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ORIGINAL PAPER

Combined effects of pre-hardening and fall fertilization on nitrogen translocation and storage in *Quercus variabilis* seedlings

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Abstract Maintaining proper seedling nitrogen status is important for outplanting success. Fall fertilization of evergreen conifer seedlings is a well-known technique for averting nitrogen (N) dilution caused by continued seedling growth during hardening. For deciduous seedlings, this technique is much less understood, and regardless of foliage type, the interaction of N status prior to fall fertilization and the rate of fall fertilization have yet to be fully explored. Therefore, we fertilized *Quercus variabilis* container seedlings with either 25, 100, or 150 mg total N seedling^{-1}, applied exponentially, during a 23-week pre-hardening regime, followed by either 0, 12, or 24 mg total N seedling^{-1} applied during hardening (i.e., fall fertilization) in equal aliquots for 4 weeks. For seedlings without supplemental N during hardening, N concentration in stems and roots increased significantly despite substantial growth. The absence of N dilution was attributed to N translocation from foliage to these tissues, which was independent of pre-hardening N status. Overall, 32 % of foliar N was translocated and accounted for 75 % of the total N increase in stems and roots. Final stem N status was a function of pre-hardening fertilization, whereas root N concentration was affected by the interaction of pre-hardening and fall fertilization. Roots appear to be the main site of N storage, and root N content was significantly affected by pre-hardening and fall fertilization, but not their interaction. A combination of pre-hardening and fall fertilizer at a rate of 100 and 24 mg total N seedling^{-1}, respectively, yielded seedlings with the largest root systems.

Keywords Pre-hardening fertilization · Fall fertilization · Nitrogen translocation · Nitrogen storage · Deciduous oaks

Introduction

Success of plantation establishment is closely associated with the availability of high-quality seedlings from nurseries (Davis and Jacobs 2005). Until newly planted seedlings grow roots into the surrounding soil, they mainly depend on their internal nutrient reserves (van den Driessche 1985; Rikala et al. 2004). New root growth is largely dependent on photosynthates originating in new shoots (van den Driessche 1987) and stored reserves must be remobilized to initiate this new growth (Timmer and Armstrong 1989). Thus, seedlings require sufficient nutrient reserves to maintain metabolic activity and initiate new root growth. During the hardening process in the nursery, however, fertilization is often reduced to encourage formation of buds (Landis et al. 1989), but continued growth can lead to nutrient dilution or internal nutrient deficiencies that may have a negative effect on subsequent field performance (Boivin et al. 2004).

Fertilization during the hardening process can improve seedling quality. Montville et al. (1996) found that adding nitrogen (N) during hardening could increase stem diameter without promoting height growth. Further, fall fertilization during hardening counters nutrient deficiencies and

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increases nutrient reserves in seedlings (van den Driessche 1985) that can improve field performance (Birchler et al. 2001). In addition to sustaining the seedling and supporting new root and shoot growth, nutrient reserves enhance the ability of seedlings to compete with natural understory vegetation (Timmer 1996; Boivin et al. 2004; Rikala et al. 2004). Therefore, fall fertilization, as a method of nutrient loading during hardening, has become a widely used technique for growing evergreen tree species including *Pinus* spp. (South and Donald 2002; Islam et al. 2009), *Picea* spp. (Boivin et al. 2002; Rikala et al. 2004; Jonsdottir et al. 2013), *Pseudotsuga menziesii* (Mirb.) Franco (Birchler et al. 2001), and *Quercus ilex* L. (Oliet et al. 2011; Andivia et al. 2012). Many researchers have demonstrated that foliage is the major N sink for evergreen seedlings and that fall fertilization increases N content of foliage (van den Driessche 1985; Margolis and Waring 1986).

In contrast to evergreen tree seedlings, most leaves of deciduous tree species abscise in the fall. Nitrogen moves from the foliar tissues to stems and roots as leaves abscise (Aerts 1996) and this might compensate for N dilution of stems and roots as they continue to add biomass during the fall. In our previous studies on *Larix olgensis* Henry, increasing N concentration of stems and roots was observed without additional fertilization despite a substantial increase in dry mass; this was attributed to translocation of N from senescing needles into perennial stems and roots (Li et al. 2012; Zhu et al. 2013). Moreover, fall fertilization further facilitated N storage in this deciduous coniferous tree through direct absorption of fall fertilization into stems and roots (Zhu et al. 2013). Considering the differences in the internal cycling of N between evergreen and deciduous trees (Aerts 1996; Millard and Grelet 2010) and the current good understanding of the relationship of fall fertilization and evergreen tree response (e.g., Rikala et al. 2004; Islam et al. 2009; Oliet et al. 2011), more information is needed on how fall fertilization affects the processes of N storage and translocation in deciduous seedlings, especially deciduous broadleaved trees.

Although research has dealt with the correlation of seedling quality with fall fertilization as it pertains to N source (van den Driessche 1985), rate (Birchler et al. 2001; Islam et al. 2009), timing (Oliet et al. 2011), and application methods (Birchler et al. 2001; Boivin et al. 2002, 2004), one area requiring more knowledge is how seedling nutrient level provided by pre-hardening fertilization influences the effectiveness of fall fertilization on seedling quality. If seedlings are grown with high rates of fertilizer and thus have stored substantial amounts of nutrients during the pre-hardening period (Timmer 1996), then fall fertilization may have little additional impact on seedling quality. Previous studies have focused on the individual effects of either pre-hardening or fall fertilization on stored nutrient reserves (Birge et al. 2006; Oliet et al. 2009, 2011; Salifu and Timmer 2001; Salifu et al. 2009; Villar-Salvador et al. 2012), not the combination. Thus, it is critical to study how pre-hardening and fall fertilization interact to affect foliar N translocation and subsequent N storage in stems and roots.

Specifically for oaks, the effects of pre-hardening fertilization on deciduous species (Birge et al. 2006; Salifu and Jacobs 2006; Salifu et al. 2009) and of fall fertilization on evergreen species (Oliet et al. 2011; Andivia et al. 2011, 2012) have been well documented. But, as is the case with deciduous species in general, pre-hardening fertilization in combination with fall fertilization has received little attention. Such information would be useful to improve the quality of Chinese cork oak (*Quercus variabilis* Blume), a source of industrial cork and one of the most valuable oak species native to China. Although it is commonly propagated in nurseries and some related cultural techniques, such as inoculation with mycorrhizal fungi on container seedlings and root pruning of bareroot seedlings, have been studied extensively (Luo et al. 2009; Zhao et al. 2009), very little is understood about the nutrient dynamics of this species. Therefore, we initiated a study to examine the individual and combined effects of pre-hardening and fall fertilization on N translocation and storage for this deciduous broadleaved species. We hypothesized that during container nursery production: (1) N dilution in stems and roots does not occur in Chinese cork oak seedlings during hardening because of N translocation from senescing leaves; (2) increased N status caused by pre-hardening fertilization will enhance N translocation during leaf abscission; (3) fall fertilization will facilitate foliar N translocation during hardening and thus increase the contribution of foliar N translocation to N increment in stems and roots; and (4) the interaction of pre-hardening and fall fertilization will affect N concentration and content in stems and roots.

Materials and methods

Plant material and treatments

Chinese cork oak acorns were collected from four parent trees in mid-September from the Chinese Cork Oak Center in Sizuolou Forest Farm (Beijing, China). On the day of collection, acorns were pooled together and immersed in hot water (50 °C) for 30 min to kill weevil larvae to prevent further damage. Acorns were then floated in water for 24 h to separate viable seeds; all floating acorns and those with visible damage were discarded. The remaining sound acorns were slightly air-dried in a single layer at ambient temperature for 2 days (Merouani et al. 2001; Tilki and

Alptekin 2006), placed in partially sealed polyethylene bags (100 μm thick, permeable to carbon dioxide and oxygen yet largely impermeable to moisture), and stored at 2 °C until the experiment began the following March (Bonner and Vozzo 1989; Kormanik et al. 1998).

The experiment was conducted in a greenhouse at the Chinese Academy of Forestry Sciences in Beijing (40°40′N, 116°14′E). To test our hypotheses, our experiment investigated the independent and interacting effects of three levels of pre-hardening N fertilization, three levels of fertilization during hardening (fall fertilization), and two sampling dates (onset of hardening and after fall fertilization). To ensure a sufficient number of seedlings would be available for sampling, we sowed 720 acorns on March 25, 2011 (one per container) at a depth of 1–2 cm in 1,050 ml containers (8 cm diameter × 20 cm deep) commonly used in China that were filled with a 3:1 (v:v) peat: vermiculite mixture. Fifteen containers were randomly assigned to each of 48 trays (44.5 cm long × 26.5 cm wide); thus, we initially employed 16 trays for each level of pre-hardening N fertilization.

Pre-hardening fertilization began on 11 April and continued for 23 weeks. Based on data for another *Quercus* species (Oliet et al. 2009), three pre-hardening fertilization regimes (25, 100, and 150 mg N per seedling) were chosen to approximate deficient, optimum, and luxury consumption, respectively, and were applied exponentially following Timmer and Aidelbaum (1996).

The rate of exponential fertilization was calculated according to Eq. (1):

$$N_T = N_s(e^{rt} - 1) \quad (1)$$

where N_s is the initial N content in each seed. In light of composite sample method (Salifu and Jacobs 2006), N_s was determined at the time of sowing using four replicates each comprising four acorns that were oven-dried (48 h at 65 °C), measured for dry mass, ground and wet-digested in a block digester using the $KMnO_4$–Fe–H_2SO_4 method modified to recover NO_3 (Bremner and Mulvaney, 1982). Subsequently, N concentration was measured by a standard Kjeldahl digestion with a distillation unit (UDK-152, Velp Scientifica, USA). N_s was calculated to be 33 mg per seed. The total number of fertilizations, t, was 23. N_T was the desired amount (25, 100, and 150 mg N) to be added over the number of fertilizer applications. Therefore, r, the relative addition rate required to increase N_s to final level $N_T + N_s$ of 58, 133, and 183 mg N was, 2.44, 6.05, and 7.43 %, respectively.

The quantity of N to apply for a specific week (N_t) was calculated using Eq. (2):

$$N_t = N_s(e^{rt} - 1) - N_{t-1} \quad (2)$$

where N_{t-1} is the cumulative amount of N applied during previous applications.

Each pre-hardening fertilization regime (hereafter, 25E, 100E, and 150E) was applied to 16 trays (240 seedlings per regime). N was supplied as urea (Xilong Chemical Co., China), a N source commonly used in Chinese nurseries. Elemental P and K were supplied as $Ca(H_2PO_4)_2{\cdot}H_2O$ (Damao Chemical Co., China) and K_2SO_4 (Xilong Chemical Co., China). We applied 2.6 mg P and 5 mg K seedling^{-1} every other week beginning in the second week of N fertigation for a total of 10 applications and a cumulative total of 26 mg P and 50 mg K. The desired amounts of N in addition to P and K were dissolved in 20 ml of water so that fertigation solution, applied by hand to each seedling, delivered the target amount of nutrients. Foliage was rinsed after each application to avoid foliar fertilizer burn. Additional irrigation was provided as necessary, about two times each week. Trays were completely randomized on raised benches and their position rotated every 2 weeks to minimize edge effect.

At the conclusion of pre-hardening fertilization (12 September), seedlings were individually surrounded by an open-topped white nylon bag in order to collect abscised leaves for periodic assessment. Following common practice, seedlings were reared under natural day-length in a greenhouse from sowing to 12 September. From 13 to 25 September, seedlings were exposed to a short-day treatment that extended the night length to 16 h. From sowing through the end of the short-day treatment, temperature, measured with a JL-18 Series thermometer (Huayan Instrument and Equipment Co., Shanghai, China) at 10-min intervals, averaged 25:18 °C (daily day: night).

Seedlings were first sampled on 26 September (week 25, *T*1 hereafter) to evaluate growth and nutritional responses resulting from the three pre-hardening N fertilization rates. Four of the 16 trays from each pre-hardening fertilization regime were randomly chosen and eight seedlings were randomly sampled from each tray (32 seedlings per pre-hardening N fertilization regime; 96 seedlings total). Seedlings were processed and composited as described below.

Of the remaining 12 trays per pre-hardening fertilization regime, groups of four trays were randomly selected and treated with one of three fall fertilization levels: 0, 12, or 24 mg N seedling^{-1} (hereafter, 0C, 12C, and 24C). These cumulative amounts were applied in four weekly, equal aliquots between 26 September and 17 October. We assumed these rates would substantially increase N reserves during the hardening period. The desired amount of N was supplied as urea; 20 ml of appropriate fertigation solution was added by hand to each seedling. Additional irrigation was applied as necessary. Because only a few blade tips were yellow on 15 October, all seedlings were moved outdoors on 24 October to hasten senescence. Within a few days (28 October), more than half of the leaves became dark yellow, and by 8 November, almost all the leaves had dried and some had abscised into the nylon

collection bags. During the N application period and until seedlings were moved outdoors, greenhouse temperatures averaged 19:14 °C (day: night). Once outside, seedlings were exposed to temperatures averaging 13:9 °C (day: night). Temperatures were monitored as described above.

Seedlings were sampled on 8 November (week 32, *T*2 hereafter) to evaluate seedling responses to the nine combinations of pre-hardening (25E, 100E, and 150E) and fall fertilization (0C, 12C, and 24C) regimes. At *T*2, eight seedlings per tray per combination of pre-hardening and fall fertilization treatments (32 seedlings per pre-hardening fertilization × fall fertilization treatment; 288 seedlings total) were randomly sampled and composited as described below.

Sampling and morphological and N assessments

At each sample date (*T*1 and *T*2), leaves (remaining on the stem and/or abscised) were collected to determine foliage dry mass. After roots were gently washed free of growing medium, seedlings were measured for height (root collar to the tip of the terminal bud) and root collar diameter (hereafter, RCD). Seedlings were then separated into stems and roots and oven-dried at 65 °C for 48 h to determine dry mass. Within each tray, each tissue fraction (stem, root, and foliage) of the eight seedlings was subsequently combined to a composite sample, ground, sieved through a 0.25 mm screen, and N concentration was determined as described above.

Net increment of N content in seedling tissues was defined as the difference (mg) observed between *T*1 and *T*2. Relative increment of N content (%) was calculated using Eq. (3):

$$\text{Relative increment} = \frac{T2 - T1}{T1} \times 100\ \% \quad (3)$$

where *T*1 was the average N content of the tissues from each pre-hardening fertilization level and *T*2 was the N content of the tissues from each tray of fall fertilization level within a pre-hardening level. For foliage, the net increment and relative increment were negative reflecting the translocation of N during hardening.

The contribution of foliar N translocation to net N increment in stems and roots (FNTC) was calculated using Eq. (4):

$$\text{FNTC} = \frac{\text{FNNI}}{S} \times 100\ \% \quad (4)$$

where FNNI was the absolute value of foliage net N increment and *S* was the total increase of N in stems and roots during hardening.

Statistical analyses

To test our hypotheses, we performed three statistical analyses using SPSS 16.0 (Chicago, Illinois, USA). First, a one-way ANOVA was used to evaluate effects of pre-hardening fertilization (25E, 100E, and 150E) on morphological and nutritional attributes pre-hardening. Second, to assess morphological and nutritional attributes pre-hardening (*T*1) and after senescence (*T*2) in the absence of fall fertilization, a *t* test was used to compare the average of all pre-hardening fertilization regimes at *T*1 with the average of the 0C treatments (no supplemental N during fall fertilization) at *T*2. Third, a two-way ANOVA was used to analyze the effects of pre-hardening fertilization, fall fertilization, and their interactions on morphological and nutritional attributes after senescence, and on the net and relative increments of N within leaves, stems, and roots, and FNTC during hardening (from *T*1 to *T*2) for a 3 × 3 factorial, randomized complete block design. When a significant interaction occurred between pre-hardening and fall fertilization, one-way ANOVA was used to examine specific significant differences among the nine treatment combinations of pre-hardening and fall fertilization. Separation of means for morphological and nutritional responses was ranked according to Duncan test at $\alpha = 0.05$. The explore function of SPSS was used to examine data prior to the *t* test and ANOVA to ensure normality and variance homogeneity requirements and no transformations were necessary.

Results

Growth and nitrogen dynamics of non-fall-fertilized seedlings during hardening

At the onset of fall fertilization (*T*1), seedlings given 25E had significantly less stem dry mass, foliar N concentration, and stem N content than seedlings receiving ≥100E, except for stem dry mass where the 25E and 100E rates were similar (Table 1).

During hardening (from *T*1 to *T*2), the non-fall-fertilized seedlings (0C; no supplemental fall N fertilization) had a significant (9.6 %) increase in stem dry mass, whereas foliage and root dry mass were unaffected (Table 2). N concentration in stems and roots significantly increased (30 and 15 %, respectively), whereas the concentration in foliage significantly decreased (28 %). A similar response was seen for N content (Table 2).

Combined fertilization effects on nitrogen translocation during hardening

The interaction of pre-hardening fertilization rate and fall fertilization rate was not significant for the net or relative N increment or the percentage of foliage N translocated to the stems or roots (Table 3). Only root net N increment was

Table 1 Means ± SE (n = 4) of Chinese cork oak seedling tissue dry mass and nitrogen (N) concentration and content at harvesting time *T*1 (26 September)

N rate (mg N seedling^{-1})	Dry mass (g)			N concentration (%)			N content (mg)		
	Foliage	Stem	Root	Foliage	Stem	Root	Foliage	Stem	Root
25	1.27 ± 0.06	0.68 ± 0.02a	3.60 ± 0.33	1.81 ± 0.03a	0.73 ± 0.02	0.98 ± 0.05	22.97 ± 1.03	4.93 ± 0.13a	34.69 ± 2.32
100	1.25 ± 0.04	0.71 ± 0.02a	3.24 ± 0.23	1.96 ± 0.04b	0.82 ± 0.01	1.24 ± 0.12	24.40 ± 0.45	5.76 ± 0.22b	39.57 ± 2.44
150	1.27 ± 0.07	0.79 ± 0.04b	3.39 ± 0.16	1.98 ± 0.01b	0.82 ± 0.03	1.15 ± 0.01	25.12 ± 1.18	6.45 ± 0.43b	38.81 ± 1.89
P value	0.866	0.028	0.090	0.008	0.064	0.087	0.259	0.007	0.255

Seedlings received pre-hardening fertilization applied exponentially to deliver 25, 100, or 150 mg N seedling^{-1}. Values within each tissue type marked with different letters are statistically different according to Duncan's test ($\alpha = 0.05$)

affected by pre-hardening N rate, with rates ≥100E having about 6 mg more N than the 25E treatment. Across the pre-hardening regimes, the average amount of foliar N translocated to stems and roots was 9.25 mg, 38 % of the original foliar N. This N accounted for, on average, 65 % of the total N stored in stems and roots.

Fall fertilization increased the amount of N translocated from foliage to stems and roots. Adding 12 or 24 mg of N during fall fertilization increased the net amount of N translocated to stems and roots, on average, 2.3 mg relative to the control (Table 3). Similar to the results for pre-hardening fertilization, fall fertilization increased the N increment in roots; the average total N increment was almost 6 mg compared with the control. Net N increments in stems and roots, on average, were 2.6 and 14.3 mg, respectively. The total N increment in roots accounted for 84 % of the N stored in stems and roots (Table 3).

Combined fertilization effects on seedling growth and nitrogen storage

At the end of growing season (*T*2), the interaction of pre-hardening and fall fertilization significantly affected root dry mass (Table 4). The combination of 100E pre-hardening and 24C fall fertilization yielded the greatest root dry mass (Fig. 1). Only height was affected by pre-hardening fertilization (Table 4), with seedlings receiving 100E having the greatest height (data not shown).

Foliar N concentration and content were affected by pre-hardening and fall fertilization, but not by their interaction (Table 4). For pre-hardening fertilization, N concentration was significantly higher in 100E and 150E than in 25E, but these two highest rates yielded similar results (Fig. 2). Interestingly, after fall fertilization, foliar N concentration was significantly lower in 12C and 24C compared with the control (Fig. 2). Foliar N content followed the same pattern as per N concentration for pre-hardening and fall fertilization (Fig. 3).

Stem N concentration and content were only affected by pre-hardening fertilization (Table 4), with the 100E and 150E rates yielding seedlings with similar concentrations and contents that were significantly greater than the 25E treatment (Figs. 2, 3).

Root N concentration was significantly affected by the interaction of pre-hardening and fall fertilization (Table 4). While under 100E and 150E, no significant differences appeared among fall fertilization treatments and seedlings receiving 25E pre-hardening plus 24C during fall yielded the highest amounts of N concentration in roots. All seedlings receiving either 100E or 150E pre-hardening fertilization had greater N concentrations than any combination of fall fertilization in combination with the 25E pre-hardening rate (Fig. 1). Pre-hardening and fall

Table 2 Means ± SE (n = 12) of Chinese cork oak seedling (without supplement nitrogen in the fall) tissue dry mass and nitrogen (N) concentration and content pre-hardening (26 September, *T*1) and after senescence (8 November, *T*2)

Harvesting time	Dry mass (g)			N concentration (%)			N content (mg)		
	Foliage	Stem	Root	Foliage	Stem	Root	Foliage	Stem	Root
*T*1	1.26 ± 0.03	0.73 ± 0.02	3.41 ± 0.14	1.91 ± 0.03	0.79 ± 0.02	1.12 ± 0.05	24.16 ± 0.56	5.71 ± 0.24	37.69 ± 1.33
*T*2	1.20 ± 0.03	0.80 ± 0.02	3.71 ± 0.11	1.37 ± 0.04	1.03 ± 0.04	1.29 ± 0.14	16.47 ± 0.78	8.33 ± 0.45	47.98 ± 1.80
P value	0.126	0.022	0.089	<0.001	<0.001	0.012	<0.001	<0.001	<0.001

P values are from a *t* test with $\alpha = 0.05$

Table 3 The net increment and relative increment of nitrogen (N) within foliage, stem, and root, and the percentage of N translocated from the foliage that contributed to stem and root N storage (FNTC) in relation to pre-hardening fertilization and fall fertilization during hardening (from *T*1 to *T*2)

Fertilization regime	N rate (mg N seedling^{-1})	Net increment (mg N)			Relative increment (%)			FNTC (%)
		Foliage	Stem	Root	Foliage	Stem	Root	
Pre-hardening	25	−9.40 ± 0.66	2.59 ± 0.51	10.95 ± 1.53a	−41.0 ± 2.81	52.9 ± 10.79	32.9 ± 4.95	79.6 ± 8.79
(PF)	100	−9.18 ± 0.60	2.89 ± 0.27	15.55 ± 2.21b	−37.6 ± 2.42	50.7 ± 4.93	40.5 ± 6.10	63.7 ± 11.98
	150	−9.16 ± 0.70	2.42 ± 0.32	16.33 ± 0.97b	−36.3 ± 2.38	38.6 ± 5.57	42.2 ± 2.38	51.0 ± 5.76
	P value	0.950	0.609	0.024	0.317	0.269	0.283	0.086
Fall fertilization	0	−7.70 ± 0.39a	2.62 ± 0.27	10.29 ± 1.48a	−32.2 ± 1.99a	45.4 ± 4.39	27.9 ± 3.92a	74.5 ± 12.38
(FF)	12	−10.32 ± 0.62b	2.30 ± 0.29	15.82 ± 1.84b	−42.8 ± 2.49b	41.3 ± 5.36	42.8 ± 5.15b	65.4 ± 8.05
	24	−9.73 ± 0.65b	2.98 ± 0.53	16.73 ± 1.38b	−40.1 ± 2.20b	55.4 ± 11.17	44.9 ± 3.79b	54.4 ± 7.40
	P value	0.012	0.378	0.006	0.007	0.310	0.019	0.278
PF × FF	*P* value	0.500	0.071	0.798	0.405	0.081	0.868	0.263

Negative values indicate a reduction in N. Mean ± SE within each fertilization regime and tissue type marked with different letters differ statistically according to Duncan's test $\alpha = 0.05$

fertilization, but not their interaction, significantly increased root N content (Table 4). The 100E and 150E pre-hardening rates and the 12C and 24C fall fertilization rates yielded similar, and significantly greater than the 25E and 0C rates, respectively, N contents (Fig. 3).

Discussion

In most evergreen tree species, fall fertilization is necessary to avoid N dilution (Rikala et al. 2004; Islam et al. 2009) because substantial growth during hardening causes N dilution (Miller and Timmer 1997; Boivin et al. 2002, 2004). During hardening of the deciduous Chinese cork oak in the absence of fall fertilization, we observed an increase in N concentration of stems and roots concurrent with significant increases in stem dry mass and slight increases in root dry mass, similar to results observed for the deciduous conifer, *Larix olgensis* (Li et al. 2012; Zhu et al. 2013). This increase in stem and root N concentration was in concert with large decreases in foliar N concentration and content, suggesting that the increases in N status in stems and roots resulted from N moving from senescing foliage to perennial tissues. In contrast to previous studies where N stored during hardening was derived more from direct N uptake from the growing medium into perennial tissues than from foliar N translocation (Weinbaum et al. 1987; Millard and Proe 1991), foliar N translocation made a greater contribution to Chinese cork oak seedlings than did fall fertilization alone. In the absence of fall fertilization, N translocation from foliage accounted for 75 % of the increase in total N in stems and roots (Table 3). Thus, we accept our first hypothesis that N dilution is avoided during hardening despite continued increases in growth because of N translocation from senescing foliage to stems and roots.

Even so, for our Chinese cork oak seedlings, only 32 % of foliar N in control seedlings (no fall fertilization) was translocated into stems and roots during hardening (Table 3). This value is similar to *Acer pseudoplatanus* L. seedlings (24–36 %) (Millard and Proe 1991) but lower than that observed in *Larix olgensis* seedlings (83 %) (Zhu et al. 2013), as well as lower than that reported by Aerts (1996) for either evergreen (47 %) or deciduous trees (54 %), suggesting that the amount of N that can be translocated from senescing foliage may be species

Table 4 *P* values derived from the ANOVA for effects of pre-hardening fertilization, fall fertilization, and their interaction on the morphological and nutritional attributes of Chinese cork oak seedlings at the end of growing season (8 November, *T*2)

Source	df	Height	RCD	Dry mass			N concentration			N content		
				Foliage	Stem	Root	Foliage	Stem	Root	Foliage	Stem	Root
Pre-hardening (PF)	2	0.033	0.737	0.432	0.404	0.518	0.002	<0.001	<0.001	0.021	0.016	<0.001
Fall fertilization (FF)	2	0.716	0.056	0.476	0.419	0.001	0.004	0.170	0.762	0.009	0.348	<0.001
PF × FF	4	0.055	0.238	0.998	0.120	0.042	0.220	0.058	0.002	0.470	0.055	0.535

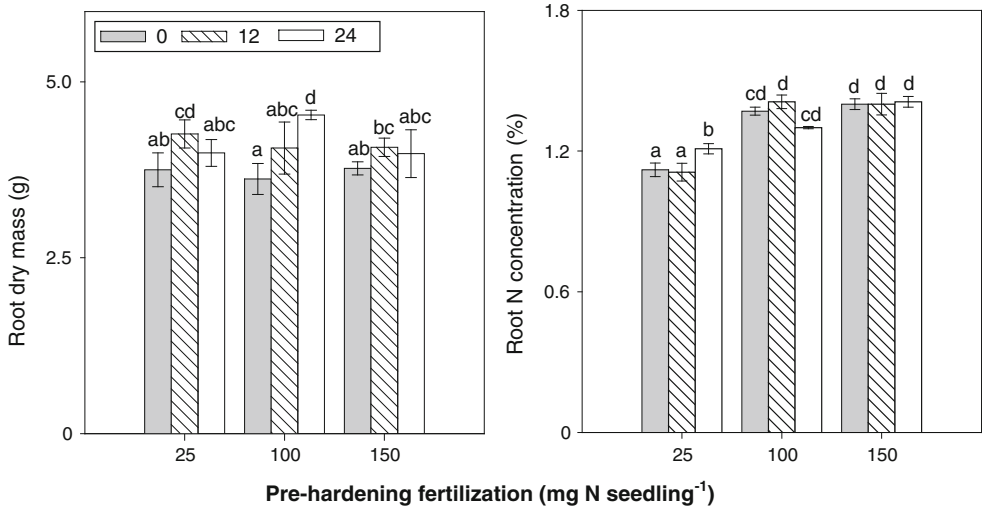

Fig. 1 The interaction of pre-hardening (25E, 100E, and 150E) and fall fertilization (0C, 12C, and 24C) on root dry mass (*left*) and N concentration (*right*) (mean ± SE) of Chinese cork oak seedlings at the end of growing season. *Bars* marked with *different letters* differ statistically according to Duncan's test $\alpha = 0.05$

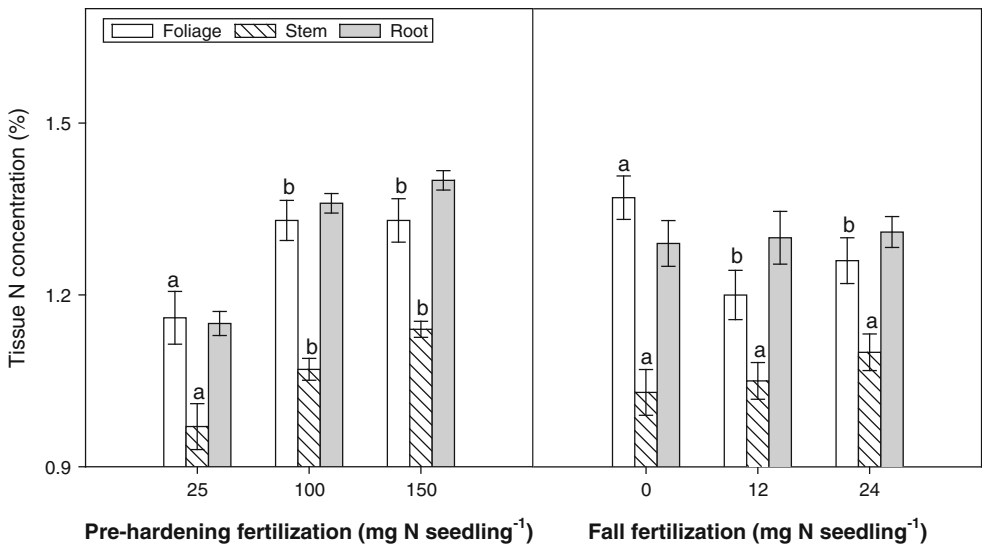

Fig. 2 Main effects of pre-hardening and fall fertilization on tissue N concentration (mean ± SE) of Chinese cork oak seedlings at the end of growing season. *Bars* marked with *different letters* differ statistically for each tissue type according to Duncan's test $\alpha = 0.05$. Because of the significant interaction effect between pre-hardening and fall fertilization, the mean separation for root N concentration response was not presented

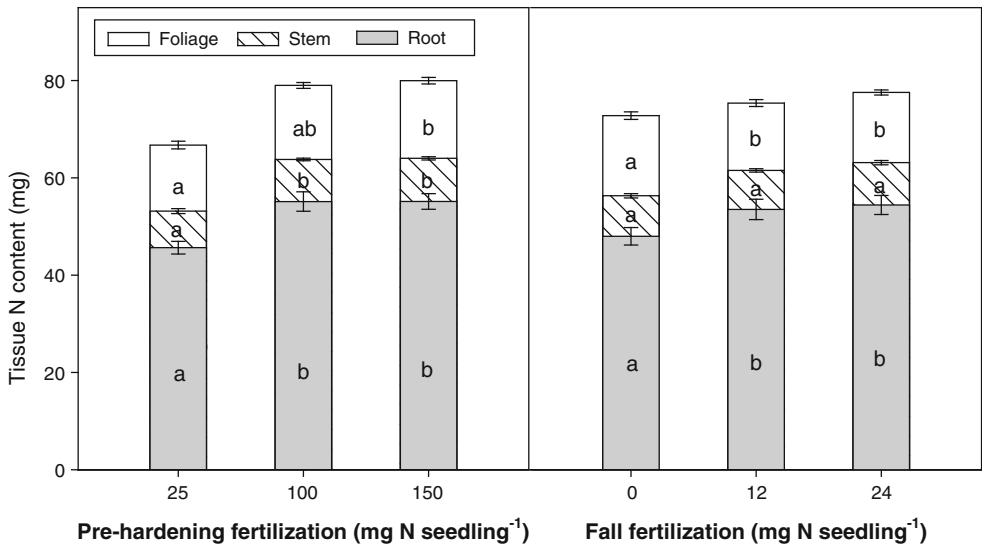

Fig. 3 Main effects of pre-hardening and fall fertilization on tissue N content (mean ± SE) of Chinese cork oak seedlings at the end of growing season. *Bars* marked with *different letters* differ statistically for each tissue type according to Duncan's test $\alpha = 0.05$

specific. In late fall and early winter, leaves of Chinese cork oak naturally become dry and eventually drop from the stem. The result is that some dried leaves may still be retained. Thus, the duration of the abscission period can be quite long, and when we sampled on 8 November, a few leaves were not completely dried. Therefore, the N recycling process may not have been completed and our values for foliar N translocation might be lower (more conservative) than if we had sampled after all leaves had finally abscised.

Several studies dealing with mature forest trees have shown that foliar N translocation is unaffected by nutrient availability (Ostman and Weaver 1982; Staaf 1982). Our seedlings shared this trait in that the amount of N supplied during pre-hardening fertilization did not influence foliar N translocation. These results, however, are not universal, as work with other deciduous tree seedlings found an opposite result. For example, *A. pseudoplatanus* seedlings had higher N translocation during fall senescence at low, rather than at high pre-hardening nutrient availability (Millard and Proe 1991). This could be a difference between tree species and age, or it may be that our foliar N contents, despite a range of N additions, were all similar prior to senescence and all within a favorable N status for Chinese cork oak. Overall, our finding that foliar N translocation during hardening was independent of pre-hardening N availability leads us to reject our second hypothesis.

Fall fertilization increased the relative and total amounts of N translocated from foliage to stems and roots. The contribution of foliar N translocation to the N increment in stems and roots was decreased to a degree with an increasing rate of fall-applied N fertilizer. High values of FNTC (54–75 %) also indicate that the increasing N status of perennial stems and roots originated from foliar N translocation. In contrast to Chinese cork oak, the increment and efficiency of N change in foliage were not significant among fall and non-fall-fertilized *Larix olgensis* seedlings; fall fertilization decreased the percentage of foliar N contributed to final stem and root N storage (Zhu et al. 2013). Thus, more comparison studies among other deciduous trees are needed to determine whether the relation between fall fertilization and N translocation is specific to foliar habit (e.g., evergreen versus deciduous). Therefore, we accept in part our third hypothesis, namely that fall fertilization facilitated foliar N translocation during hardening but did not impact the total contribution of foliar N translocation to N increment in stems and roots.

Similar to other deciduous oaks (Birge et al. 2006; Salifu and Jacobs 2006; Salifu et al. 2009), exponential fertilization of Chinese cork oak during the pre-hardening period benefited N storage of seedlings within a range of external N supply. Contrary to pre-hardening fertilization, fall fertilization improved N storage in roots, demonstrating that roots are a major N sink for fall fertilization in deciduous trees (Li et al. 2012; Zhu et al. 2013). For evergreen trees, foliage is the major sink of N storage (van den Driessche 1985; Millard and Grelet 2010), and the benefit of fall fertilization on enhancement of foliar N content is linked to field performance (Margolis and Waring 1986; Sung et al. 1997). There has been a general consensus that root reserves in deciduous trees play a fundamental role in establishment success (Aerts 1996). Therefore, a deeper understanding of the subsequent role of nursery fall fertilization calls for further work on deciduous

trees, with an emphasis on N remobilization during leaf development the following spring and field performance after several seasons.

Additional fertilization in the fall has been widely used in evergreen tree species (e.g., Birchler et al. 2001; Rikala et al. 2004; Islam et al. 2009; Oliet et al. 2011) and deciduous conifers (Li et al. 2012; Zhu et al. 2013) to avoid nutrient dilution during hardening. Although it appears to have merit for deciduous hardwood trees based on this study of Chinese cork oak, fall fertilization has had mixed effects on N concentration in stems and roots of deciduous species. In bareroot *Larix olgensis*, fall fertilization had no effect on stem N concentration but increased it in a container stocktype, whereas root N concentration was enhanced in both stocktypes (Li et al. 2012; Zhu et al. 2013). For Chinese cork oak, fall fertilization did not affect stem N concentration, but the interaction of pre-hardening and fall fertilization had a significant effect on root N concentration. These inconsistent results suggest that the effect of fall fertilization on N concentration may be dependent on not only stocktype and tissue type, but also the pre-hardening fertilization regimes as reported by Boivin et al. (2002) for the evergreen tree *Picea mariana* Mill. Therefore, our fourth hypothesis that the interaction of pre-hardening and fall fertilization affects N status in stems and roots is only partially correct: The interaction only influenced root N concentration and had no effect on N storage.

In practice, intensive nursery fertilization is viewed as a strategy to increase the nutrient content and improve potential field performance (Villar-Salvador et al. 2012; Oliet et al. 2013). At the end of the growing season, the studied Chinese cork oak seedlings had higher N content in stem and roots at 100E and 150E than at 25E, concurrent with a lack of a phytotoxic effect on dry mass at 100E and 150E. Thus, our highest pre-hardening fertilization treatment contributed to nutrient loading of the seedlings as defined by the conceptual model (Timmer 1996). Fall fertilization did increase N reserves in roots and thus could be regarded as a method to induce optimum seedling N status, especially for tree species with an indeterminate growth strategy during hardening (Schott et al. 2013).

Conclusions

For non-fall-fertilized seedlings, dry mass significantly increased in stems and to a lesser degree in roots during hardening. Meanwhile, N concentration in stems and roots was observed to increase significantly, indicating that continued growth did not contribute to their N concentration dilution. The lack of N dilution without supplemental fertilization was associated with foliar N translocation into stems and roots during senescence: 32.2 % of foliar N was translocated and accounted for 74.5 % of total N increment in stems and roots. Foliar N translocation was independent of pre-hardening N availability. In contrast, fall fertilization facilitated it. At the end of growing season, stem N concentration and content were influenced by pre-hardening fertilization, whereas root N concentration was impacted by the interaction of pre-hardening and fall fertilization. Root N content was significantly affected by pre-hardening and fall fertilization. Overall, nursery managers may wish to grow container Chinese cork oak seedlings with a 100E pre-hardening fertilizer regime followed with 24C applied as fall fertilization during hardening.

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