement Qingxue Guo had the main responsibility for data collection, analysis and writing, Haifeng Song and Jieyu Kang had a significant contribution to data collection and analysis, Helena Korpelainen had a significant contribution to the interpretation of data and manuscript preparation, and Chunyang Li (the corresponding author) had the overall responsibility for experimental design and project management.

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Conflict of interest The authors declare that they have no conflicts of interest.

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Table 1 Leaf pigments of the two *Larix* species in early September, 2015. *Chl a*: chlorophyll *a*, *Chl b*: chlorophyll *b*, *Caro*: carotenoid, *Tchl*: chl *a* + chl *b*.

Species	Treatment	*Chl a* (mg·g^{-1}FW)	*Chl b* (mg·g^{-1}FW)	*Caro (*mg·g^{-1}FW)	*Tchl* (mg·g^{-1}FW)	*Chl a*: *Chl b*
L. kaempferi	Inter	0.64 ± 0.05**AB**	0.15 ± 0.01cd**BC**	0.03 ± 0.00ab**AB**	0.79 ± 0.07bc**ABC**	4.34 ± 0.07bc**C**
		0.66 ± 0.04AB	**0.10 ± 0.01C**	**0.03 ± 0.00AB**	**0.75 ± 0.05BC**	**6.94 ± 0.34A**
	Intra	0.50 ± 0.01**B**	0.09 ± 0.01e**C**	0.03 ± 0.00b**B**	0.59 ± 0.02d**C**	5.83 ± 0.39a**AB**
		0.60(0.03)B	**0.13 ± 0.01BC**	**0.03 ± 0.00AB**	**0.73 ±0.05C**	**4.51 ± 0.26BC**
	Inter-N	0.76 ± 0.04	0.18± 0.01bc	0.04 ± 0.01ab	0.94 ± 0.05ab	4.20 ± 0.13bc
	Intra-N	0.67 ± 0.03	0.15 ± 0.00cd	0.04 ± 0.00ab	0.81 ± 0.03bc	4.57 ± 0.17bc
L. olgensis	Inter	0.77 ± 0.02**A**	0.20 ± 0.01b**A**	0.04 ± 0.01ab**A**	0.97 ± 0.02ab**A**	3.83 ± 0.22cd**C**
		0.65(0.05)AB	**0.14 ± 0.02BC**	**0.03 ± 0.00AB**	**0.79 ± 0.07ABC**	**5.03 ± 0.47BC**
	Intra	0.61 ± 0.04**AB**	0.12 ± 0.01de**BC**	0.03 ± 0.00ab**AB**	0.73 ± 0.05c**C**	5.08 ± 0.32ab**BC**
		0.78(0.02)A	**0.17 ± 0.02AB**	**0.05 ± 0.00A**	**0.95 ± 0.03AB**	**4.65 ± 0.35BC**
	Inter-N	0.80 ± 0.02	0.17 ± 0.00bc	0.04 ± 0.00ab	0.98 ± 0.01ab	4.66 ± 0.20bc
	Intra-N	0.81 ± 0.08	0.28 ± 0.01a	0.05 ± 0.00a	1.09 ± 0.08a	2.95 ± 0.31d
	P: F$_{S}$	0.001 **0.001**	0.000 **0.000**	0.039 **0.018**	0.000 **0.000**	0.002 **0.003**
	P: F$_{Int}$	0.004 **0.041**	0.016 **0.091**	0.105 **0.440**	0.001 **0.040**	0.054 **0.937**
	P: F$_{N\ (So)}$	0.000 **0.147**	0.000 **0.542**	0.009 **0.227**	0.000 **0.329**	0.001 **0.033**
	P: F$_{S\times Int}$	0.518 **0.147**	0.000 **0.491**	0.387 **0.327**	0.144 **0.341**	0.003 **0.057**
	P: F$_{S\times N\ (\times So)}$	0.693 **0.584**	0.208 **0.770**	0.750 **0.927**	0.526 **0.612**	0.897 **0.582**
	P: F$_{Int\times N\ (\times So)}$	0.078 **0.002**	0.000 **0.000**	0.030 **0.002**	0.002 **0.000**	0.000 **0.000**
	P: F$_{S\times Int\times N\ (\times So)}$	0.301 **0.063**	0.000 **0.633**	0.238 **0.191**	0.102 **0.112**	0.014 **0.018**

Inter, interspecific interaction; Intra, intraspecific interaction; Inter-N, interspecific interaction under N fertilization; Intra-N, intraspecific interaction under N fertilization. F_S, species effect; F_{Int}, plant-plant interaction effect; $F_{N(So)}$, N fertilization or soil type effect; $F_{S\times Int}$, species × plant-plant interaction effect; $F_{S\times N(\times So)}$, N fertilization or soil type × species effect; $F_{Int\times N(\times So)}$, N fertilization or soil type × plant-plant interaction effect; $F_{S\times Int\times N(\times So)}$, N fertilization or soil type × species × plant-plant interaction. In Experiment 1 (two soil types), different capital letters in the same column indicate significant differences at $P < 0.05$ based on Tukey's *b* (values in bold). In Experiment 2 (N fertilization), values followed by different lowercase letters in the same column are significantly different at $P < 0.05$ based on Tukey's *b* analysis.

1 **Table 2** Amino acids concentrations of the two *Larix* species in each treatment in early September, 2015.

Amino acid	*L. kaempferi*				*L. olgensis*			
(g·100g^{-1}DW)	Inter	Intra	Inter-N	Intra-N	Inter	Intra	Inter-N	Intra-N
Aspartate	1.14 ± 0.08**A**	0.99 ± 0.08**A**	1.07 ± 0.11	1.11 ± 0.12	1.22 ± 0.10**A**	1.11 ± 0.00**A**	1.05 ± 0.13	1.11 ± 0.06
	1.07 ± 0.10A	**1.21 ± 0.06A**			**1.09 ± 0.04A**	**1.21 ± 0.08A**		
Threonine	0.58 ± 0.04	0.49 ± 0.04	0.55 ± 0.05	0.57 ± 0.06	0.61 ± 0.05	0.57 ± 0.00	0.54 ± 0.07	0.56 ± 0.03
	0.55 ± 0.04	**0.57 ± 0.03**			**0.58 ± 0.03**	**0.60 ±0.02**		
Serine	0.55 ± 0.04	0.46 ± 0.03	0.51 ± 0.05	0.53 ± 0.06	0.55 ± 0.05	0.53 ± 0.00	0.50 ± 0.06	0.52 ± 0.03
	0.48 ± 0.04	**0.51 ± 0.02**			**0.51 ± 0.02**	**0.54 ± 0.03**		
Glutamate	1.29 ± 0.10	1.09 ± 0.08	1.20 ± 0.12	1.26 ± 0.14	1.33 ± 0.11	1.32 ± 0.03	1.21 ± 0.15	1.25 ± 0.07
	1.20 ± 0.08	**1.28 ± 0.06**			**1.31 ± 0.05**	**1.29 ± 0.05**		
Glycine	0.69 ± 0.06**A**	0.60 ± 0.05**A**	0.64 ± 0.06	0.67 ± 0.08	0.74 ± 0.06**A**	0.66 ± 0.01**A**	0.64 ± 0.08	0.66 ± 0.04
	0.65 ± 0.06A	**0.73 ± 0.03A**			**0.68 ± 0.03 A**	**0.72 ± 0.05A**		
Alanine	0.79 ± 0.06	0.66 ± 0.05	0.73 ± 0.07	0.76 ± 0.09	0.81 ± 0.07	0.80 ± 0.02	0.73 ± 0.09	0.75 ± 0.04
	0.75 ± 0.06	**0.78 ± 0.04**			**0.82 ± 0.04**	**0.80 ± 0.03**		
Cysteine	0.05 ± 0.01a**A**	0.04 ± 0.01a**A**	0.04 ± 0.01a	0.04 ± 0.01a	0.05 ± 0.01a**A**	0.04 ± 0.00a**A**	0.03 ± 0.00a	0.04 ± 0.00a
	0.03 ± 0.01A	**0.04 ± 0.00A**			**0.03 ± 0.01A**	**0.04 ± 0.01A**		
Valine	0.74 ± 0.06	0.64 ± 0.05	0.69 ± 0.07	0.73 ± 0.08	0.80 ± 0.06	0.74 ± 0.01	0.69 ± 0.09	0.72 ± 0.04
	0.72 ± 0.05	**0.80 ± 0.04**			**0.76 ± 0.02**	**0.78 ± 0.04**		
Methionine	0.17 ± 0.04**A**	0.07 ± 0.01**A**	0.10 ± 0.02	0.08 ± 0.01	0.10 ± 0.02**A**	0.10 ± 0.04**A**	0.06 ± 0.01	0.06 ± 0.00
	0.07 ± 0.02A	**0.07 ± 0.00A**			**0.05 ± 0.01A**	**0.11 ± 0.04A**		
Isoleucine	0.61 ± 0.05**A**	0.53 ± 0.04**A**	0.56 ± 0.05	0.59 ± 0.06	0.66 ± 0.05**A**	0.58 ± 0.00**A**	0.56 ± 0.07	0.57 ± 0.03
	0.59 ± 0.06A	**0.65 ± 0.03A**			**0.60 ± 0.02A**	**0.63 ± 0.04A**		
Leucine	1.16 ± 0.09	0.98 ± 0.08	1.08 ± 0.11	1.12 ± 0.13	1.22 ± 0.10	1.16 ± 0.02	1.07 ± 0.14	1.10 ± 0.06
	1.09 ± 0.08	**1.14 ± 0.05**			**1.19 ± 0.04**	**1.16 ± 0.04**		
Tyrosine	0.45 ± 0.04**A**	0.36 ± 0.04**A**	0.40 ± 0.05	0.42 ± 0.06	0.49 ± 0.05**A**	0.43 ± 0.01**A**	0.37 ± 0.04	0.40 ± 0.02

	0.39 ± 0.03A	**0.45 ± 0.02A**			**0.45 ± 0.02A**	**0.46 ± 0.03A**		
Phenylalanine	0.73 ± 0.06	0.62 ± 0.05	0.69 ± 0.07	0.70 ± 0.08	0.78 ± 0.07	0.72 ± 0.01	0.67 ± 0.08	0.70 ± 0.03
	0.69 ± 0.05	**0.74 ± 0.03**			**0.75 ± 0.03**	**0.74 ± 0.03**		
Lysine	0.57 ± 0.04**A**	0.55 ± 0.03**A**	0.56 ± 0.07	0.62 ± 0.07	0.65 ± 0.05**A**	0.58 ± 0.03**A**	0.58 ± 0.07	0.58 ± 0.05
	0.62 ± 0.06A	**0.71 ± 0.04A**			**0.58 ± 0.02A**	**0.58 ± 0.03A**		
NH_4^+	0.16 ± 0.01	0.13 ± 0.01	0.14 ± 0.01	0.15 ± 0.01	0.16 ± 0.01	0.15 ± 0.00	0.14 ± 0.01	0.16 ± 0.02
	0.14 ± 0.01	**0.16 ± 0.01**			**0.15 ± 0.00**	**0.15 ± 0.01**		
Histidine	0.29 ± 0.02	0.25 ± 0.02	0.27 ± 0.03	0.28 ± 0.03	0.30 ± 0.02	0.29 ± 0.00	0.27 ± 0.03	0.28 ± 0.02
	0.29 ± 0.02	**0.29 ± 0.01**			**0.31 ± 0.01**	**0.32 ± 0.02**		
Arginine	0.77 ± 0.07	0.64 ± 0.05	0.74 ± 0.08	0.82 ± 0.15	0.80 ± 0.07	0.77 ± 0.01	0.74 ± 0.10	0.81± 0.04
	0.71 ± 0.05	**0.75 ± 0.04**			**0.76 ± 0.03**	**0.81 ± 0.05**		
Proline	0.62 ± 0.04	0.53 ± 0.04	0.59 ± 0.06	0.60 ± 0.07	0.65 ± 0.05	0.67 ± 0.04	0.59 ± 0.07	0.58 ± 0.04
	0.61 ± 0.06	**0.58 ± 0.03**			**0.71 ± 0.06**	**0.57 ± 0.02**		
Total amino acid	11.36 ± 0.87	9.64 ± 0.74	10.56 ± 1.07	11.04 ± 1.28	11.93 ± 1.01	11.21 ± 0.12	10.42 ± 1.29	10.87 ± 0.57
	10.67 ± 0.80	**11.46 ± 0.54**			**11.33 ± 0.41**	**11.49 ± 0.51**		

Inter, interspecific interaction; Intra, intraspecific interaction; Inter-N, interspecific interaction under N fertilization; Intra-N, intraspecific interaction under N fertilization. Tukey's *b* tests were conducted as post hoc tests to discover differences among treatments, when a significant factor interaction was observed after ANOVA analysis. Values in bold indicate results among treatments between the two soil types (Experiment 1); same capital letters indicate no significant difference.

Figure legends

Figure 1 Relative growth rates as diameter (a) and stem height (b) in two *Larix* species. Inter and Intra refer to inter- and intraspecific interaction, respectively; Inter-N and Intra-N refer to inter- and intraspecific interaction under N fertilization, respectively. N: N fertilization effect, S: species effect; Int: plant-plant interaction effect, N×S: N fertilization × species effect, N×Int: N fertilization × plant-plant interaction effect, S×Int: species × plant-plant interaction effect, N×S×Int: N fertilization × species × plant-plant interaction effect, So: soil type effect, S×So: species × soil type effect, S×So×Int: species × soil type × plant-plant interaction. In Experiment 1 (two soil types), different capital letters indicate significant differences at $P < 0.05$. The red frame indicates treatments in the mixed forest soil. In Experiment 2 (N fertilization), values followed by different lowercase letters are significantly different at $P < 0.05$. Black and white bars indicate *L. kaempferi* and *L. olegensis*, respectively.

Figure 2 Net photosynthetic rate *Pn* (a and c) and photosynthetic nitrogen use efficiency (PNUE, b and d) in two *Larix* species in late July and early September. Detailed information of symbols shown in **Fig. 1**.

Figure 3 Non-structural carbohydrates of leaves including starch, soluble sugars and total non-structural carbohydrates (TNC) in two *Larix* species in early September, 2015. (a) Leaf starch concentration, (b) leaf soluble sugar concentration, (c) leaf TNC.

Detailed information of symbols shown in **Fig. 1**.

Figure 4 Ultrastructure of mesophyll cells in two *Larix* species in early September, 2015. (a) - (d): *L. kaempferi* in larch soil, (a): Inter, (b): Intra, (c): Inter-N, (d): Intra-N; (e) - (h): *L. olegensis* in larch soil, (e): Inter, (f): Intra, (g): Inter-N, (h): Intra-N; (i) - (j): *L. kaempferi* in mixed forest soil, (i): Inter, (j): Intra, (k) - (l): *L. olegensis* in mixed forest soil, (k): Inter, (l): Intra. The bar indicates 1 μm (c). CW: cell wall, Ch: chloroplast, SG: starch granum. Detailed information of symbols shown in **Fig. 1**.

Figure 5 $\delta^{13}C$ (a), $^{15}NO_3^-$-N (b) and $^{15}NH_4^+$-N (c) of two *Larix* species in early September, 2015. Detailed information of symbols shown in **Fig. 1**.

Figure 6 Leaf nitrogen concentrations in two *Larix* species at the end of July (a) and in early September (b), and variation in leaf nitrogen concentration from late July to early September in 2015 (c). Calculations of nitrogen concentrations explained in the Methods and materials section. Detailed information of symbols shown in **Fig. 1**.

Figure 7 Glutamate dehydrogenase (GDH) (a), glutamine synthetase (GS) (b) and nitrate reductase (NR) concentrations (c) in two *Larix* species in early September, 2015. GDH: Glutamate dehydrogenase, GS: glutamine synthetase, NR: nitrate reductase. Detailed information of symbols shown in **Fig. 1**.

Figure 1

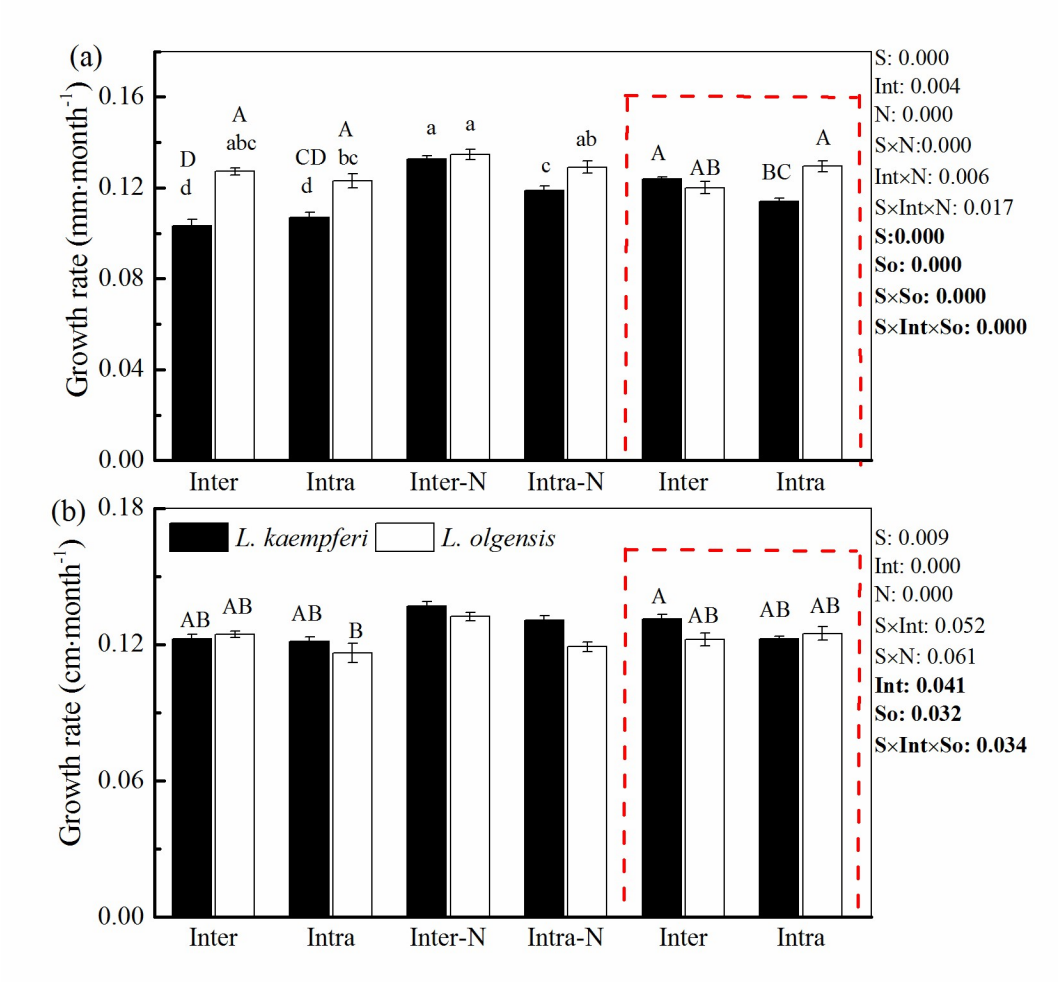

(a)
Growth rate (mm·month-1)
0.16
0.12
0.08
0.04
0.00
D
d
A
abc
CD
d
A
bc
a
a
c
ab
A
AB
BC
A
Inter
Intra
Inter-N
Intra-N
Inter
Intra
S: 0.000
Int: 0.004
N: 0.000
S×N:0.000
Int×N: 0.006
S×Int×N: 0.017
S:0.000
So: 0.000
S×So: 0.000
S×Int×So: 0.000
(b)
Growth rate (cm·month-1)
0.18
0.12
0.06
0.00
L. kaempferi
L. olgensis
AB
AB
AB
B
A
AB
AB
AB
Inter
Intra
Inter-N
Intra-N
Inter
Intra
S: 0.009
Int: 0.000
N: 0.000
S×Int: 0.052
S×N: 0.061
Int: 0.041
So: 0.032
S×Int×So: 0.034

Figure 2

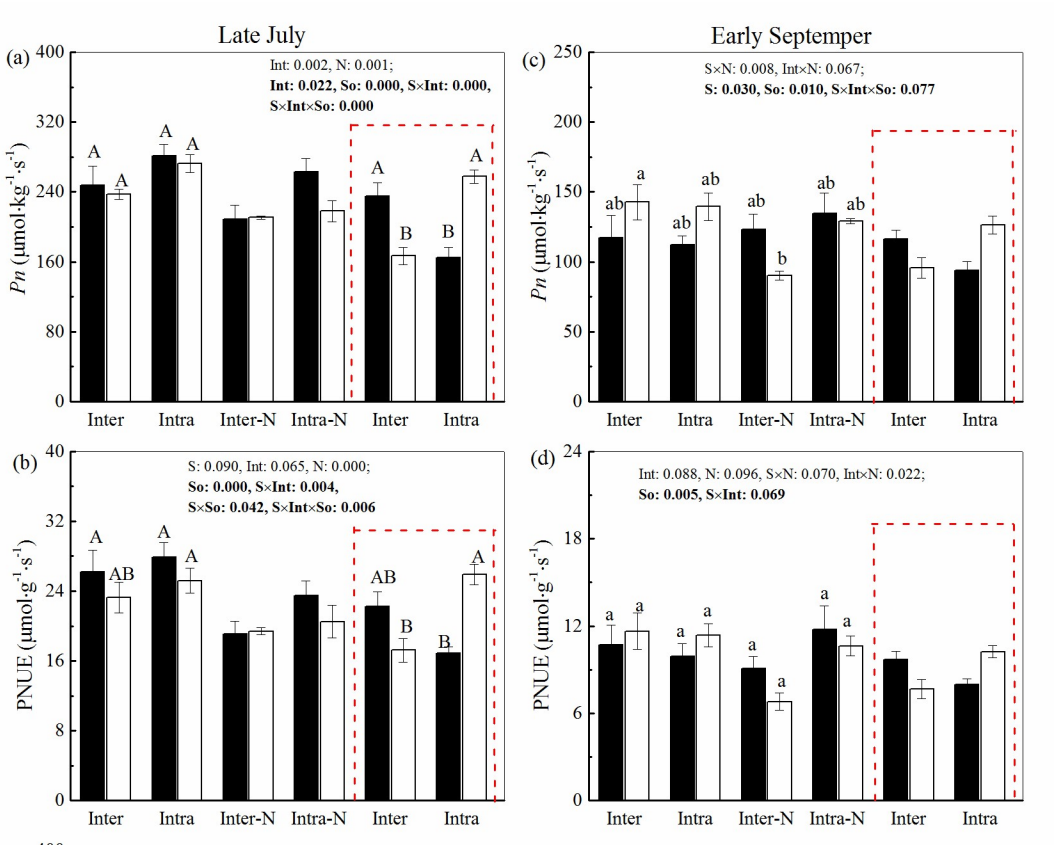

Late July
Early Septemper
(a)
Int: 0.002, N: 0.001;
Int: 0.022, So: 0.000, S×Int: 0.000,
S×Int×So: 0.000
Pn (μmol·kg-1·s-1)
(c)
S×N: 0.008, Int×N: 0.067;
S: 0.030, So: 0.010, S×Int×So: 0.077
Pn (μmol·kg-1·s-1)
(b)
S: 0.090, Int: 0.065, N: 0.000;
So: 0.000, S×Int: 0.004,
S×So: 0.042, S×Int×So: 0.006
PNUE (μmol·g-1·s-1)
(d)
Int: 0.088, N: 0.096, S×N: 0.070, Int×N: 0.022;
So: 0.005, S×Int: 0.069
PNUE (μmol·g-1·s-1)
Inter
Intra
Inter-N
Intra-N

Figure 3

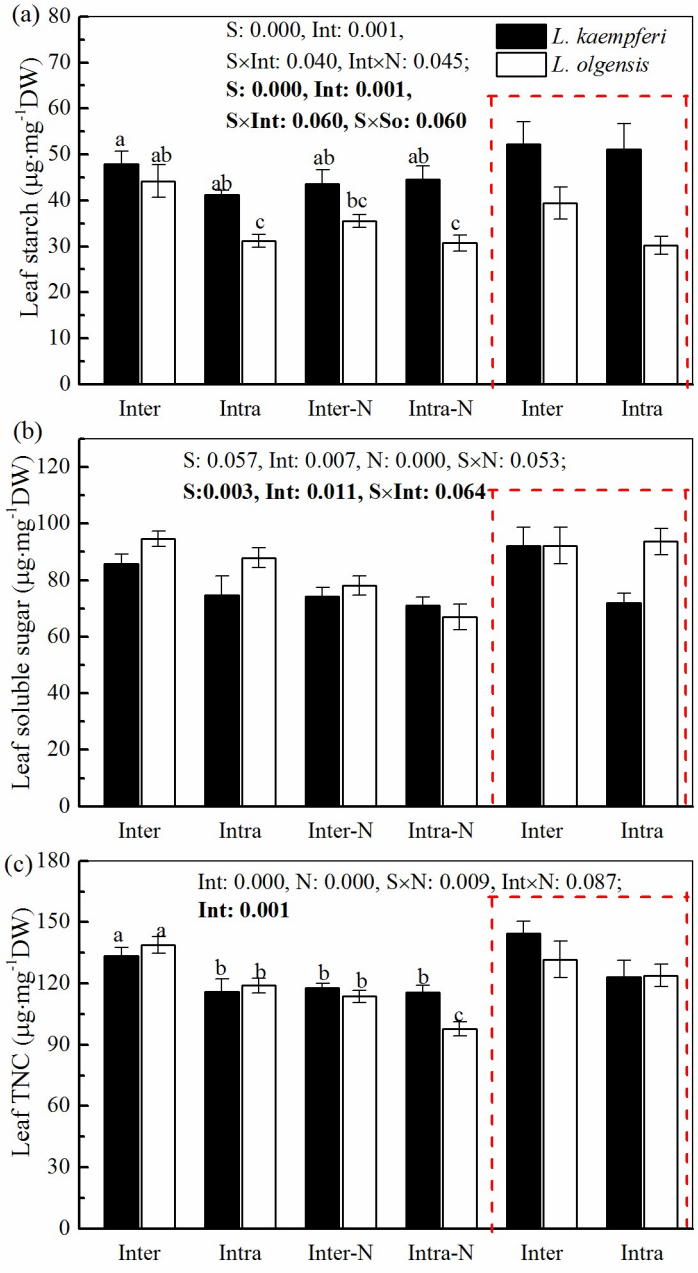

(a)
S: 0.000, Int: 0.001,
S×Int: 0.040, Int×N: 0.045;
S: 0.000, Int: 0.001,
S×Int: 0.060, S×So: 0.060
L. kaempferi
L. olgensis
Leaf starch (μg·mg-1DW)
(b)
S: 0.057, Int: 0.007, N: 0.000, S×N: 0.053;
S:0.003, Int: 0.011, S×Int: 0.064
Leaf soluble sugar (μg·mg-1DW)
(c)
Int: 0.000, N: 0.000, S×N: 0.009, Int×N: 0.087;
Int: 0.001
Leaf TNC (μg·mg-1DW)
Inter
Intra
Inter-N
Intra-N

Figure 4

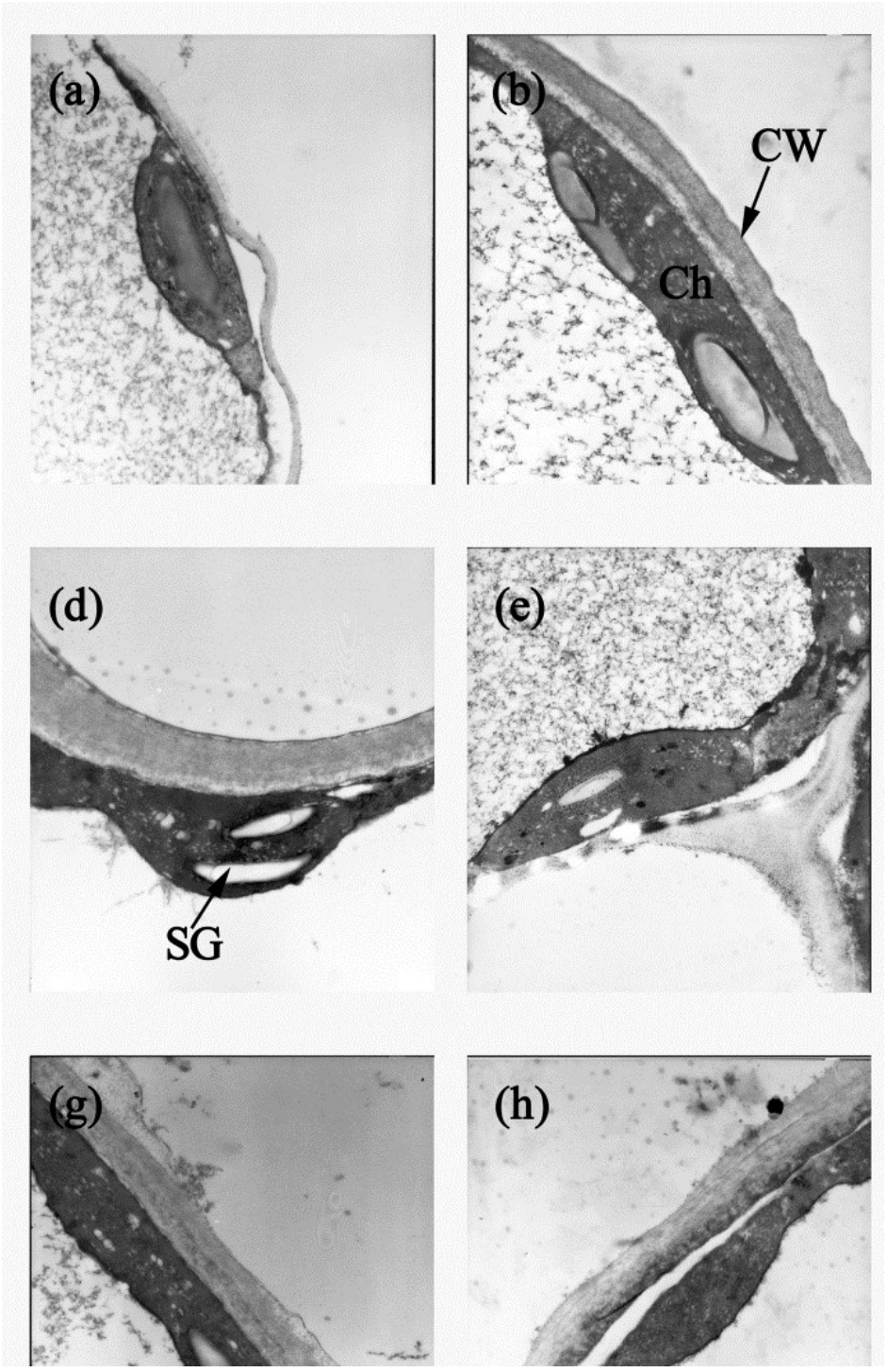


Figure 5

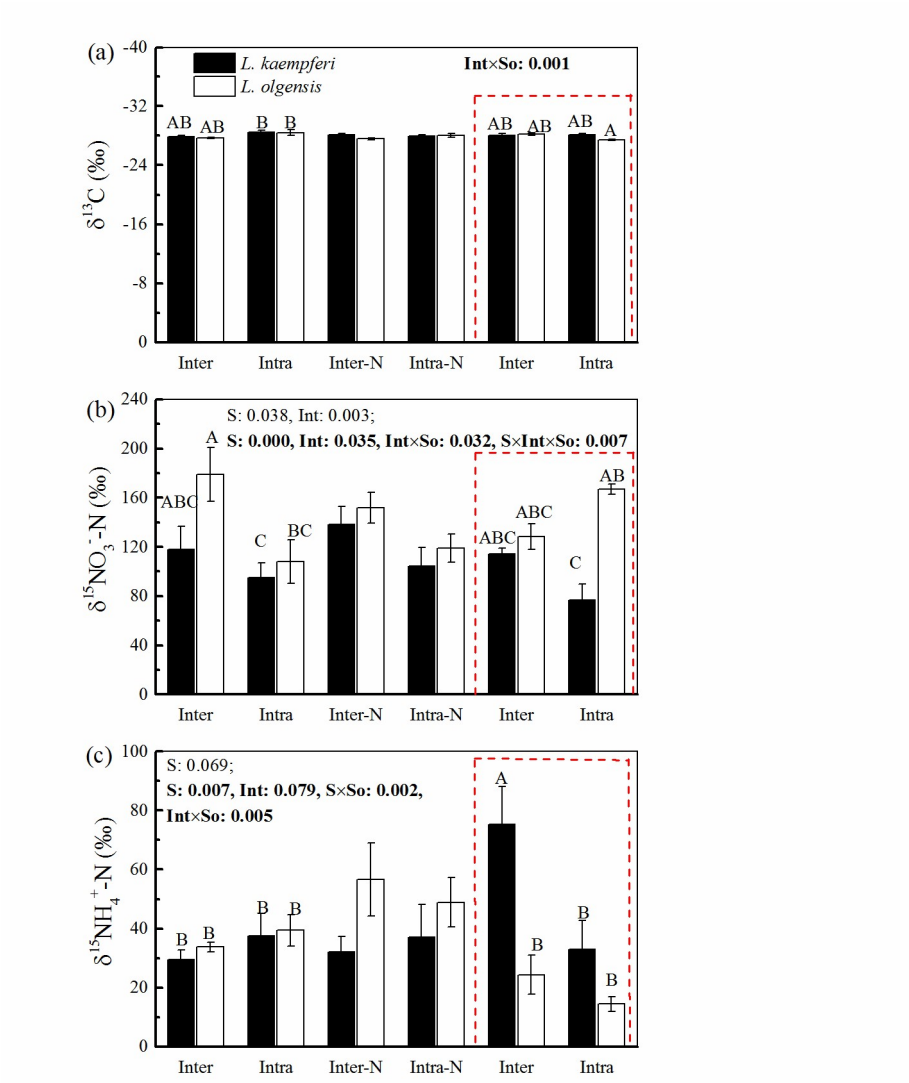

(a)
L. kaempferi
L. olgensis
Int×So: 0.001
δ13C (‰)
-40
-32
-24
-16
-8
0
AB AB B B AB AB AB A
Inter Intra Inter-N Intra-N Inter Intra
(b)
S: 0.038, Int: 0.003;
S: 0.000, Int: 0.035, Int×So: 0.032, S×Int×So: 0.007
δ15NO3--N (‰)
240
200
160
120
80
40
0
ABC A C BC ABC ABC C AB
Inter Intra Inter-N Intra-N Inter Intra
(c)
S: 0.069;
S: 0.007, Int: 0.079, S×So: 0.002,
Int×So: 0.005
δ15NH4+-N (‰)
100
80
60
40
20
0
B B B B A B B B
Inter Intra Inter-N Intra-N Inter Intra

Figure 6

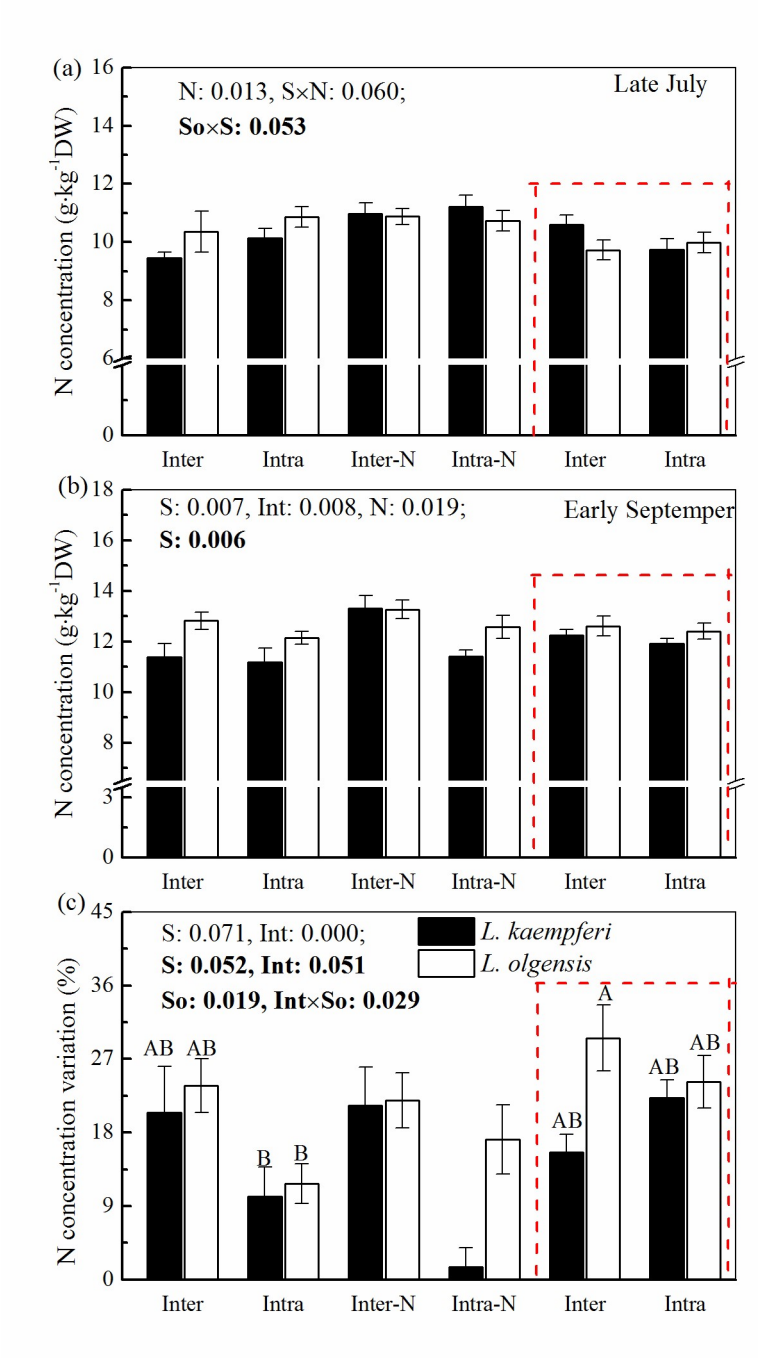
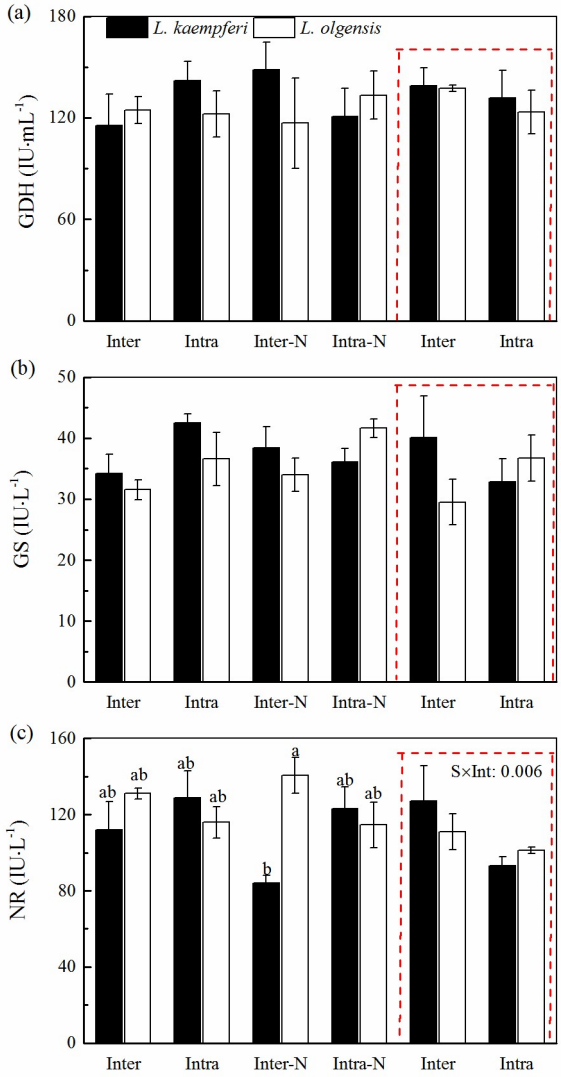

(a)
N: 0.013, S×N: 0.060;
So×S: 0.053
Late July
N concentration (g·kg-1DW)
(b)
S: 0.007, Int: 0.008, N: 0.019;
S: 0.006
Early Septemper
N concentration (g·kg-1DW)
(c)
S: 0.071, Int: 0.000;
S: 0.052, Int: 0.051
So: 0.019, Int×So: 0.029
L. kaempferi
L. olgensis
N concentration variation (%)
AB AB
B B
AB
A
AB AB
Inter
Intra
Inter-N
Intra-N
Inter
Intra

Figure 7


(a)
L. kaempferi
L. olgensis
GDH (IU·mL-1)
180
120
60
0
Inter
Intra
Inter-N
Intra-N
Inter
Intra
(b)
GS (IU·L-1)
50
40
30
20
10
0
(c)
NR (IU·L-1)
160
120
80
40
S×Int: 0.006
ab
a
b