



Impacts of competition and nitrogen addition on plant stoichiometry and non-structural carbohydrates in two larch species

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Received: 13 March 2020 / Accepted: 17 July 2020 / Published online: 24 October 2020
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Abstract Previous research has shown that competition between plants can have differential effects on leaf stoichiometry and non-structural carbohydrate (NSC) in different environments. However, little attention has been given to understanding these effects on non-photosynthetic organs, particularly of deciduous tree species. Here we assess the impact of competition on below and aboveground biomass, stoichiometry, nutrient composition and NSC in pure and mixed forests of two Larch species, *Larix kaempferi* and *L. olgensis* under nitrogen (N) addition. Nitrogen enrichment did not result in stronger intraspecific competition for both species and *L. olgensis* benefited from the presence of *L. kaempferi* under different N levels. Stems kept relatively stable C/N compared to roots and branches in response to competition, while N addition imposed stronger impacts on

N/P of different organs rather than competition. In contrast to stable C concentrations, starch and soluble sugar concentrations were more easily impacted by competition and the addition of nitrogen. Competition forced *L. kaempferi* and *L. olgensis* to allocate more carbon into storage by increasing their starch concentration and starch/soluble sugar of stems under competition. However, no significant differences in stoichiometry and NSC concentration between intra- and interspecific competition were found. NSC and nutrient pools of *L. kaempferi* stems, branches and coarse roots consistently declined due to competition regardless of N addition. Coarse and fine roots of *L. kaempferi* accumulated more N when in competition with *L. olgensis* than with a conspecific neighbor under N addition. Our results show that NSC was more sensitive to competition relative to stoichiometric traits (N and P) of non-photosynthetic organs.

Project funding: This work was supported by Zhejiang Provincial Natural Science Foundation of China (LQ18C030003, LQ18C160004) and Starting Research Fund from Hangzhou Normal University (2018QDL006).

The online version is available at <http://www.springerlink.com>

Corresponding editor: Yanbo Hu

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s11676-020-01236-1>) contains supplementary material, which is available to authorized users.

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Keywords Intraspecific and interspecific competition · Non-photosynthetic organs · Resource competition · Remobilization and storage · Mixed forest plantations

Introduction

Competition is an important biotic factor in impacting elemental concentrations of nitrogen and phosphorus and physiological processes in carbon allocation or phytohormone levels in different environments (Hommel et al. 2016; Guo et al. 2020). Competitive intensity or outcome relies on neighbor identity and environment conditions, for example, soil nitrogen (N) availability (Guiz et al. 2018; Wang et al. 2019; Jin et al. 2020). Yu et al. (2017) demonstrated that Faber's fir [*Abies fabri* (Mast.) Craib] had higher leaf N and phosphorus (P) concentrations under interspecific competition than intraspecific competition in soil collected from

an *A. fabri*-dominated community. The opposite was found when *A. fabri* was grown in soil collected from a community dominated by Sargent's spruce [*Picea brachytyla* (Franch.) E. Pritz]. Therefore, plant species have some degree of flexibility to alter their stoichiometric ratios in response to changes in neighbor identity and/or in environmental conditions, such as with the addition of nitrogen.

Nitrogen addition results in significant changes in plant stoichiometry (e.g. Jing et al. 2017; Song et al. 2017). In most studies, leaves are usually selected to study plant stoichiometry because, for example, increasing leaf C/N ratios are not associated with the dilution of N due to the biomass accumulation of C-rich organs (e.g. stems) (Sardans et al. 2015; Guiz et al. 2018). With regards to responses of leaf stoichiometry to intraspecific and interspecific competition, research has shown that plants with lower leaf C/N ratios showed superior competition capacity relative to neighboring plants (Sardans et al. 2015; Song et al. 2017; Broadbent et al. 2018). Non-photosynthetic organs have different nutrient requirements relative to leaves due to the different functions they perform (Fortunel et al. 2012; Zhao et al. 2019), thus their stoichiometry may also be expected to differ in response to competition by conspecific and heterospecific neighbors. Differences in N and P concentrations, as well as their ratios in different plant tissues are a consequence of metabolic, morphological and physiological functions (Peñuelas et al. 2010). However, little attention has been given to understanding these responses by non-photosynthetic plant organs, especially between co-existing heterospecific species.

The status of non-structural carbohydrates (NSC, the sum of starch and soluble sugars) reflects the balance between photosynthesis and carbon demand (growth, defense, respiration) and is impacted by soil N availability (Koch 2004; Guo et al. 2016). Soluble sugars provide the building blocks needed for growth, while starch functions mainly as a carbon reserve that can be broken down into sugars through glycolysis (Koch 2004; Simard et al. 2013; Guo et al. 2016). Andersen et al. (2001) found that soluble sugar concentrations of ponderosa pine (*Pinus ponderosa* Douglas ex C. Lawson) were significantly reduced and became more susceptible to ozone when in competition with grasses. This implies that competition and environment or their interactions may affect the NSC status. Research also shows that NSC is affected by different N levels in the soil, and the increase in N in tissues results in low starch levels (Druege et al. 2004; Hermans et al. 2006). Therefore, differences in nitrogen concentrations caused by intra- and interspecific competition may change the NSC status of different plant organs.

Recent research has shown that the interaction between nutrient concentrations and biomass, which represents the nutrient pool size and NSC, are associated with plant growth

(Druege et al. 2004; Li et al. 2018a). Lower levels of plant tissue NSC and nutrient pools are assumed to indicate carbon and nutrient limitations for the growth needed (Walker et al. 2017; Li et al. 2018a), and this can be exacerbated by increasing competition for resources. In order to prioritize the ability to capture soil resources, competition may force plants to allocate more carbon to develop their root systems. If one plant competing with its neighbor allocates more carbon to root growth, it would likely suppress the growth of its neighbor, causing the neighbor to produce relatively fewer roots (Wang et al. 2010; Broadbent et al. 2018). In this study, we explore how the addition of N and type of competition (intraspecific and interspecific), as well as their interactive effects, influences stoichiometry, NSC traits and nutrient pools in two Larch species: *Larix kaempferi* (Lamb.) Carr. and *L. olgensis* A. Henry.

Plant competition relationships would be much stronger in a productive environment than in a relatively resource-limited surrounding (Bertness and Callaway 1994). Kunstler et al. (2016) demonstrated that competition among conspecific neighbors for resources is higher than with heterospecific species and is intensified by the density of neighbors and available resources. In the present study, we hypothesized that competition from conspecific neighbors would be stronger than from heterospecific neighbors under N addition. Stoichiometry, NSC traits and pool sizes of nutrients and NSC of different plant tissues were explored to determine whether growing with a neighbor would impact nutrient and NSC distribution. It was also hypothesized that plants that were more suppressed were more likely to have smaller nutrient pools due to the competition pressure.

Materials and methods

This study was conducted at the Qingyuan Experimental Station of the Forest Ecology, Institute of Applied Ecology, Chinese Academy of Sciences, Liaoning Province, Northeast China (41°51' N, 124°54' E). The soil was homogenized before planting and properties were: pH 5.6, C 18.6 g kg⁻¹, N 1.8 g kg⁻¹. All seedlings were planted in late October 2013.

The selected *Larix* species are two important species used for monoculture and mixed plantations (Zhu et al. 2011; Li et al. 2018a, b). One-year seedlings *Larix kaempferi* and *Larix olgensis* with similar crown size and height were grown in plastic 56-cm diameter, 33-cm tall pots. There were two N concentrations and three competition levels (Fig. 1). Nitrogen levels were prepared using urea (46.3% N) (Zhu et al. 2011), applied at 5.1 g twice a year in May and June during 2014 and 2015. Two seedlings reflecting intra- and interspecific competition were planted 10 cm apart in each plastic pot.

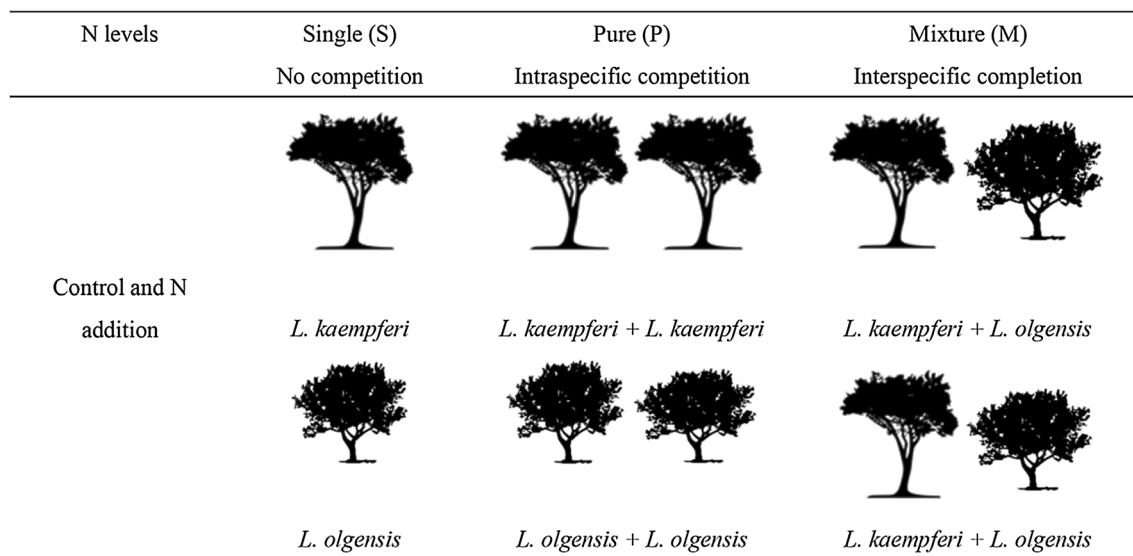


Fig. 1 Experimental design of the study investigating the treatment of *Larix kaempferi* and *L. olgensis* to two N levels under three competition states; S: single-grown plant; P: intraspecific competition; M:

interspecific competition; SN: single-grown plant with N addition; PN: intraspecific competition under N addition; MN: interspecific competition under N addition

Harvest and measurements

The plants were harvested August 29, 2014 and September 4, 2015. During the first harvest, roots were not divided into coarse and fine roots, and branches were not separated from stems (Guo et al. 2016). For the second harvest, four to five pots of each treatment were harvested. As plants increase in size, the functions between coarse and fine roots, branches and stems are considerably different, and therefore, these organs were separated during the second harvest. Coarse roots were defined as roots > 2 mm

rapid dichromate oxidation method (Nelson and Sommers 1982), the semi-micro Kjeldahl method (K9860, Hanon Instruments, Shanghai, China) described by (Mitchell (1998), and the vanadate-molybdate yellow colorimetric method (Yang et al. 2011).

Statistical analysis

Neighbor effects on above- and below-ground growth were estimated using the following formulas described by Manea and Leishman (2011):

$$\text{Neighbor effect}_{\text{pure}} = (\text{Biomass}_{\text{pure}} - \text{Biomass}_{\text{single}}) / (\text{Biomass}_{\text{pure}} + \text{Biomass}_{\text{single}})$$

$$\text{Neighbor effect}_{\text{mixed}} = (\text{Biomass}_{\text{mixed}} - \text{Biomass}_{\text{single}}) / (\text{Biomass}_{\text{mixed}} + \text{Biomass}_{\text{single}}),$$

diameter and fine roots < 2 mm. The harvested materials were dried at 70 °C for 72 h.

Nutrient composition and concentrations

All dried samples were grounded into powder and 50 mg extracted in 80% (v/v) ethanol at 80 °C for 30 min and centrifuged at 7000 g for 5 min. The supernatant was used to test soluble sugars at 625 nm (Yemm and Willis 1954) (UV2100, LabTech, Inc., Hopkinton, MA, USA). The residues were used to test for starch according Chen et al. (2015). The details for measuring C, N, P, starch and soluble sugars were described by Guo et al. (2016). Carbon, N and P concentrations were measured using the

where the neighbor effect_{pure} and neighbor effect_{mixed} indicate intraspecific competition and interspecific competition, respectively. Biomass_{pure}, biomass_{mixed} and biomass_{single} indicate plant biomass in pure- (intra-competition), mixed- (inter-specific competition) and singly grown, respectively. Aboveground and belowground biomass of each treatment were used to estimate the impact of competition on growth of plants. In the formula, the lower neighbor effect scores the stronger competition effect from the neighbor under the different conditions tested.

All data were checked for normality and homogeneity of variances. Tamhane's T2 *posthoc* comparisons were applied if the variances were still not homogeneous after log-transformation. A two-factor ANOVA was used to assess the effects of competition, N addition and their interactions.

If a significant interactive effect was found, pairwise comparison with Tukey's *posthoc* tests was conducted to clarify significant differences among treatments. Data were analyzed using Statistical Package for the Social Science (SPSS) version 20.0 and statistical tests were considered significant at $P < 0.05$.

Results

Neighbor effect on above- and belowground biomass allocation

The aboveground neighbor effect of *L. olgensis* was less affected by the presence or interspecific competition from *L. kaempferi* in all treatments, M (interspecific) and MN, (interspecific competition under N addition) (Fig. 2a). The belowground competition of *L. kaempferi* was significantly reduced when competing with *L. olgensis* under N addition (Fig. 2b). Belowground allocation of *L. kaempferi* was significantly influenced by competition and nitrogen addition with higher belowground allocation in MN than in PN (intraspecific competition under N) (Fig. S1a). Fine/coarse root ratios of *L. kaempferi* were significantly impacted by competition, nitrogen addition and their interaction. The fine/coarse root ratios of *L. olgensis* in M were significantly lower than in S (single-grown plant), whereas it became significantly higher in MN than in SN (single-grown plant with N addition) (Fig. S1d).

Plant stoichiometry response to competition and nitrogen addition

Carbon concentrations of stems, branches and coarse roots showed little variations in both species (Fig. S2). Intraspecific competition significantly promoted fine root N concentration of *L. kaempferi* than in single-grown plants, and similarly for *L. olgensis* when nitrogen was added (Fig. S3d, h). Phosphorus concentrations of the two species were mainly impacted by N addition (Fig. S4). For *L. kaempferi*, C/N ratios of stems, branches and coarse roots did not significantly vary to competition and N addition (Table 1). The fine root C/N ratios were strongly affected by intraspecific and interspecific competition, and were especially lower in MN than in single-grown plants with nitrogen added (Table 1). For *L. olgensis*, the C/N ratios of coarse and fine roots were more affected by N addition than by competition (Table 1). Nitrogen addition strongly affected N/P ratios of stems, coarse and fine roots than competition for both species (Table 2).

Nitrogen and phosphorus storage response to competition and nitrogen

In *L. kaempferi*, nitrogen addition significantly enhanced branch and coarse root nitrogen pools compared with corresponding treatments without the addition of nitrogen (Fig. 3b, c). Nitrogen also significantly promoted coarse and fine root nitrogen pools when *L. olgensis* was in competition with neighbors (PN and MN, respectively) (Fig. 3g, h). Competition strongly influenced nitrogen storage capacities

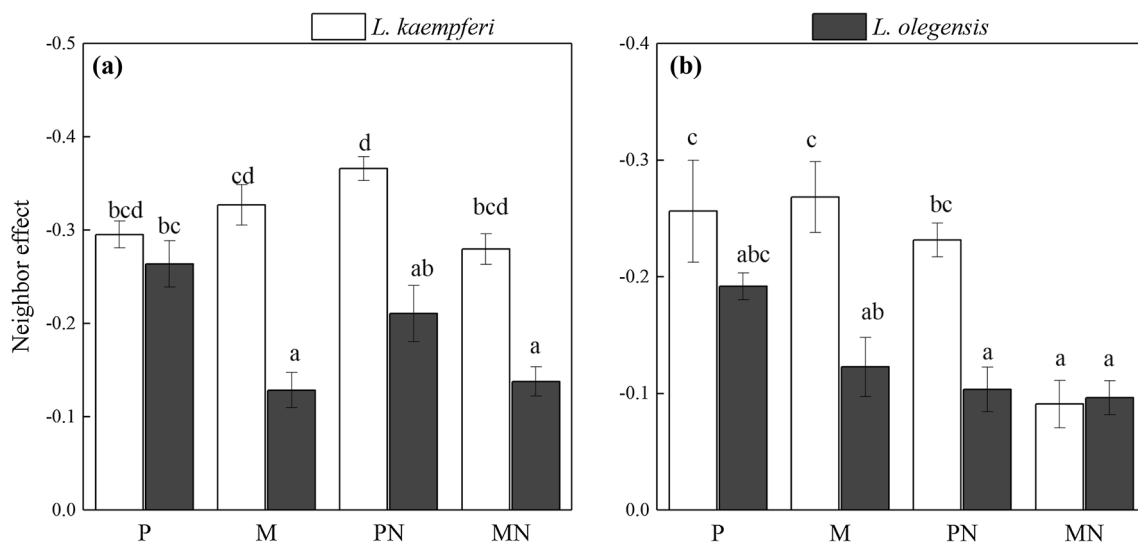


Fig. 2 Neighbor effect of *Larix kaempferi* and *L. olgensis* under different nitrogen levels: **a** Neighbor effect on aboveground, **b** neighbor effect on belowground. P: intraspecific competition; M: interspecific competition; PN: intraspecific competition under N addition; MN:

interspecific competition under N addition. Tukey's *posthoc* tests were conducted at a significant level $P < 0.05$. Different letters indicate significant differences among treatments

Table 1 The C/N of non-photosynthetic organs of *Larix kaempferi* and *L. olgensis* under different competition and nitrogen levels

	S	P	M	SN	PN	MN	F_N	F_{Com}	$F_{N \times Com}$
<i>Larix kaempferi</i>									
Stem	167.4 ± 13.9	119.3 ± 4.3	143.8 ± 12.7	138.4 ± 7.3	141.8 ± 7.5	140.1 ± 9.5	0.171 ^{n.s}	2.218 ^{n.s}	2.963 ^{n.s}
Branch	90.1 ± 3.2	120.4 ± 13.7	119.1 ± 9.0	105.9 ± 10.1	106.0 ± 5.9	94.5 ± 5.9	1.152 ^{n.s}	1.325 ^{n.s}	2.526 ^{n.s}
Coarse root	99.9 ± 2.8 ^b	121.2 ± 3.4 ^{ab}	112.9 ± 6.7 ^{ab}	125.5 ± 7.4 ^a	110.8 ± 3.4 ^{ab}	108.1 ± 4.9 ^{ab}	0.648 ^{n.s}	0.638 ^{n.s}	5.711*
Fine root	75.7 ± 1.4 ^{ab}	66.6 ± 3.1 ^{bc}	69.5 ± 2.1 ^{bc}	85.1 ± 4.0 ^a	72.9 ± 4.1 ^{abc}	61.6 ± 2.2 ^c	0.992 ^{n.s}	10.606 ^{***}	4.662*
<i>Larix olgensis</i>									
Stem	140.1 ± 10.1	129.6 ± 7.0	114.0 ± 4.3	143.2 ± 2.2	131.1 ± 6.3	135.1 ± 6.0	2.304 ^{n.s}	2.481 ^{n.s}	1.187 ^{n.s}
Branch	73.1 ± 5.9 ^b	94.2 ± 3.4 ^a	87.6 ± 3.2 ^{ab}	89.0 ± 2.2 ^a	88.3 ± 1.8 ^{ab}	92.5 ± 3.3 ^a	3.188 ^{n.s}	4.631*	4.726*
Coarse root	112.4 ± 3.3	116.0 ± 6.7	121.5 ± 11.6	105.0 ± 3.9	102.0 ± 7.6	89.9 ± 4.6	7.056*	0.115 ^{n.s}	1.235 ^{n.s}
Fine root	61.2 ± 1.1	69.9 ± 1.2	66.8 ± 4.5	57.4 ± 3.1	56.2 ± 1.6	63.6 ± 3.2	7.240*	1.692 ^{n.s}	1.937 ^{n.s}

F value and P value of two-way ANOVA in responding to N addition and competition were displayed. Labels: F_N : N addition effect; F_{Com} : competition effect; $F_{N \times Com}$: interactive effect of N addition and competition. Each value is the mean ± SE. S: single-grown plant; P: intraspecific competition; M: interspecific competition; SN: single-grown plant with N addition; PN: intraspecific competition under N addition; MN: interspecific competition under N addition. Different letters denote significant differences among treatments according to Tukey's *posthoc* tests at a significance level of $P < 0.05$. *** $P \leq 0.001$, ** $0.01 \leq P < 0.001$, * $0.05 \leq P < 0.01$, ^{n.s} $P > 0.05$ indicate significance of competition, N addition and their interactive effect

Table 2 The N/P ratios of non-photosynthetic organs of *Larix kaempferi* and *L. olgensis* under different competition and nitrogen levels

	S	P	M	SN	PN	MN	F_N	F_{Com}	$F_{N \times Com}$
<i>Larix kaempferi</i>									
Stem	26.1 ± 2.9	26.1 ± 0.8	26.1 ± 2.4	34.7 ± 3.8	29.7 ± 3.1	27.4 ± 2.7	3.767 ^{n.s}	0.786 ^{n.s}	0.801 ^{n.s}
Branch	23.9 ± 0.9	17.1 ± 1.8	19.7 ± 1.9	24.9 ± 0.7	21.2 ± 1.7	24.3 ± 1.7	4.861*	4.010*	0.497 ^{n.s}
Coarse root	25.0 ± 0.3	26.4 ± 1.2	28.1 ± 1.7	27.9 ± 1.7	31.2 ± 1.9	38.0 ± 2.8	12.133**	5.360*	1.634 ^{n.s}
Fine root	35.7 ± 1.0	35.3 ± 2.7	35.9 ± 0.9	52.9 ± 0.8	49.6 ± 5.7	58.4 ± 6.2	24.078***	0.674 ^{n.s}	0.502 ^{n.s}
<i>Larix olgensis</i>									
Stem	21.4 ± 1.5	24.4 ± 2.7	27.8 ± 1.9	38.6 ± 3.6	36.5 ± 5.3	29.6 ± 4.8	9.577*	0.119 ^{n.s}	1.89 ^{n.s}
Branch	27.3 ± 0.1	20.6 ± 0.4	26.6 ± 2.7	26.4 ± 1.3	24.5 ± 1.3	23.5 ± 1.0	0.001 ^{n.s}	3.068 ^{n.s}	2.596 ^{n.s}
Coarse root	28.4 ± 2.2	27.8 ± 3.5	25.8 ± 3.7	27.3 ± 1.8	46.3 ± 5.9	41.2 ± 6.7	8.091**	1.391 ^{n.s}	2.212 ^{n.s}
Fine root	51.5 ± 3.1	38.8 ± 3.7	38.9 ± 3.0	62.8 ± 9.6	63.5 ± 7.5	48.0 ± 3.1	11.133*	3.100 ^{n.s}	1.298 ^{n.s}

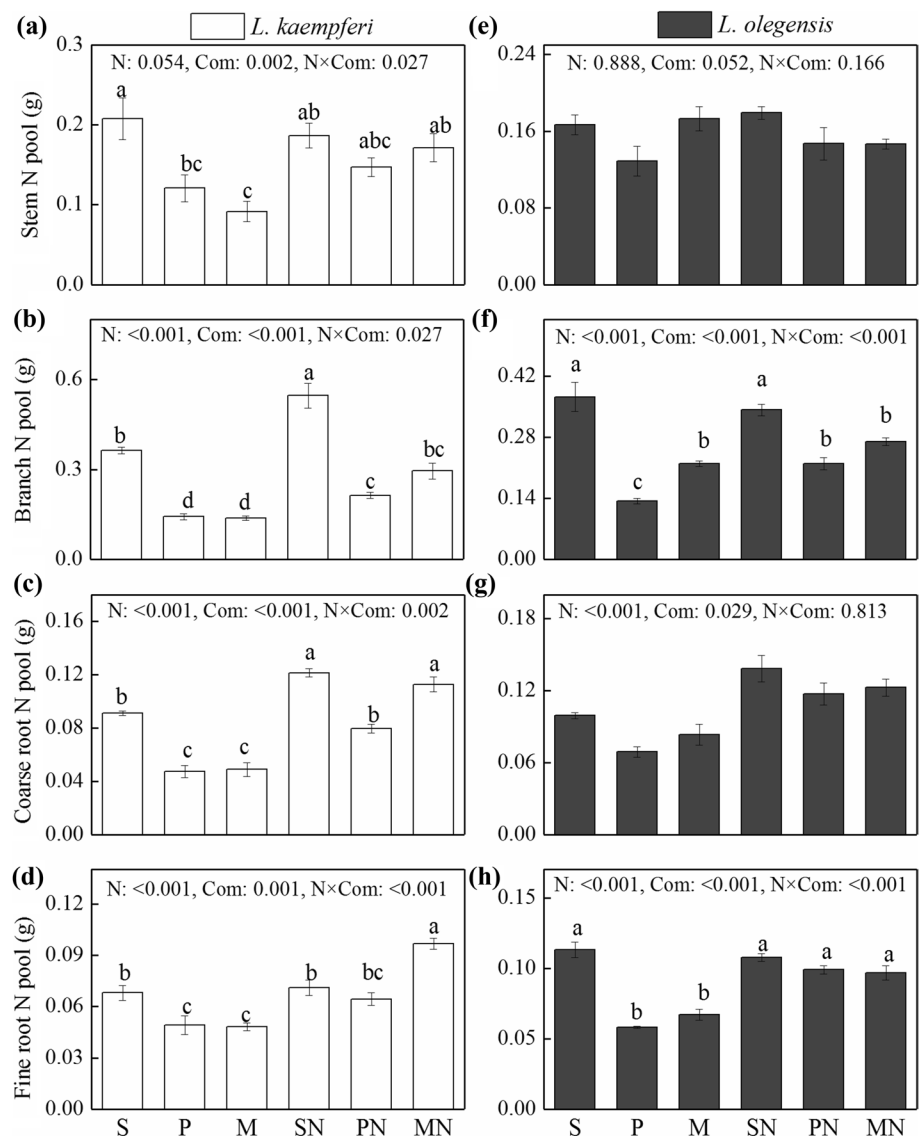
F value and P value of two-way ANOVA in responding to N addition and competition were displayed. Labels: F_N : N addition effect; F_{Com} : competition effect; $F_{N \times Com}$: interactive effect of N addition and competition. Each value is the mean ± SE. S: single-grown plant; P: intraspecific competition; M: interspecific competition; SN: single-grown plant with N addition; PN: intraspecific competition under N addition; MN: interspecific competition under N addition. *** $P \leq 0.001$, ** $0.01 \leq P < 0.001$, * $0.05 \leq P < 0.01$, ^{n.s} $P > 0.05$ indicate significance of competition, N addition and their interactive effect

of branches, and coarse and fine roots for both species (Fig. 3). Specifically, nitrogen storage in branches and fine roots in intraspecific competition and interspecific competition were significantly lower than in single-grown plants, while of that of coarse and fine roots of *L. kaempferi* in inter-specific competition were significantly higher than in intra-specific competition under nitrogen addition (Fig. 3c, d). In addition, competition significantly reduced phosphorus storage in branches and coarse roots of *L. kaempferi* (Fig. 4b, c), while the phosphorus storage of the two species increased in interspecific completion with N addition than in intraspecific completion under N treatments (Fig. 4).

NSC response to competition and nitrogen addition

For the two species, the effect of competition on starch concentration was much stronger than to the addition of nitrogen (Table 3). Competition significantly enhanced the starch concentrations of stems and fine roots for both species at each N level. Stem sugar concentrations for both species declined significantly as a result of competition. Moreover, the sugar concentrations of *L. kaempferi* in intraspecific and interspecific competition with nitrogen added were significantly higher than those in SN (Table S1). However, the fine root sugar concentration of *L. olgensis* was improved by competition (Table S1). Similar to *L. kaempferi*, competition showed stronger effects on starch/sugar ratios than the addition of nitrogen in *L.*

Fig. 3 Nitrogen pools of non-photosynthetic organs of *Larix kaempferi* and *L. olegensis* under different competition and nitrogen additions. Each value is the mean \pm SE. N: addition effect; Com: competition effect; N \times Com: interactive effect of N addition and competition. S: single-grown plant; P: intraspecific competition; M: interspecific competition; SN: single-grown plant with N addition; PN: intraspecific competition under N addition; MN: interspecific competition under N addition. Different letters denote significant differences among treatments according to Tukey's *posthoc* tests at a significance level of $P < 0.05$



olegensis (Fig. S4). Competition significantly increased stem starch/sugar ratios of both species, especially under N addition (Fig. S5a, e), while coarse root starch/sugar ratios of *L. olegensis* was significantly reduced by competition (Fig. S5g).

NSC storage responses to nitrogen addition and competition

Competition significantly reduced branch and coarse root starch storage of both species (Fig. 5). Fine and coarse root starch storage of *L. kaempferi* was significantly enhanced by nitrogen addition (Fig. 5c, d). Sugar storage in stems, branches and coarse roots of *L. kaempferi* in single-grown plants without and with nitrogen added were significantly higher than when in competition (Fig. 6a–c), showing a clear impact of competition on *L. kaempferi*'s ability to store sugars in these organs. For *L. olegensis*, competition also tended to reduce stem

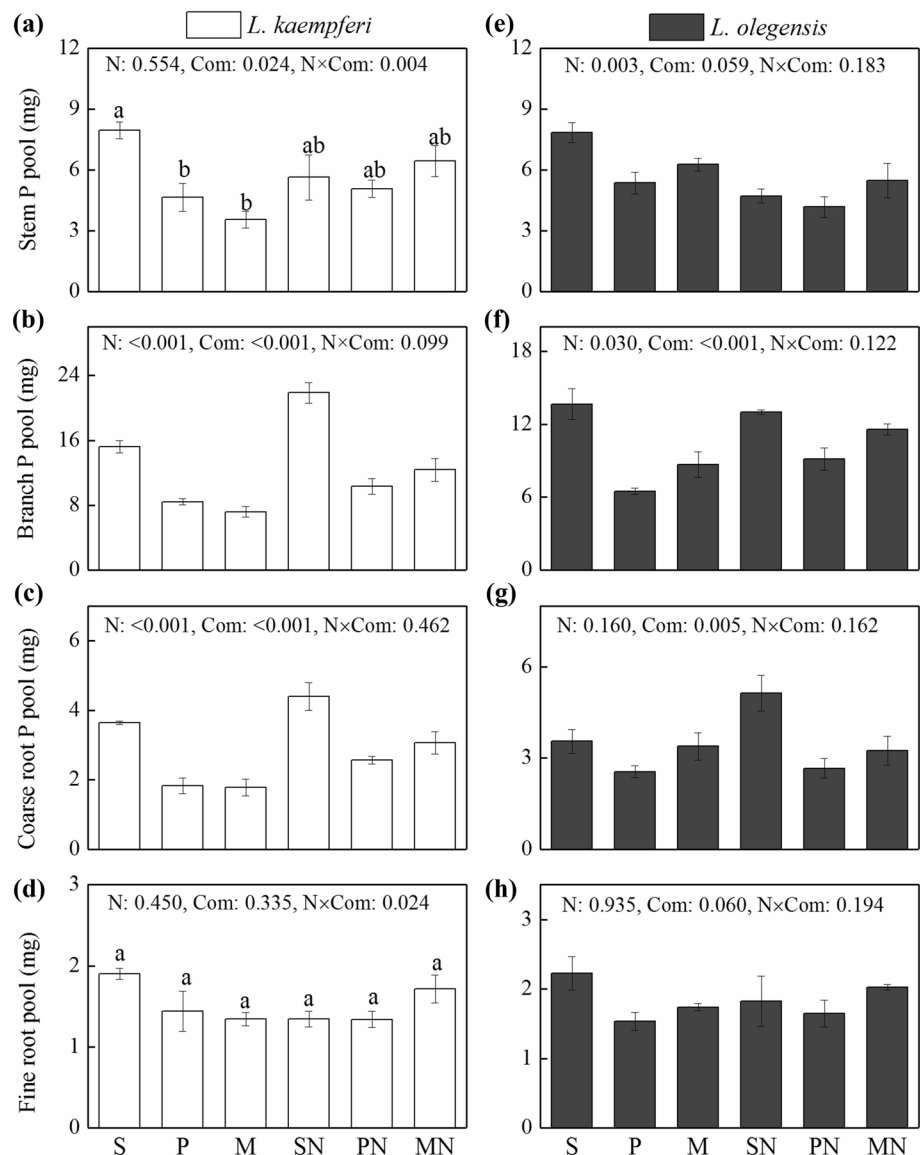
and branch sugar storage (Fig. 6e, f), while N addition tended to increase coarse and fine root sugar storage (Fig. 6g, h).

Discussion

Nitrogen addition alleviated interspecific competition

The addition of nutrients such as N enhances the competitive ability of neighboring plants, whose impacts may be negative or positive depending on whether it is a hetero or conspecific species (Bertness and Callaway 1994; Wang et al. 2010; Trinder et al. 2012; Guo et al. 2019). Bertness and Callaway (1994) suggested that plant competition relationships would be much stronger in a productive environment than in a relatively resource-limited one. However, we found that *L. olegensis* benefited from the presence of *L. kaempferi* under different N

Fig. 4 Phosphorus pools of non-photosynthetic organs of *Larix kaempferi* and *L. olegensis* under different competition and nitrogen additions. Each value is the mean \pm SE. N: addition effect; Com: competition effect; N \times Com: interactive effect of N addition and competition. S: single-grown plant; P: intraspecific competition; M: interspecific competition; SN: single-grown plant with N addition; PN: intraspecific competition under N addition; MN: interspecific competition under N addition. Different letters denote significant differences among treatments according to Tukey's *posthoc* tests at a significance level of $P < 0.05$



levels and also showed less belowground competition from heterospecific neighbors than from conspecific individuals after N addition (Fig. 2). Therefore, nitrogen enrichment reduced interspecific competition intensity for both species.

Effects of N addition and competition on stoichiometric traits

Lovelock et al. (2007) found a negative relationship between growth rate and N/P, whereas other studies were inconsistent (e.g., Matzek and Vitousek 2009; Jing et al. 2017). Nitrogen addition was the primary factor affecting N/P ratios of different non-photosynthetic organs for both species because it strongly drove changes in P levels (Table 2, Fig. S4). Nitrogen addition significantly promoted growth rates of both species but the N/P ratios of different organs showed no significant decline in the present study. We speculated that

plants can store nutrients and alter the relationship between N/P ratios and growth rate.

Variation in stoichiometric ratios, both within and across species, reflects a trade-off between adaptive capacity (flexibility) and stability (homeostasis) (Guiz et al. 2018). The observed stable stem C/N ratio, relative to root and branch ratios, suggest that stems were strongly capable in maintaining nutrient balance and were less likely to vary in response to competition (Myers and Kitajima 2007). Roots can detect and directly respond to changes in their neighbor's identity and in soil nutrient availability by changing root traits (biomass, fine and/or coarse roots) (Broadbent et al. 2018; Wang et al. 2019). Competition significantly reduced N concentration of fine root for both species (Fig. S2d, h). However, C/N ratios of *L. olegensis* fine root were not affected by competition, indicating a stronger ability to maintain stable stoichiometry than *L. kaempferi* (Table 1).

Table 3 The starch concentration of non-photosynthetic organs of *Larix kaempferi* and *L. olgensis* under different competition and nitrogen levels

	S	P	M	SN	PN	MN	F_N	F_{Com}	$F_{N \times Com}$
<i>Larix kaempferi</i>									
Stem	71.8 ± 4.6	94.5 ± 5.1	86.5 ± 3.7	85.7 ± 7.2	104.4 ± 4.4	107.8 ± 2.7	16.365***	10.718***	0.962 ^{n.s}
Branch	38.3 ± 2.1	30.2 ± 1.0	33.3 ± 1.9	39.0 ± 3.0	34.9 ± 2.4	36.0 ± 1.5	2.603 ^{n.s}	3.993*	0.438 ^{n.s}
Coarse root	149.8 ± 9.2 ^a	85.1 ± 6.2 ^b	91.8 ± 5.4 ^b	111.6 ± 5.4 ^b	94.5 ± 5.8 ^b	96.8 ± 6.2 ^b	2.236 ^{n.s}	20.426***	7.037**
Fine root	72.3 ± 5.7	74.3 ± 6.1	76.3 ± 5.8	71.8 ± 3.2	92.6 ± 8.1	87.3 ± 5.4	0.090 ^{n.s}	0.252 ^{n.s}	0.416 ^{n.s}
<i>Larix olgensis</i>									
Stem	74.3 ± 4.8	97.6 ± 3.0	112.4 ± 5.5	78.0 ± 6.0	93.9 ± 4.7	108.8 ± 6.5	0.072 ^{n.s}	17.978***	0.247 ^{n.s}
Branch	39.3 ± 3.8	33.8 ± 0.9	33.0 ± 1.4	51.7 ± 2.0	33.2 ± 1.7	41.1 ± 3.0	10.045**	10.707***	3.535 ^{n.s}
Coarse root	113.0 ± 2.9	91.1 ± 4.9	101.9 ± 7.7	132.4 ± 14.8	111.3 ± 9.4	105.1 ± 5.8	4.138 ^{n.s}	3.499 ^{n.s}	0.669 ^{n.s}
Fine root	71.9 ± 4.2	107.4 ± 7.6	111.2 ± 5.8	73.6 ± 9.1	98.2 ± 7.2	89.3 ± 7.2	2.682 ^{n.s}	8.853**	1.267 ^{n.s}

F value and P value of two-way ANOVA in responding to N addition and competition were displayed. Labels: F_N : N addition effect; F_{Com} : competition effect; $F_{N \times Com}$: interactive effect of N addition and competition. Each value is the mean ± SE. S: single-grown plant; P: intraspecific competition; M: interspecific competition; SN: single-grown plant with N addition; PN: intraspecific competition under N addition; MN: interspecific competition under N addition. Different letters denote significant differences among treatments according to Tukey's *posthoc* tests at a significance level of $P < 0.05$. *** $P \leq 0.001$, ** $0.01 \leq P < 0.001$, * $0.05 \leq P < 0.01$, ^{n.s} $P > 0.05$ indicate significance of competition, N addition and their interactive effect

Leaf stoichiometry of species in interspecific competition tends to become more deviant than in their monoculture counterparts, often showing increasing trait dissimilarities when intense competition occurs (Guiz et al. 2018). *L. kaempferi* and *L. olgensis* differ in nutrient uptake and use efficiency, with the growth of *L. kaempferi* requiring more nitrogen (Li et al. 2016, 2018b). However, there was no obvious difference between intra- and interspecific competition in C/N and N/P ratios of different plant organs in the present study. Song et al. (2017) also found that growth of *Populus purdomii* Rehder was enhanced by the presence of a heterospecific neighbor, *Salix rehderiana* C. K. Schneider, under N addition but the C/N ratios of their organs showed little variation under the influence of intraspecific and interspecific competition. The competition intensity, under both intra- and interspecific competition between two individuals, may not enough to drive differences in the stoichiometry of different organs.

Effects of N addition and competition on NSC

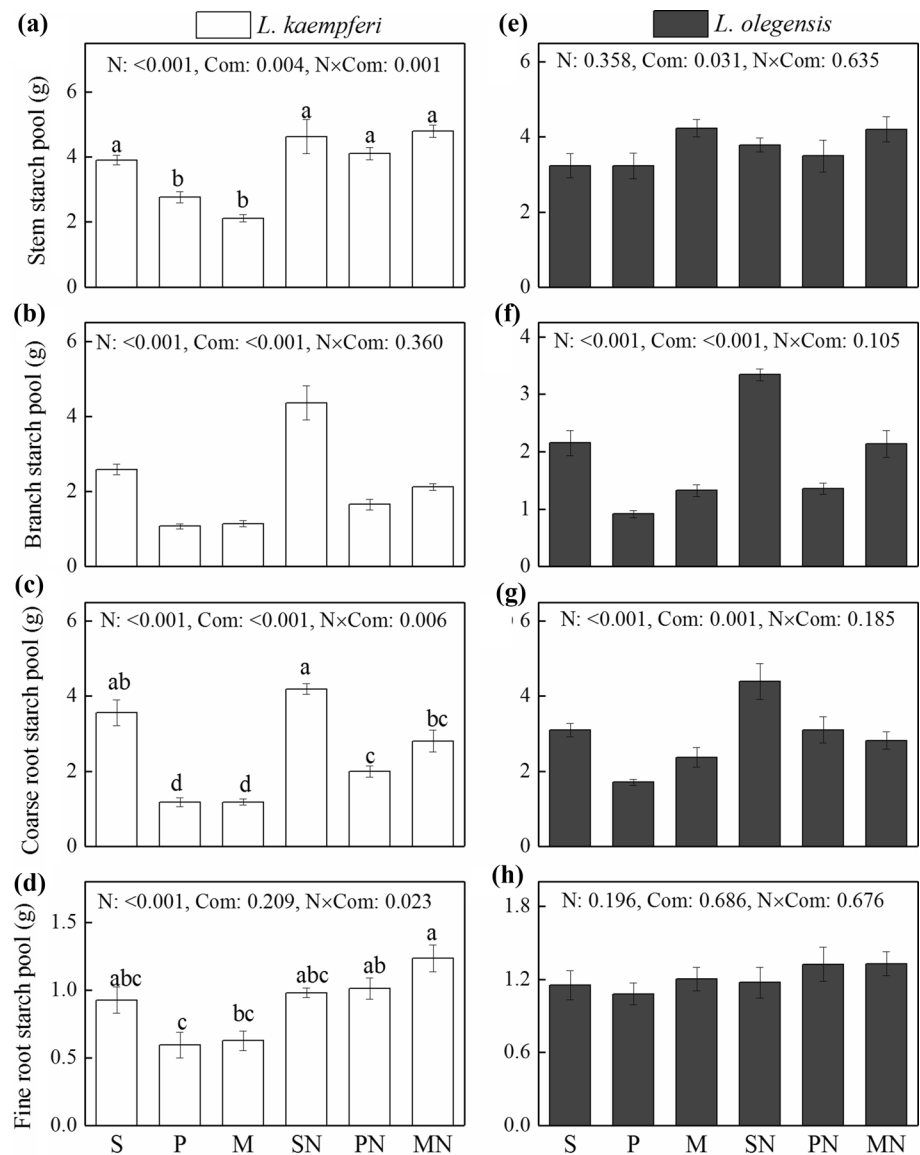
The carbon concentrations of different plant organs showed less variation relative to N and P under different treatments. Minden and Kleyer (2014) noted that plant C gain needs a more stable and optimal stoichiometric ratio than absorbing function. By contrast, we found that starch and soluble sugar concentrations, as well as their ratios, were more easily affected by competition, N addition and/or their interactions. Stem and fine root soluble sugar concentrations of *Pinus ponderosa* were reduced when competing

with blue wild-rye grass, *Elymus glaucus* Buckley, while C concentration was less affected (Andersen et al. 2001). Our results suggest that the effects of competition on non-structural carbohydrates imply potential changes in growth, storage or allocation. Hoch and Körner (2003) suggested that the NSC stored in aboveground biomass of mature deciduous trees is possibly sufficient to enable the plant to replace its entire leaf canopy four times over without photosynthesis. In this study, as crucial stored energy, more starch was stored in the stems as indicated by increasing starch concentrations and starch/soluble sugar under competition for both species. Therefore, competition declined the growth of *L. kaempferi* and *L. olgensis* but forced allocation of more carbon into storage.

Effects of N addition and competition on carbon and nutrient reserves

One of the unique findings of this study was that carbohydrate and nutrients pools of branches and coarse roots were more sensitive to competition or to nitrogen addition. Several studies have confirmed the crucial roles of carbon and nutrient reserves in budbreak, leaf growth, shoot expansion and root development (e.g. Kagawa et al. 2006; Millard and Grelet 2010; Palacio et al. 2018). Tree branches function as important carbohydrate and nutrient storage sites prior to budbreak (Palacio et al. 2018), and the stored carbohydrate can be remobilized from coarse roots to maintain functions of fine roots and to

Fig. 5 Starch pools of non-photosynthetic organs of *Larix kaempferi* and *L. olegensis* under different competition and nitrogen addition. N: addition effect; Com: competition effect; N×Com: interactive effect of N addition and competition. Each value is the mean ± SE. S: single-grown plant; P: intraspecific competition; M: interspecific competition; SN: single-grown plant with N addition; PN: intraspecific competition under N addition; MN: interspecific competition under N addition. Different letters denote significant differences among treatments according to Tukey's *posthoc* tests at a significance level of $P < 0.05$

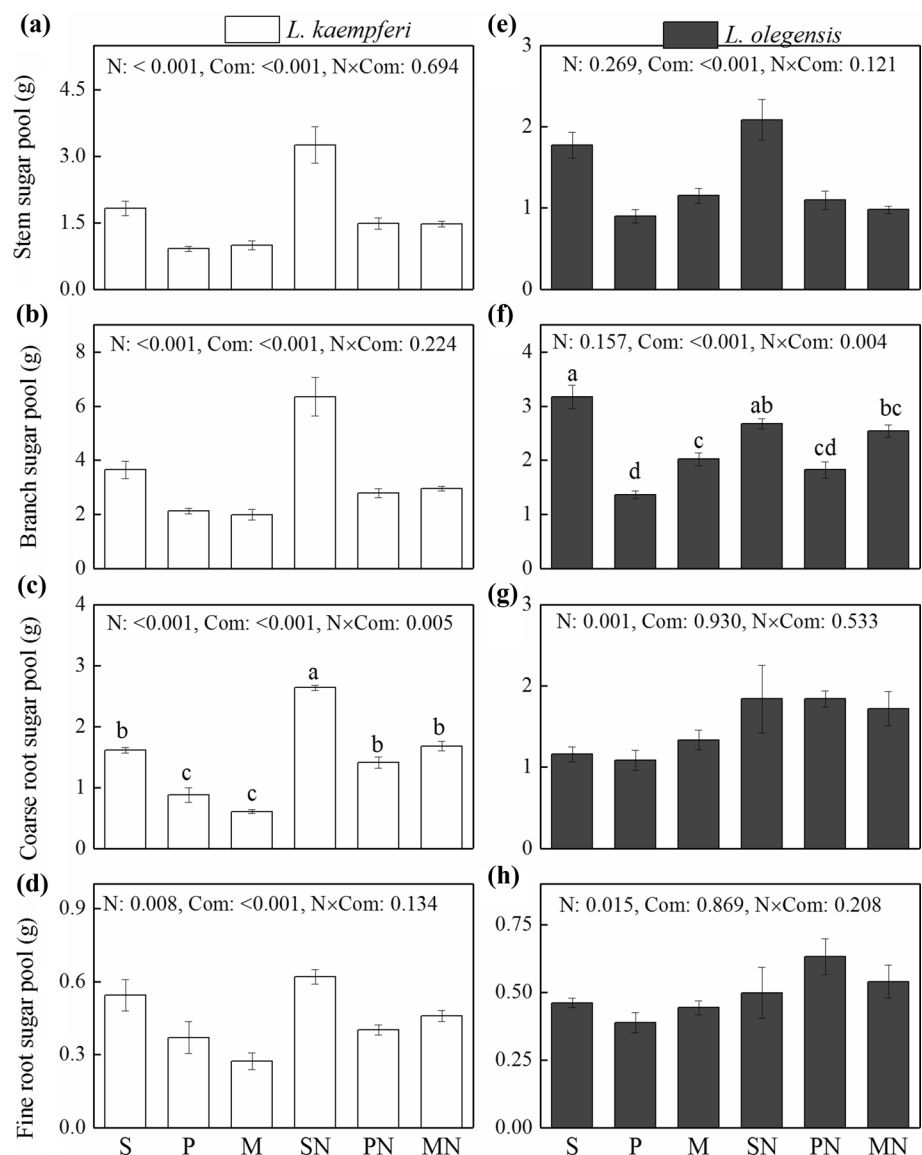


affect root development (Druege et al. 2004; Kobe et al. 2010; Aubrey and Teskey 2018). *Larix olegensis* enhanced branch nutrients and carbohydrate pools when competing with *L. kaempferi* because neighbor competition showed less effects on aboveground growth of *L. olegensis*. This implies that *L. olegensis* may have more intense budbreak and develop more branches in mixed than in pure forests the following spring, making it more able to outcompete *L. kaempferi*. The coarse and fine roots of *L. kaempferi* accumulated more nitrogen when in competition with *L. olegensis* than with conspecific neighbors under N addition because of less intraspecific competition from *L. olegensis* neighbors and more belowground biomass allocation.

Conclusions

Nitrogen addition alleviated interspecific competition intensity and was the primary factor in impacting N/P ratios of different non-photosynthetic organs for both species. Competition reduced the growth of *L. kaempferi* and *L. olegensis* but forced them to allocate more carbon to storage. However, competition between two individuals was not strong enough to drive changes in stoichiometry under intra- and interspecific competition. If neighbor competition intensity became stronger, deciduous species with higher nutrients and non-structural carbon pools in stored organs would keep competitive superiority and enhanced survival, relative to

Fig. 6 Soluble sugar pools of non-photosynthetic organs of *Larix kaempferi* and *L. olegensis* under different competition and nitrogen additions. Each value is the mean \pm SE. N: addition effect; Com: competition effect; N \times Com: interactive effect of N addition and competition. S: single-grown plant; P: intraspecific competition; M: interspecific competition; SN: single-grown plant with N addition; PN: intraspecific competition under N addition; MN: interspecific competition under N addition



neighbors. More research is needed in different forest types, under different climates and between different species, in order to understand further how competition drives nutrient and NSC allocation in plants. Moreover, changing global climatic conditions, as well as increasing atmospheric nitrogen deposition, especially from anthropogenic sources, will increase impacts on plant productivity, which will in turn affect forest productivity. These concepts should be explored further with studies on plant competition, particularly in mixed environments.

Acknowledgements We are grateful to the Qingyuan Experimental Station, Institute of Applied Ecology, Chinese Academy of Science for providing experimental sites and support. We also thank Dr. Deliang Lu with helping us in data collection.

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