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Resorption-related nitrogen changes in the leaves and roots of *Larix kaempferi* seedlings under nutrient-sufficient and nutrient-starvation conditions

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Abstract

Aims
Larch is the dominant timber species in Northeast China. However, compared with the adjacent secondary forests, soil available nitrogen (N) significantly declined in ~40-year-old larch plantations. Thus, it is of great importance to determine how N use strategies in larch change in response to declining soil N availability.

Methods
We investigated the changes in N concentration and ^{15}N natural abundance ($\delta^{15}N$) from 18 August to 25 October in the leaves, stems, branches and roots of 1-year-old *Larix kaempferi* seedlings under nutrient-sufficient (NSu) and nutrient-starvation (NSt) conditions with a pot experiment in Northeast China.

Important Findings
Stem and branch N concentrations exhibited upward trends, and leaf N concentration exhibited a downward trend. Root N concentration exhibited an upward trend under NSu conditions, but a downward trend under NSt conditions. These results suggested that stems and branches were served as N storage organs, but roots shifted from storage to resorption organs when switched from NSu to NSt. Leaf nutrient resorption was intensely occurred on 11 October, as indicated by the sharply decreased leaf N concentration and increased stem, root and branch N concentrations. The $\delta^{15}N$ of roots, branches and leaves overlapped between NSu and NSt approximately on 11 October, which may be regulated by isotope discrimination during N resorption. Leaf N resorption efficiency under NSt (76.33%) was significantly higher than that of NSu (56.76%), indicating that nutrient stress stimulates leaf N resorption. Taken together, larch seedlings enhance leaf nutrient resorption and shift roots from nutrient storage to nutrient resorption to adapt to NSt conditions. These changes might relieve the adverse effects of declining soil nutrient availability on seedling survival and regeneration.

Keywords: nutrient stress, storage organ, resorption organ, redistribution, stable nitrogen isotope

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INTRODUCTION

Larix spp., mainly including *L. olgensis*, *L. principis-rupprechtii* and *L. kaempferi*, are the most important commercial timber species in Northeast China (Mason and Zhu 2014; Yan *et al.* 2017). However, in comparison with the adjacent secondary forests, soil available nitrogen (N) significantly declined (~30%) in the ca. 40-year-old larch plantations (Yang *et al.* 2013). Thus, it would be imperative to know how nutrient use strategies of larch seedlings response to declining soil nutrient availability, which will be beneficial for a better understanding of the survival and regeneration of larch seedlings.

Senescence process is regarded as a form of programmed cell death that occurs in all areas of plant anatomy, and the

immolation of organs and the well-regulated developmental process is for the maximum benefit of the entire plant (Brant and Chen 2015; Humbeck 2014). During the period of senescence, one of the most important nutrient conservation mechanisms is nutrient resorption, which refers to the process that withdraws a proportion of nutrients from senescing organs to storage organs (Aerts 1996; Brant and Chen 2015). Remobilization of the stored nutrients can be directly used in the subsequent growing season, in particular the leaf regrowth in the early spring for deciduous tree species (Martínez-Alcántara *et al.* 2011; Villar-Salvador *et al.* 2015), which can reduce the dependence of the plant on soil nutrient supplies (Kobe *et al.* 2005). Furthermore, autumntal N nutrition affects carbon and N storage and the architecture of young tress (Jordan *et al.* 2011). However, much less is known about resorption-related N dynamics and resorption variations in other organs (e.g. fine roots, stems or branches) than leaves. These organs also play an essential role in the whole-plant nutrient budget and biogeochemical cycles (Freschet *et al.* 2010; Kunkle *et al.* 2009; Lü *et al.* 2012). To better understand the nutrient use strategy and nutrient economy of a plant, it is important to use the whole-plant system during the process of nutrient resorption.

Nitrogen is the most limiting element for temperate plantation forests (LeBauer and Treseder 2008; Magnani *et al.* 2007), particularly for younger stands (e.g. larch plantations, Yan *et al.* 2018a). Globally, 62% of leaf N is resorbed during senescence, which could meet up to 31% of the annual plant N demands (Cleveland *et al.* 2013; Vergutz *et al.* 2012). In addition to leaves, other organs (e.g. twigs and fine roots) could also occur different levels of resorption during senescence (Chen *et al.* 2015; Freschet *et al.* 2010). Unfortunately, the few studies published to date have mainly focused on non-woody plants (Lü *et al.* 2012; Mao *et al.* 2013), but little on woody plants, and the resorption by other organs (e.g. roots) has been largely overlooked (Freschet *et al.* 2010; Kunkle *et al.* 2009).

Changes in resource availability are expected to trigger variations in plant functional traits (e.g. phenotypic and/or nutrient use strategy) to mitigate the constraints caused by the limiting resources (Freschet *et al.* 2015; Lü *et al.* 2012; Reich *et al.* 2014). In this case, two main nutrient strategies (optimizing nutrient acquisition and reducing nutrient loss) have developed for plants to grow in nutrient limitation conditions (Freschet *et al.* 2010; Yan *et al.* 2018a). Plants can respond to nutrient deficiency by internal nutrient redistribution to support sink demands (Tully *et al.* 2013; Yan *et al.* 2018a). Due to the important role nutrient resorption plays in nutrient conservation, it is generally assumed that species in nutrient-poor sites are more proficient at resorption than those in nutrient-rich sites (See *et al.* 2015; Yan *et al.* 2016). Most previous studies have investigated plant performance, e.g. competition (Guo *et al.* 2016), nutrient resorption (Yuan and Chen 2015) and remobilization (Martínez-Alcántara *et al.* 2011) under N fertilization treatments (Jordan *et al.* 2011). To our knowledge, however, few studies have been conducted on resorption-related N changes under nutrient-sufficient and nutrient-starvation conditions. Thus, it is still not well understood how the nutrient use strategies of larch seedling respond to nutrient deficiency.

In this study, N dynamic changes from 18 August to 25 October of *L. kaempferi* seedling organs (stem, branch, root and leaf) were investigated under nutrient-sufficient and nutrient-starvation conditions in Northeast China. The objectives of the present study were to identify (i) when leaf resorption was intensively occurred, (ii) whether nutrient resorption also occurred in other organs (e.g. root), and (iii) how the nutrient use strategies of larch seedlings change in response to nutrient starvation. We hypothesized that larch seedlings would change nutrient use strategies (e.g. increase leaf nutrient resorption efficiency and/or shift root from nutrient storage to nutrient resorption) under soil nutrient-starvation conditions.

MATERIALS AND METHODS

Study site

This study was conducted at the Qingyuan Forest CERN (Chinese Ecosystem Research Network), Chinese Academy of Sciences, located in the mountainous region of Liaoning Province, Northeast China (124°54′E, 41°51′N, elevation 500–1100 m a.s.l.). The site is subject to a continental monsoon type with a monsoon spring with strong winds, a humid and rainy summer, and a cold and dry winter. Mean annual air temperature varies between 3.9 and 5.4°C, the minimum temperature is −37.6°C in January, and the maximum temperature is 36.5°C in July. Annual precipitation ranges from 700 to 850 mm and was mainly distributed (~80%) from June to August. On average, the frost-free period lasts for 130 days, with the first frost occurs in October and late frost in April (Zhu *et al.* 2007). The soil in this area is typical brown forest soil (Yang *et al.* 2013).

Experimental design

One-year-old *L. kaempferi* seedlings were acquired from a nursery in April 2015. Forest soil (0–20 cm) was obtained from a mature larch plantation (ca. 40 years old) within the study site and passed through a 2-mm sieve (Qu *et al.* 2004). The N concentration and C/N ratio of initial soil obtained from the mature larch plantation were 0.35% and 10.25%, respectively. Larch seedlings with similar initial heights (~22.1 ± 0.8 cm) and root collar diameters (~0.35 ± 0.02 cm) were carefully transplanted into plastic pots (4.5 l, per pot) filled with the forest soil. In total, 180 individual seedlings were planted (one seedling per pot) and randomly placed outdoors. In mid-August (18 August), when at peak biomass, all seedlings were removed from the pots and carefully rinsed with deionized water. Then, the soil from one half of the pots (90 pots) was replaced by washed fine sand (treated as nutrient starvation, NSt, i.e. forest soil replaced by washed fine sand with no nutrients, e.g. N), while the remaining half of the pots (90 pots) were refilled with forest soil

(treated as nutrient sufficient, NSu). After that, the seedlings were immediately replanted in these pots (i.e. 90 pots were filled with soil, and the other 90 pots were filled with sand). All the pots were transferred to a greenhouse (5 m × 12 m, 2 m high), which was covered with a transparent plastic sheet to prevent exposure to rain and had open sides to allow free air circulation to ensure the same temperature inside and outside (Ueda *et al.* 2011; Ueda 2012). These pots were arranged in three blocks; each block had 30 pots filled with soil and another 30 pots filled with sand. All seedlings were watered daily with deionized water to guarantee sufficient soil moisture for survival of the seedlings (Kunkle *et al.* 2009; Zhang *et al.* 2013).

Sample collection

In mid-August (18 August) of 2015 (taken as the mature stage, see Yan *et al.* 2016), we sampled the seedlings for the first time. Thereafter, sampling was performed every 10 days and continued until the end of the growing season (25 October). In total, seven sampling sessions were conducted from 18 August to 25 October. For each sampling, we collected three replicates for both soil-pot and sand-pot treatments (representing nutrient sufficient and nutrient starvation, respectively) from the three blocks, and each replicate contained four to six randomly selected individual seedlings. Thus, 12–18 individuals per treatment were destructively harvested at each sampling time. After harvest, each seedling was divided into needles, branches, stem, fine roots (<2 mm in diameter, hereafter referred to roots) and coarse roots (≥2 mm in diameter). Thus, all parts of each organ (e.g. all needles) were sampled at each sampling time. The entire root system was washed with deionized water to remove any remnants of soil. The biomasses of different organs were measured (oven dried at 65°C for at least 48 h to a constant weight) at the first and last sampling time.

Laboratory experiment

All samples were immediately transferred to the laboratory and then oven dried at 65°C for at least 48 h to a constant weight. The samples (leaves, branches, stem and roots) were subsequently ground to pass through a 100-mesh sieve for leaf C, N and $\delta^{15}N$ analysis. Leaf C (%) and N (%) and $\delta^{15}N$ (‰) were analyzed by an elementary analyzer (Elementar vario MICRO cube, Germany) coupled to a stable isotope ratio mass-spectrometer (IsoPrime 100, UK).

Calculation

Nitrogen resorption efficiency (NRE) was calculated as the ratio of the difference in N content [defined as the product of organ weight (g) and the corresponding N concentration (%)] between mature material and litter to mature material N content during senescence (Aerts 1996; Brant and Chen 2015; Chen *et al.* 2015; Freschet *et al.* 2010). NRE was calculated as follows:

$$\mathrm{NRE}\ (\%) = [(N_m - N_s) / N_m] \times 100 \quad (1)$$

where N_m and N_s represent mean N contents in mature and senesced organs (e.g. leaves and roots), respectively.

Leaf senescence was defined as when the color of the leaves turned from green to yellow, while fine root senescence was defined as when the color of fine roots turned from gray to dark and/or black (Freschet *et al.* 2010; Kunkle *et al.* 2009; Yan *et al.* 2016). It should be noted that the concept of 'resorption' is not suitable for large/coarse parts of the larch species (e.g. stem and branches) because there are no 'dead' parts corresponding to their live parts. Therefore, the calculated NRE of stems and branches in the present study was served to explore the N storage capacity during autumn, and was not a true measure of NRE.

The N isotope ratio ($\delta^{15}N$) of the plant sample was calculated as follows:

$$\delta^{15}N\ (\%) = [(R_{sample} / R_{standard}) - 1] \times 1000 \quad (2)$$

where R_{sample} and $R_{standard}$ represent the isotope ratios ($^{15}N/^{14}N$) of samples and the standard, respectively. The overall analytical precision for $\delta^{15}N$ was better than 0.2‰.

Statistical analysis

Data were tested for normality by the Kolmogorov–Smirnov test and for equality of error variances by the Levene's test. One-way analysis of variance (ANOVA) was used to test for the differences in N resorption efficiency of different organs between NSu and NSt treatments. We used one-way ANOVA to test for the differences in N concentration, C/N ratio and $\delta^{15}N$ in each organ for the two soil types (NSu and NSt) before and after senescence. Additionally, we used one-way ANOVA to test for the differences in N concentration, C/N ratio and $\delta^{15}N$ among organs under each soil type (NSu and NSt) before and after senescence; if the difference was significant, then *post hoc* multiple comparisons were conducted using the least significant difference test. Repeated-measures ANOVA was used to assess the effects of soil type, sampling time and their interactions on the N concentration, C/N ratio and $\delta^{15}N$ in the organs. Paired *t*-tests were used to assess the difference of N concentration, C/N ratio or $\delta^{15}N$ for each organ between NSu and NSt conditions after senescence. All statistical analyses were conducted in SPSS 13.0 for Windows (SPSS Inc., Chicago, IL) and the considered significance was set as $P < 0.05$.

RESULTS

Organ N concentration, C/N ratio and $\delta^{15}N$ dynamics

Similar variations were observed for the N concentration in different organs between NSu and NSt conditions (Fig. 1). Specifically, branch and stem N concentration showed an upward trend, while leaf N concentration showed a downward trend under both NSu and NSt conditions (Fig. 1b–d), whereas root N concentration showed an upward trend under NSu, but a downward trend under NSt (Fig. 1a). Leaf N concentration sharply decreased, whereas root, branch and stem N concentrations increased on 11 October (Fig. 1).

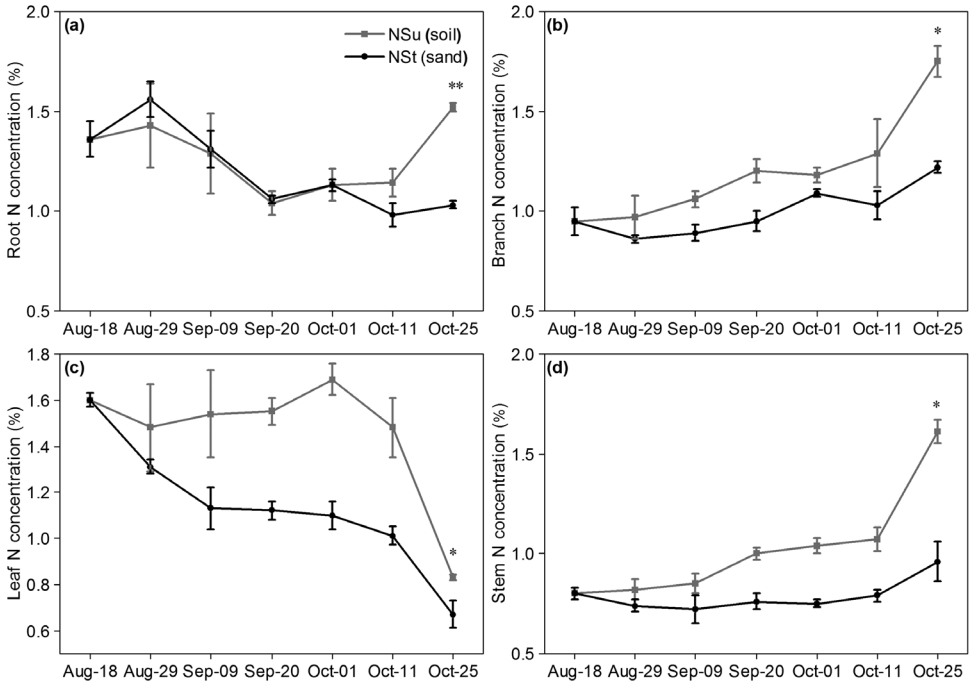

Figure 1: the N concentrations of (**a**) roots, (**b**) branches, (**c**) leaves and (**d**) stems under NSu (soil) and NSt (sand) conditions. The asterisks represent significant differences in N status of the same organ between NSu and NSt conditions on 25 October (*$P < 0.05$ and **$P < 0.001$). Mean values are given ± SE ($n = 3$).

In general, the C/N ratios in different organs (i.e. root, branch, leaf and stem) showed the opposite trends with those of N concentrations (Fig. 2). The values of $\delta^{15}N$ in different organs tended to show downward trends under both NSu and NSt conditions (Fig. 3). There existed overlap for $\delta^{15}N$ in organs (i.e. roots, branches and leaves) between NSu and NSt conditions approximately on 11 October, whereas the intersection for stem $\delta^{15}N$ was between 29 August and 9 September (Fig. 3).

Significant resorption-related variations were observed for organ N concentrations, C/N ratios and $\delta^{15}N$ (Table 1). Soil types (NSu and NSt) and sampling time had significant effects on both organ N concentrations and C/N ratios, with the exception of the effect of soil type on root N concentration ($P = 0.163$). Sampling time had significant effect on $\delta^{15}N$ in stem, leaf and root (Table 1). In addition, sampling time significantly interacted with soil type to affect organ N concentrations and C/N ratios ($P \leq 0.001$) and only $\delta^{15}N$ in stems ($P = 0.040$) (Table 1).

Organ N status after leaf senescence

After leaf senescence (on 25 October), there were significant differences for N concentration and C/N ratio between NSu and NSt conditions, while no remarkable differences were observed for $\delta^{15}N$ (Figs 1–3). For each soil type (NSu and NSt), significant differences were observed in N concentrations, C/N ratios and $\delta^{15}N$ in the different organs ($P < 0.05$).

Organ N resorption efficiency

Leaf NRE under NSt was significantly higher than that under NSu (76.33% and 56.76%, respectively). In addition, root NRE was present under NSt (7.02%), whereas root N concentration accumulation was present under NSu (Fig. 4). By contrast, no positive NRE (i.e. N accumulation was present) was observed for branch and stem under either NSu or NSt conditions (Fig. 4).

DISCUSSION

Nitrogen dynamics of organs during the process of resorption

Nitrogen (N) redistribution and transfer among organs within a plant reflects an adaption to environmental conditions (Brant and Chen 2015; Martínez-Alcántara *et al.* 2011). In the present study, larch seedlings exhibited significant resorption-related N changes (from 18 August to 25 October) under both NSu and NSt conditions (Figs 1–3, Table 1). Generally, during the period of leaf senescence, stem and branch N concentrations showed an upward trend; in contrast, leaf N concentrations showed a downward trend under both NSu and NSt conditions (Fig. 1). Similar findings were found in young *Salix dasyclados* that were grown under low and high nutrient availabilities (von Fircks *et al.* 2001). Root N concentration showed an upward trend under NSu conditions, but a downward

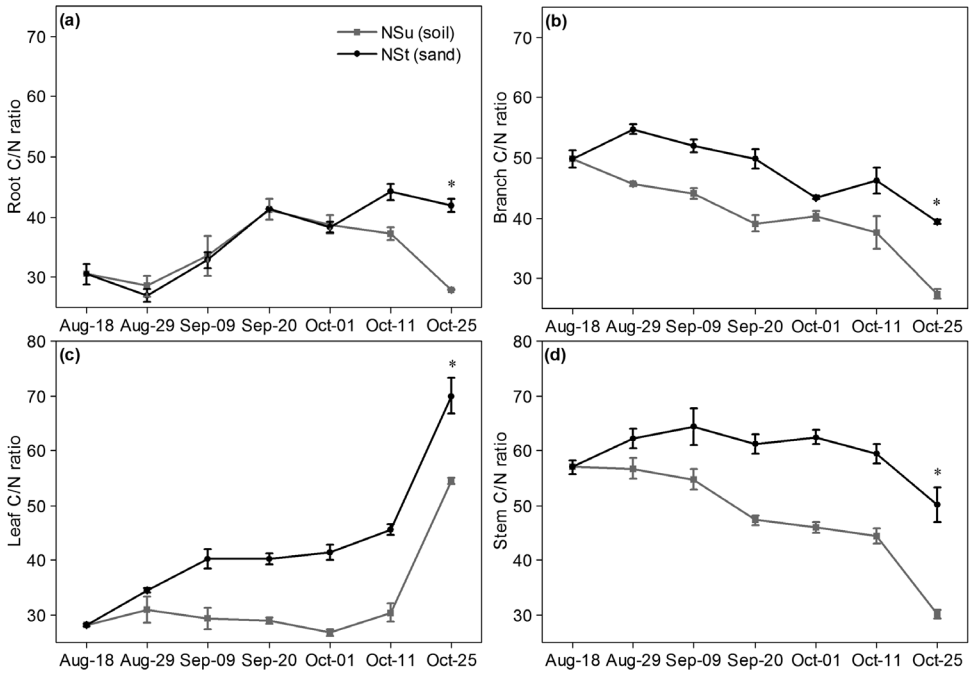

Figure 2: the C/N ratios of (**a**) roots, (**b**) branches, (**c**) leaves and (**d**) stems under NSu (soil) and NSt (sand) conditions. The asterisks represent significant differences in C/N status of the same organ between NSu and NSt conditions on 25 October (*$P < 0.05$). Mean values are given ± SE ($n = 3$).

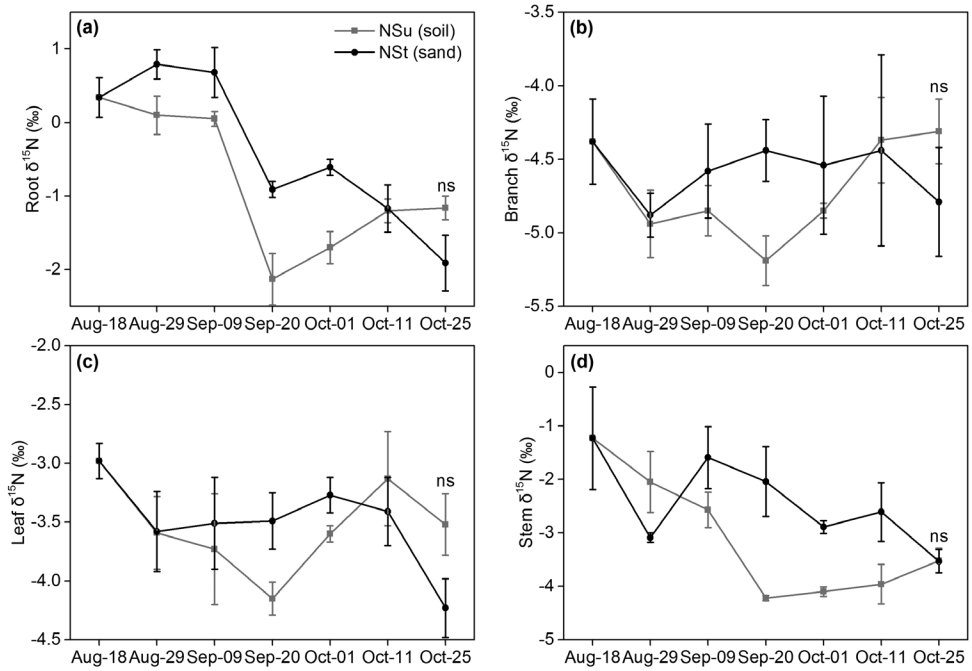

Figure 3: the $\delta^{15}N$ values of (**a**) roots, (**b**) branches, (**c**) leaves and (**d**) stems under NSu (soil) and NSt (sand) conditions. ns represents no significant difference in $\delta^{15}N$ of the same organ between NSu and NSt conditions on 25 October. Mean values are given ± SE ($n = 3$).

trend under NSt condition (Fig. 1). These results suggest that, under NSu conditions, leaves were acting as a resorption organ (nutrient source), whereas stems, branches and roots were acting as storage organs (nutrient sinks) during leaf senescence. By contrast, roots were acting as a resorption organ under NSt conditions. This is consistent with our hypothesis.

Table 1: results (*P* values) of repeated-measures ANOVA on the effects of soil type, sampling time, and their interactions on the N concentration, C/N ratio and $\delta^{15}N$ of different organs

	N			C/N ratio			$\delta^{15}N$		
	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*
Stem									
Soil type	1	202.43	**<0.001**	1	61.357	**0.001**	1	3.091	0.154
Sampling time	6	73.17	**<0.001**	6	34.089	**<0.001**	6	6.536	**<0.001**
Soil type × Sampling time	6	26.60	**<0.001**	6	9.182	**<0.001**	6	2.661	**0.040**
Branch									
Soil type	1	55.34	**0.002**	1	71.077	**0.001**	1	0.312	0.606
Sampling time	6	47.614	**<0.001**	6	42.891	**<0.001**	6	0.925	0.495
Soil type × sampling time	6	9.671	**<0.001**	6	5.606	**0.001**	6	0.866	0.534
Leaf									
Soil type	1	139.524	**<0.001**	1	170.020	**<0.001**	1	0.019	0.896
Sampling time	6	49.394	**<0.001**	6	109.759	**<0.001**	6	3.818	**0.008**
Soil type × sampling time	6	7.933	**<0.001**	6	8.150	**<0.001**	6	1.794	0.143
Root									
Soil type	1	2.909	0.163	1	61.357	**0.001**	1	2.679	0.177
Sampling time	6	20.538	**<0.001**	6	34.089	**<0.001**	6	21.930	**<0.001**
Soil type × sampling time	6	7.820	**<0.001**	6	9.182	**<0.001**	6	1.769	0.148

Numbers in bold indicate significant difference at $P < 0.05$

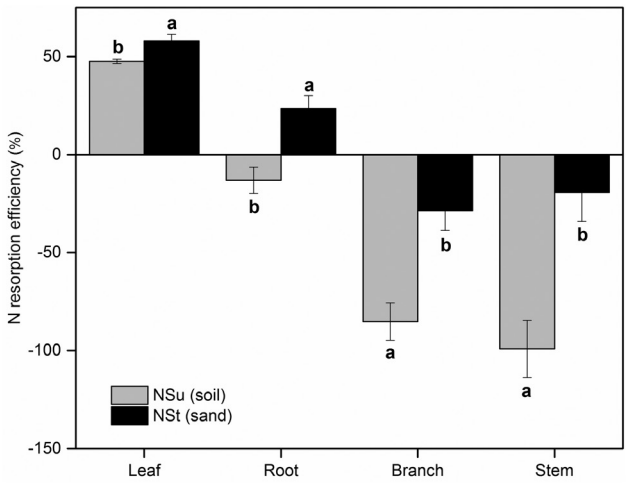

Figure 4: nitrogen resorption efficiency of leaves, roots, branches and stems under NSu (soil) and NSt (sand) conditions. Different letters indicate significant differences between NSu and NSt ($P < 0.05$). Mean values are given ± SE ($n = 3$).

The alteration in N concentrations also resulted in concomitant changes in the observed C/N ratios (Figs 1 and 2, Table 1). The opposite trends of C/N ratios of different organs compared with those of N concentrations were primarily mediated by the stable C but changed N concentrations of different organs. The N concentrations in branch, stem and root sharply increased, while the leaf N concentration sharply decreased on 11 October (Fig. 1), suggesting that nutrient resorption was intensely occurred on 11 October.

^{15}N natural abundance ($\delta^{15}N$) can potentially reveal how disturbances affect N cycles (Craine *et al.* 2015; Yan *et al.* 2018b). Generally, larch seedlings under NSu conditions were slightly less ^{15}N enriched than those under NSt conditions (Fig. 3). The most plausible explanation for this phenomenon may be that seedlings grown under NSu conditions can uptake N from soil via roots, and thus discrimination during this process would cause relatively more lighter in ^{15}N or less ^{15}N enriched in plant organs (Kahmen *et al.* 2008; Yan *et al.* 2018b). There existed overlap in $\delta^{15}N$ in roots, branches and leaves between NSu and NSt conditions around 11 October (Fig. 3). This pattern is probably because during the later stage, the more intensive occurrence of nutrient resorption (Fig. 4), and thus nutrient retransfer under NSt conditions resulted in more isotope discrimination (Yue *et al.* 2013). This agrees with the findings by Yue *et al.* (2013), who reported remarkable isotope discrimination associated with leaf resorption, but differs from the findings by Kolb and Evans (2002), who reported no detectable isotope discrimination during the process of leaf resorption. Roots were significantly more enriched in ^{15}N than the other organs (Fig. 3), which may be due to a lower nitrate reduction capacity in roots and/or substantial efflux of organic N from roots (Kalcsits *et al.* 2014; Kolb and Evans 2002). Similar results were also obtained by Kolb and Evans (2002) for *Quercus rubra* and *Q. alba*. In the condition of NSu, significant differences were only observed for root $\delta^{15}N$ before and after leaf senescence, whereas both root and leaf $\delta^{15}N$ exhibited significant differences before and after leaf senescence under NSt conditions (Fig. 3). These results suggest that detectable isotope discrimination occurred in root

under both NSu and NSt conditions, and for leaves under NSt condition during leaf senescence. Because both roots and leaves were resource-acquiring tissues, and they can perform physiological and biochemical activities (e.g. the assimilation and hydrolysis of organic materials), especially during leaf senescence, which would cause substantial N isotope discrimination (Kalcsits *et al.* 2014; Luo *et al.* 2015; Newman and Hart 2006).

Organ N resorption efficiency

In the present study, leaf NRE in NSt conditions (76.33%) was significantly higher than that NSu conditions (56.76%) (Fig. 4). This is consistent with our hypothesis and agrees with the general consensus, which holds that plants in nutrient-poor sites are expected to have higher nutrient resorption efficiency than those in nutrient-rich sites (See *et al.* 2015; Yan *et al.* 2016). Roots are capable for acquiring resources from soil and, accordingly, enabling plants to adapt to variations in environmental conditions (Zadworny *et al.* 2015). Fine roots can act as a sink as well as a source during the process of senescence (Gordon and Jackson 2000; Kunkle *et al.* 2009), but N resorption from senescing roots is frequently negligible (Silla and Escudero 2006). In the present study, larch seedlings occurred N resorption in both leaves and roots in response to NSt treatment, and the average NRE of leaves (76.33%) was 10.9 times higher than that of roots (7.02%). However, roots displayed resorption only under NSt conditions, which implied that roots have an immense positive effect on whole-plant nutrient strategies, especially during extraordinarily nutrient limiting conditions. This kind of nutrient redistribution is of tremendous importance in the economics, survival and growth of plants (Freschet *et al.* 2010; Kunkle *et al.* 2009).

The majority of the resorbed N was stored in woody tissues (von Fircks *et al.* 2001; Silla and Escudero 2006). During leaf senescence, stems are generally considered as a major sink for resorbed nutrients, which will be used for growth in the next growing season (Freschet *et al.* 2010; Milla *et al.* 2005; Silla and Escudero 2006). In our present study, leaves were priority N sources (resorption organs), whereas stems, branches and roots were acting as sinks (storage organs) during leaf senescence in NSu conditions (Figs 1 and 4). This is in agreement with the findings of Pregitzer *et al.* (1990) and Mei *et al.* (2015), who found that the majority of resorbed N from leaves were stored in roots, stems and branches following leaf fall, and with the findings of Kolb and Evans (2002), who found that roots and stems served as storage tissues for four woody deciduous species. In addition to leaves, roots also exhibited N resorption (7.02%) in the NSt condition, indicating that when subject to nutrient-deficiency conditions, roots play a similar role to leaves to retain more nutrients within larch individuals. That is, under NSt condition, the harsh situation triggered a shift of roots from nutrient sink to source during leaf senescence and demonstrated the plasticity of larch seedling to maximize nutrient conservation and minimize nutrient-deficiency stress.

Implications for seedling nutrient use strategy and regeneration

Taken together, nutrient resorption is an efficient nutrient conservation strategy, and the process of nutrient resorption in roots also plays an important role in plant economics when they are subjected to extreme nutrient stress. Such functions of leaf and root N use strategies reflect the buffering capacity of larch seedlings against soil nutrient stress (at least in the short term). Seedling establishment and first-year survival are crucial processes to the early performance of plantation forests, e.g. larch plantations (Villar-Salvador *et al.* 2015; Zhu *et al.* 2008). The resorbed nutrients are of critical importance for leaf regrowth and biomass growth of deciduous species in the following spring, particularly when the soil temperature is unfavorable for adequate N uptake by root systems (Martínez-Alcántara *et al.* 2011; Tomlinson *et al.* 2013). The potential implication of our present study is that larch seedlings might improve nutrient use efficiency and strategy to respond to the extreme stress of poor soil nutrients (at least in the short term) to relieve adverse effects on seedling survival and regeneration.

CONCLUSION

We investigated the resorption-related N changes of larch seedlings growing in two contrasting soil fertility conditions (NSu and NSt). Our results indicated that stems and branches serve as N storage organs (nutrient sinks) during leaf senescence; by contrast, the roots shifted from a storage organ to a resorption organ when switched from NSu to NSt conditions. The contrasting responses of roots to soil nutrient supplies indicated the plasticity in roots functions to adapt to nutrient starvation. The overlap in organ (roots, branches and leaves) $\delta^{15}N$ between the NSu and NSt conditions around 11 October may be regulated by more intensive isotope discrimination under NSt condition during the process of N resorption. Leaf NRE in NSt was significantly higher than in NSu. In summary, our findings highlight the importance of nutrient resorption (both by leaves and by roots) for plant nutrient economy and could improve our understanding of the adaptation mechanism and nutrient strategies of *L. kaempferi* under nutrient-starvation conditions. Our study supplies some clues for the management of seedling survival and regeneration in the field.

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Conflict of interest statement. None declared.

REFERENCES

Aerts R (1996) Nutrient resorption from senescing leaves of perennials: are there general patterns? *J Ecol* **84**:597–608.

Brant AN, Chen HYH (2015) Patterns and mechanisms of nutrient resorption in plants. *Crit Rev Plant Sci* **34**:471–86.

Chen FS, Niklas KJ, Liu Y, *et al*. (2015) Nitrogen and phosphorus additions alter nutrient dynamics but not resorption efficiencies of Chinese fir leaves and twigs differing in age. *Tree Physiol* **35**:1106–17.

Cleveland CC, Houlton BZ, Smith WK, *et al*. (2013) Patterns of new versus recycled primary production in the terrestrial biosphere. *Proc Natl Acad Sci USA* **110**:12733–7.

Craine JM, Brookshire ENJ, Cramer MD, *et al*. (2015) Ecological interpretations of nitrogen isotope ratios of terrestrial plants and soils. *Plant Soil* **396**:1–26.

Freschet GT, Cornelissen JH, van Logtestijn RS, *et al*. (2010) Substantial nutrient resorption from leaves, stems and roots in a subarctic flora: what is the link with other resource economics traits? *New Phytol* **186**:879–89.

Freschet GT, Swart EM, Cornelissen JH (2015) Integrated plant phenotypic responses to contrasting above- and below-ground resources: key roles of specific leaf area and root mass fraction. *New Phytol* **206**:1247–60.

Gordon WS, Jackson RB (2000) Nutrient concentrations in fine roots. *Ecology* **81**:275–80.

Guo QX, Li JY, Zhang YX, *et al*. (2016) Species-specific competition and N fertilization regulate non-structural carbohydrate contents in two *Larix* species. *Forest Ecol Manag* **364**:60–9.

Humbeck K (2014) Senescence in plants. *J Plant Growth Regul* **33**:1–3.

Jordan MO, Vercambre G, Le Bot J, *et al*. (2011) Autumnal nitrogen nutrition affects the C and N storage and architecture of young peach trees. *Trees Struct Funct* **25**:333–44.

Kahmen A, Wanek W, Buchmann N (2008) Foliar $\delta^{15}N$ values characterize soil N cycling and reflect nitrate or ammonium preference of plants along a temperate grassland gradient. *Oecologia* **156**:861–70.

Kalcsits LA, Buschhaus HA, Guy RD (2014) Nitrogen isotope discrimination as an integrated measure of nitrogen fluxes, assimilation and allocation in plants. *Physiol Plant* **151**:293–304.

Kobe RK, Lepczyk CA, Iyer M (2005) Resorption efficiency decreases with increasing green leaf nutrients in a global dataset. *Ecology* **86**:2780–92.

Kolb KJ, Evans RD (2002) Implications of leaf nitrogen recycling on the nitrogen isotope composition of deciduous plant tissues. *New Phytol* **156**:57–64.

Kunkle JM, Walters MB, Kobe RK (2009) Senescence-related changes in nitrogen in fine roots: mass loss affects estimation. *Tree Physiol* **29**:715–23.

LeBauer DS, Treseder KK (2008) Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. *Ecology* **89**:371–9.

Lü XT, Freschet GT, Flynn DFB, *et al*. (2012) Plasticity in leaf and stem nutrient resorption proficiency potentially reinforces plant–soil feedbacks and microscale heterogeneity in a semi-arid grassland. *J Ecol* **100**:144–50.

Luo J, Zhou J, Li H, *et al*. (2015) Global poplar root and leaf transcriptomes reveal links between growth and stress responses under nitrogen starvation and excess. *Tree Physiol* **35**:1283–302.

Magnani F, Mencuccini M, Borghetti M, *et al*. (2007) The human footprint in the carbon cycle of temperate and boreal forests. *Nature* **447**:848–50.

Mao R, Song CC, Zhang XH, *et al*. (2013) Response of leaf, sheath and stem nutrient resorption to 7 years of N addition in freshwater wetland of Northeast China. *Plant Soil* **364**:385–94.

Martínez-Alcántara B, Quiones A, Primo-Millo E, *et al*. (2011) Nitrogen remobilization response to current supply in young citrus trees. *Plant Soil* **342**:433–43.

Mason WL, Zhu JJ (2014) Silviculture of planted forests managed for multi-functional objectives: lessons from Chinese and British experiences. In Fenning T (ed). *Challenges and Opportunities for the World's Forests in the 21st Century* (pp. 37–54). New York, NY: Springer.

Mei L, Xiong Y, Gu J, *et al*. (2015) Whole-tree dynamics of non-structural carbohydrate and nitrogen pools across different seasons and in response to girdling in two temperate trees. *Oecologia* **177**:333–44.

Milla R, Castro-Díez P, Maestro-Martínez M, *et al*. (2005) Relationships between phenology and the remobilization of nitrogen, phosphorus and potassium in branches of eight Mediterranean evergreens. *New Phytol* **168**:167–78.

Newman GS, Hart SC (2006) Nutrient covariance between forest foliage and fine roots. *Forest Ecol Manag* **236**:136–41.

Pregitzer KS, Dickmann DI, Hendrick R, *et al*. (1990) Whole-tree carbon and nitrogen partitioning in young hybrid poplars. *Tree Physiol* **7**:79–93.

Qu LY, Shinano T, Quoreshi AM, *et al*. (2004) Allocation of ^{14}C-carbon in two species of larch seedlings infected with ectomycorrhizal fungi. *Tree Physiol* **24**:1369–76.

Reich PB, Luo Y, Bradford JB, *et al*. (2014) Temperature drives global patterns in forest biomass distribution in leaves, stems, and roots. *Proc Natl Acad Sci USA* **111**:13721–6.

See CR, Yanai RD, Fisk MC, *et al*. (2015) Soil nitrogen affects phosphorus recycling: foliar resorption and plant–soil feedbacks in a northern hardwood forest. *Ecology* **96**:2488–98.

Silla F, Escudero A (2006) Coupling N cycling and N productivity in relation to seasonal stress in *Quercus pyrenaica* Willd. saplings. *Plant Soil* **282**:301–11.

Tomlinson KW, van Langevelde F, Ward D, *et al*. (2013) Deciduous and evergreen trees differ in juvenile biomass allometries because of differences in allocation to root storage. *Ann Bot* **112**:575–87.

Tully KL, Wood TE, Schwantes AM, *et al*. (2013) Soil nutrient availability and reproductive effort drive patterns in nutrient resorption in *Pentaclethra macroloba*. *Ecology* **94**:930–40.

Ueda MU (2012) Gross nitrogen retranslocation within a canopy of *Quercus serrata* saplings. *Tree Physiol* **32**:859–66.

Ueda MU, Mizumachi E, Tokuchi N (2011) Foliage nitrogen turnover: differences among nitrogen absorbed at different times by *Quercus serrata* saplings. *Ann Bot* **108**:169–75.

Vergutz L, Manzoni S, Porporato A, *et al*. (2012) Global resorption efficiencies and concentrations of carbon and nutrients in leaves of terrestrial plants. *Ecol Monogr* **82**:205–20.

Villar-Salvador P, Uscola M, Jacobs DF (2015) The role of stored carbohydrates and nitrogen in the growth and stress tolerance of planted forest trees. *New Forest* **46**:813–39.

von Fircks Y, Ericsson T, Sennerby-Forsse L (2001) Seasonal variation of macronutrients in leaves, stems and roots of *Salix dasyclados* Wimm. grown at two nutrient levels. *Biomass Bioenerg* **21**:321–34.

Yan T, Lü XT, Yang K, *et al*. (2016) Leaf nutrient dynamics and nutrient resorption: a comparison between larch plantations and adjacent secondary forests in Northeast China. *J Plant Ecol* **9**:165–73.

Yan T, Lü XT, Zhu JJ, *et al*. (2018a) Changes in nitrogen and phosphorus cycling suggest a transition to phosphorus limitation with the stand development of larch plantations. *Plant Soil* **422**:385–96.

Yan T, Zhu JJ, Fang YT, *et al*. (2018b) Effects of thinning on nitrogen status of a larch plantation, illustrated by ^{15}N natural abundance and N resorption. *Scan J For Res* **33**:357–64.

Yan T, Zhu JJ, Yang K, *et al*. (2017) Nutrient removal under different harvesting scenarios for larch plantations in northeast China: implications for nutrient conservation and management. *Forest Ecol Manag* **400**:150–8.

Yang K, Shi W, Zhu JJ (2013) The impact of secondary forests conversion into larch plantations on soil chemical and microbiological properties. *Plant Soil* **368**:535–46.

Yuan ZY, Chen HY (2015) Negative effects of fertilization on plant nutrient resorption. *Ecology* **96**:373–80.

Yue YQ, Guo DL, Xiong YM, *et al*. (2013) The changes of $\delta^{15}N$ during leaf nitrogen resorption of tree species in Liangshui and Baotianman areas, China. *Ecol Environ Sci* **22**:379–86.

Zadworny M, McCormack ML, Rawlik K, *et al*. (2015) Seasonal variation in chemistry, but not morphology, in roots of *Quercus robur* growing in different soil types. *Tree Physiol* **35**:644–52.

Zhang M, Zhu JJ, Li MC, *et al*. (2013) Different light acclimation strategies of two coexisting tree species seedlings in a temperate secondary forest along five natural light levels. *Forest Ecol Manag* **306**:234–42.

Zhu JJ, Liu ZG, Wang HX, *et al*. (2008) Effects of site preparation on emergence and early establishment of *Larix olgensis* in montane regions of northeastern China. *New Forest* **36**:226–47.

Zhu JJ, Mao ZH, Hu LL, *et al*. (2007) Plant diversity of secondary forests in response to anthropogenic disturbance levels in montane regions of northeastern China. *J Forest Res* **12**:403–16.