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Combined pre-hardening and fall fertilization facilitates N storage and field performance of *Pinus tabulaeformis* seedlings

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Exponential fertilization during the pre-hardening stage and fall fertilization during the hardening stage have each been used independently to nutrient load seedlings. However, nursery and field responses of seedlings to the combination of exponential fertilization and fall fertilization have received little attention. Chinese pine (*Pinus tabulaeformis* Car.) container seedlings were exponentially fertilized with accumulated totals of 40, 80 or 120 mg N per seedling during pre-hardening, and fall-fertilized with 0, 12, 24 or 48 mg N per seedling, and were subsequently outplanted and followed for two growing seasons. Interactions of exponential and fall fertilization had significant effects on plant N content in the nursery and first-year height after outplanting. Fall fertilization promoted additional nutrient loading during hardening for the 40-80 mg N per seedling pre-hardening regimes. The highest exponential fertilization rate enhanced N concentration in foliage and roots compared to the other two rates. Maximum diameter was observed in the lowest exponential fertilization rate at the second year after outplanting. Fall fertilization enhanced foliar N concentration. Supplemental 12 and 24 mg N per seedling during fall were more effective in increasing height increment at the second year after outplanting. Our results indicate that pre-hardening fertilization is a useful tool to nutrient load Chinese pine in the nursery and facilitate outplanting performance in the field. In combination, fall fertilization has potential to further augment this response, although further research is needed to precisely match rates of pre-hardening and fall fertilization to optimize seedling performance.

Keywords: Exponential Fertilization, Fall Fertilization, Nutrient-loading, Field Performance, Chinese Pine

Introduction

More nitrogen (N) is retranslocated from perennial tissues to new apical growth tissues in seedlings with rich N reserves, especially when seedlings are outplanted in poor fertility soil (Salifu & Timmer 2003a, Oliet et al. 2009, Villar-Salvador et al. 2012). Thus, additional N fertilization during nursery culture may provide an effective solution for enhancing field performance and has received worldwide attention (Timmer 1996, Andivia et al. 2011, Villar-Salvador et al. 2012, Oliet et al. 2013).

Exponential fertilization during the pre-hardening stage and fall fertilization during the hardening stage are two approaches to facilitate N storage in seedlings (Timmer 1996, Sung et al. 1997, Dumroese 2003). Exponential fertilization reflects application of fertilizer at exponential rates corresponding closer to the desired relative growth rate of plants during their exponential phase of growth (Timmer 1996, Birge et al. 2006). It synchronizes nutrient supply with crop demand, referred to as steady-state nutrition (Imo & Timmer 1992), and has proven to facilitate nutrient storage in a variety of tree species (Timmer & Armstrong 1987, Miller & Timmer 1994, Quoreshi & Timmer 2000, Salifu & Timmer 2001, 2003b, Qu et al. 2003). Previous studies comparing one exponential fertilization rate with one or more conventional fertilization rates, have effectively demonstrated that greater plant N reserves induced by exponential fertilization have the potential to promote seedling field performance over conventional fertilization (Dumroese et al. 2005, Close et al. 2005, Way et al. 2007). When outplanted onto a field site, how seedlings respond to varying exponential fertilization rates in the nursery has been studied on several species, including western hemlock (*Tsuga heterophylla* (Raf.) Sarg. – Hawkins et al. 2005), northern red oak (*Quercus rubra* L.) and white oak (*Q. alba* L. – Salifu et al. 2009). However, less attention has been given to pines (*Pinus* spp.), which are among the most widely distributed and planted species worldwide.

Fall fertilization is another approach to facilitate nutrient loading. Fall fertilization could counter nutrient dilution due to biomass accumulation and promote storage of nutrient reserves in seedlings (Van Den

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Driessche 1985). Therefore, it has been implemented in a variety of tree species such as loblolly pine (*Pinus taeda* L. – Sung et al. 1997), slash pine (*Pinus elliottii* var. *elliottii* Engelm. – Irwin et al. 1998), red pine (*Pinus resinosa* Ait. – Islam et al. 2009), Norway spruce (*P. abies* (L.) Karsten – Rikala et al. 2004, Heiskanen et al. 2009), black spruce (*Picea mariana* Milli.B.S.P. – Boivin et al. 2004), Lutz spruce (*Picea x lutzii* Littl. – Jonsdottir et al. 2013), Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco – Van Den Driessche 1985, Margolis & Waring 1986a, Margolis & Waring 1986b), holm oak (*Q. ilex* spp. *ballota* Desf. – Oliet et al. 2011, Andivia et al. 2011), Olga Bay larch (*Larix olgensis* Henry – Li et al. 2012, Zhu et al. 2013).

In addition, the efficacy of fall fertilization on seedling nutrient reserves is dependent on pre-hardening nutrient status (Boivin et al. 2004, Li et al. 2014). If seedlings are provided with high rates of fertilizer and thus have substantial amounts of stored nutrients during the pre-hardening period, then fall fertilization has little additional impact on nutrient reserves (Boivin et al. 2004). For example, in the deciduous Chinese cork oak (*Quercus variabilis* Blume), root N concentration was affected by the interaction of pre-hardening and fall fertilization (Li et al. 2014). Due to variable strategies of nursery fertilization among tree species (Valdecantos et al. 2006), little information is available on how the combination of pre-hardening and fall fertilization impacts nutrient storage of *Pinus* spp. seedlings.

Stored nutrients in nursery seedlings are often correlated with early seedling survival after outplanting (Van Den Driessche 1985, Villar-Salvador et al. 2012), and nutrient differences among fall fertilization treatments are influenced by pre-hardening fertilization (Boivin et al. 2004, Li et al. 2014). Earlier papers have primarily dealt with the relationship between field performance and pre-hardening or fall fertilization independently (Quoreshi & Timmer 2000, Rikala et al. 2004, Hawkins et al. 2005, Close et al. 2005, Dumroese et al. 2005, Oliet et al. 2011, 2013), but expanded study aimed at understanding how their interactive effects impact nursery and field performance are needed, especially for species of widely planted genera such as *Pinus*.

Chinese pine (*Pinus tabulaeformis* Carr.) is a native evergreen coniferous species and the most widely planted species in northern China. Because of its resistance to drought and low soil fertility, it is planted across a wide range of site types and is often exposed to environmental stresses (Xu et al. 1981, Wu & Feng 1994). Nursery fertilization has been shown to promote Chinese pine seedling nutrient reserves (Huang et al. 2004, Wang 2014), suggesting potential to increase stress resistance and improve outplanting performance (Villar-Salvador et al. 2012, Oliet et al. 2013). Thus, our objectives were to: (1) examine the combined effects of these two nutrient loading approaches on Chinese pine seedling nutrient storage in the nursery; and (2) assess how field performance responds to the application of combined exponential and fall fertilizations in the nursery. We focused on N because this nutrient is often the most limiting factor for seedling growth in peat-growing media (Folk et al. 1992), is required in the largest amount for intensive nursery culture of conifers (Salifu & Timmer 2003b), and is highly correlated with field performance after outplanting (Villar-Salvador et al. 2012, Oliet et al. 2013). We hypothesized that: (1) pre-hardening and fall fertilization would each promote nutrient reserves in Chinese pine seedling yet vary in their effectiveness; (2) pre-hardening and fall fertilization would be more advantageous in combination than individually; and (3) outplanting performance would be strongly correlated with seedling growth and nutrient storage from the nursery phase.

Materials and methods

Nursery fertilization treatments and outplanting trial

The experiment was conducted in a greenhouse at the Chinese Academy of Forestry Sciences in Beijing (40° 40′ N, 116° 14′ E). Our experiment investigated the main and interacting effects of three rates of pre-hardening N fertilization and four rates of N fertilization during hardening (fall fertilization). On 21 February 2010, seeds from a regional seed orchard (National Seed Orchard for Chinese Pine, Qigou Forest Farm, 41° 00′ N, 118° 27′ E, 526 m a.s.l.) were sown into plastic containers (8 cm diameter × 8 cm deep, 187 ml capacity) filled with a 3:1 (v:v) peat:vermiculite medium. Thirty containers were randomly assigned to a tray (53 cm long × 44.5 cm wide), providing a density of 127 seedlings m^{-2}.

Three pre-hardening fertilization rates based on another *Pinus* species study (Miller & Timmer 1994), starting 1 week after germination and continuing for 25 weeks, were tested: (i) 40 mg N per seedling; (ii) 80 mg N per seedling; and (iii) 120 mg N per seedling following exponential (E) functions (McAlister & Timmer 1998, Dumroese et al. 2005). A total of 48 trays were arranged in a completely randomized design with sixteen trays for each rate.

In this study, the average initial N content per seed (N_S) was determined to be 1.58 mg N. The total number of fertilizations (*t*) was 25. N was supplied as urea (Xilong Chemical Co., China), a N source commonly used in Chinese nurseries. Elemental P and K were supplied as KH_2PO_4 (Guangdong Guanghua Sci-Tech Co. Ltd., China). On 16 August the pre-hardening fertilization was terminated, each seedling received total amounts of 26.2 mg P and 32.9 mg K which were evenly split and applied weekly in addition to the N fertilizer (1 March-16 August, 2010). The desired amounts of N, P and K were dissolved in distilled water and added manually to each seedling once per week with a hand-sprayer. No micronutrients were applied during the experiment. Seedlings were rinsed after each application to avoid foliar fertilizer burn. Additional irrigation was provided as necessary, about two times each week. The trays on raised benches were rotated every week to minimize edge effect.

Fall fertilization started two weeks after the pre-hardening fertilization ended when most seedlings had already formed the terminal buds, and lasted for four weeks (30 August-20 September, 2010). The doses of N were evenly split and applied weekly as four fall fertilization rates: (i) control (no additional fertilization); (ii) 12 mg N per seedling; (iii) 24 mg N per seedling; and (iv) 48 mg N per seedling. Fall fertilization was applied within each of the three pre-hardening regimes (40, 80, 120 mg N per seedling) as a factorial 3×4 design that was randomly arranged with four replications per treatments combination. The desired amount of N was supplied as urea; 20 ml of appropriate fertigation solution was added by hand to each seedling. On 24 October, all seedlings were moved outdoors to hasten hardening. After 28 November, seedlings were stored under snow cover over winter.

Temperature was measured with a JL-18 Series thermometer (Huayan Instrument and Equipment Co., Shanghai, China) at 10-min intervals. From sowing throughout the end of the pre-hardening fertilization, the daily average for day:night temperature in the greenhouse was 25:18 °C, respectively. During the fall N application period and until the 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).

On 1 May 2011, seedlings from the twelve fertility treatments were shipped and outplanted in a previously cultivated land at the Beijing Forestry University Northern Experimental Base at Pingquan, Hebei province (41° 13′ N, 118° 40′ E). The soil was ploughed before outplanting. Weeds were removed by hand. The site has an average elevation of 765 m a.s.l. with a small slope (< 2%). The depth of soil varied between 45 and 60 cm. The surface soil (0-20 cm) is 73% sand, 11% silt, and 16% clay (a sandy clay loam) with a pH of 6.2 and soil organic carbon of 0.7%. Average total N, available P, and available K were 628.7, 139.5, and 113.5 mg kg^{-1}, respectively. The soil was considered to have moderate fertility according to the macronutrient classification criteria (Bao 2000). The area has a temperate continental monsoon climate, characterized by dry winter and spring seasons. During 2011 and 2012, mean annual air temperature was 7.4 °C and 6.8 °C, respectively. Annual rainfall was 486 mm in 2011 and 626 mm in 2012.

The field experiment was arranged as a

Tab. 1 - P-values derived from ANOVA of the main effects pre-hardening fertilization (PF), fall fertilization (FF) and of their interaction effect (PF×FF) on Chinese pine seedling biomass, N concentration and N content prior to plating.

Source	df	Biomass				N concentration				N content			
		Foliage	Stem	Root	Plant	Foliage	Stem	Root	Plant	Foliage	Stem	Root	Plant
PF	2	0.829	0.417	0.360	0.273	<0.001	0.191	<0.001	<0.001	0.070	0.076	0.061	<0.001
FF	3	0.152	0.784	0.892	0.624	0.001	0.434	0.120	0.867	0.373	0.493	0.248	0.137
PF × FF	6	0.407	0.205	0.113	0.346	0.147	0.219	0.832	0.919	0.520	0.375	0.083	0.010

randomized complete block experimental design with four replicates. Each block measured 14×16.5 m^2 and was separated from adjacent blocks by 1 m buffers. Fifteen randomly selected seedlings from a tray per treatment were planted in single parallel rows within each block, resulting in a total of 720 seedlings planted. Seedlings were planted in 0.4×0.4×0.4 m manually dug planting holes with a 1×1.5 m^2 spacing.

Harvesting and measurements

Seedlings were sampled prior to planting to evaluate biomass allocation and N storage responses to nursery fertilization. Thirty-two seedlings per nursery fertilization treatment (eight seedlings per replicated tray) were randomly chosen. After carefully washing, seedlings were separated into needles, stems and roots. Organs of seedlings were oven-dried at 65 °C for 48 h to determine biomass. Organ N concentration was determined from a composite sample of eight seedlings for each treatment from a tray using semi-micro Kjeldahl distillation (Bremner & Mulvaney 1982).

Immediately after planting on 2 May 2011, seedling height and diameter were measured (T_1). When seedling growth ceased in late October of 2011 (T_2) and 2012 (T_3), survival, height and diameter were measured. Survival was calculated as the number of saplings of the original fifteen remaining alive for each nursery-treatment replicate at the time of each annual measurement. Diameter was measured at ground rate on each tree stem, and total seedling height was measured from the root collar to the tip of the terminal bud. Net increment of height in cm (or diameter in mm) was defined as the difference observed between T_2 and T_1, and between T_3 and T_2.

Statistical analyses

Effects of pre-hardening fertilization (3 rates), fall fertilization (4 rates), and their interactions were assessed using a two-way analysis of variance (ANOVA) applied on a completely randomized design at the nursery experiment and on a randomized complete block design in the outplanting trial. When ANOVA results showed a significant effect, *post-hoc* Duncan's test was carried out for multiple comparisons among treatments (α=0.05). Statistical analyses were performed using SPSS® 16.0 (Chicago, Illinois, USA). The "explore" function of SPSS was used to examine data prior to ANOVA, and survival was arsine-transformed to fulfill normality and variance homogeneity requirements.

Results

Nursery phase

Fertilization factors and its interaction did not significantly affect biomass of specific organs or the whole plant (Tab. 1). The foliage, stem, and root biomass of seedlings averaged 0.33 ± 0.01, 0.16 ± 0.001, and 0.40 ± 0.01 g, respectively. N concentration in foliage, root and whole plant was higher at 120 than at 40 and 80 mg N per seedling of per-hardening fertilization. Fall fertilization enhanced foliar N concentration relative to the control (Tab. 1, Fig. 1). Organ N content did not differ among pre-hardening fertilization or fall fertilization, although pre-hardening fertilization marginally affected N content of three organs (Tab. 1). Overall mean of N content was 7.7 ± 0.16, 3.5 ± 0.08, and 6.7 ± 0.13 mg in foliage, stem and root, respectively. The N content of whole plants, however, was influenced by the interaction of pre-hardening fertilization and fall fertilization (Tab. 1, Fig. 2). At the lowest pre-hardening fertilization regime (40 mg N per seedling), 24 mg N per seedling of fall fertilization significantly increased the N content. At 80 mg N per seedling of pre-hardening fertilization regime, 12 mg N per seedling of fall fertilization yielded a similar effect.

Field performance

For all field attributes, the interaction of pre-hardening fertilization and fall fertilization only had a significant effect on total height at the first growing season after outplanting (Tab. 2). The combination of pre-hardening at 40 mg N per seedling and 24 mg N per seedling of fall fertilization (24 mg) yielded the maximum height (Fig. 2). Within the highest pre-hardening fertiliza-

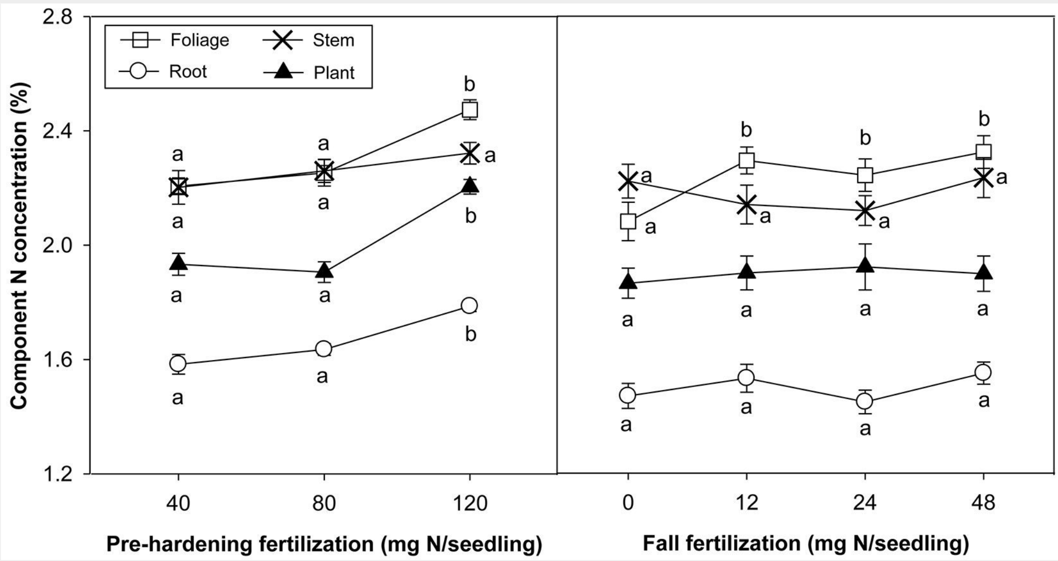

Fig. 1 - Chinese pine seedling organ N concentration (means ± SE) in relation to pre-hardening and fall fertilization prior to planting. No significant interaction between pre-hardening and fall fertilization occurred in N concentration. Bars marked with different letters significantly differed for each organ and the whole plant after Duncan's test (α = 0.05).

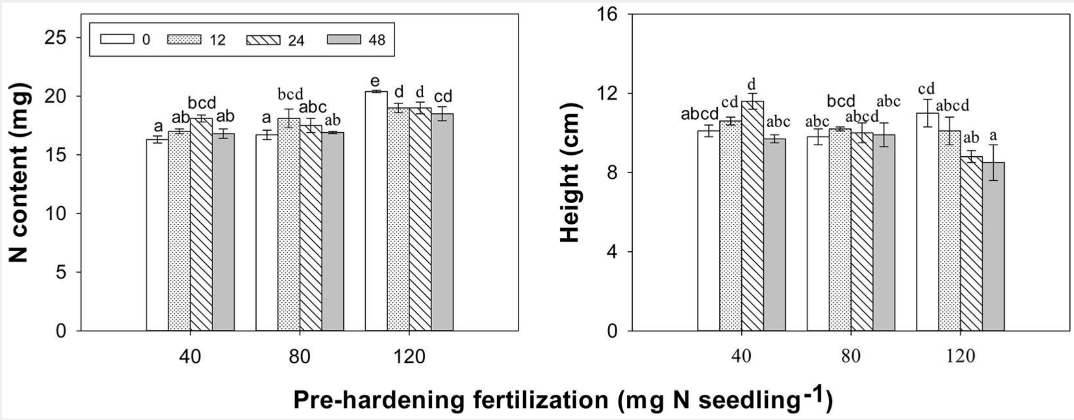

Fig. 2 - Effect of combination treatments between pre-hardening fertilization and fall fertilization (0, 12, 24, 48) on Chinese pine seedling N content (mg N/seedling) prior to planting and total height (cm) at the end of first growing season (2011) after planting. Means ± SE marked with different letters significantly differed after Duncan's test (α = 0.05).

Tab. 2 - P-values derived from ANOVA of main effects pre-hardening fertilization (PF), fall fertilization (FF) and of their interaction effect (PF×FF) on Chinese pine seedlings survival, total height, diameter and their increments prior to planting (Initial), at the end of first (2011) and second (2012) growing seasons after planting.

Source	Height			Height increment		Diameter			Diameter increment		Survival	
	Initial	2011	2012	2011	2012	Initial	2011	2012	2011	2012	2011	2012
PF	0.028	0.054	0.079	0.005	0.231	0.002	0.088	0.004	0.022	0.005	0.161	0.373
FF	0.458	0.087	0.031	0.021	0.029	0.303	0.201	0.309	0.138	0.366	0.458	0.793
PF×FF	0.780	0.026	0.682	0.077	0.699	0.094	0.677	0.786	0.937	0.834	0.847	0.598

tion regime, 24 and 48 mg N per seedling of fall fertilization decreased height compared to the control.

Pre-hardening fertilization had no significant effect on survival either during the first or second growing season (Tab. 2). Survival of 40, 80 and 120 mg N per seedling of pre-hardening fertilization was 90%, 95%, and 89% in the first growing season, and was 79%, 79%, and 70% in the second growing season, respectively. Pre-planting height was superior for the highest pre-hardening fertilization rate. However, first-

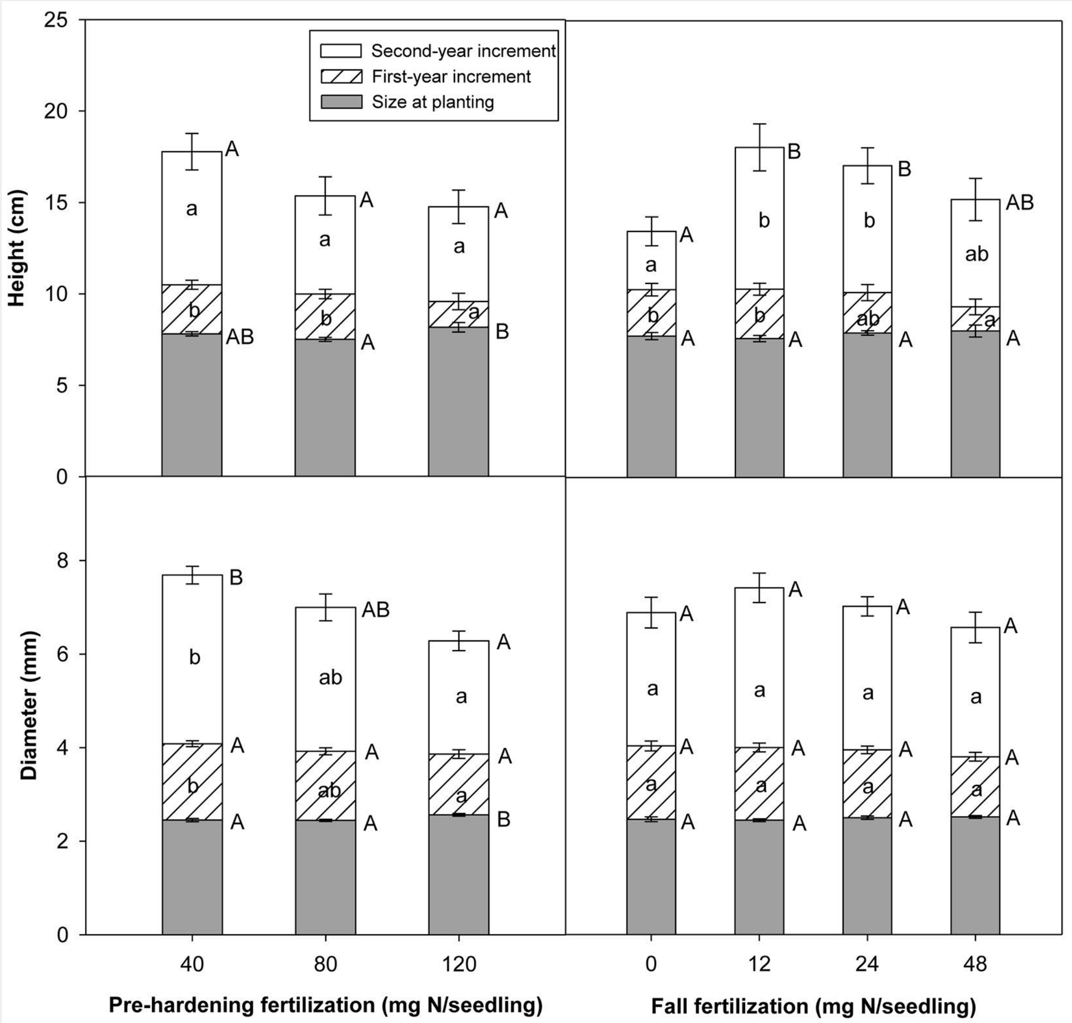

Fig. 3 - Chinese pine seedlings total height, diameter and their increments in relation to pre-hardening and fall fertilization at planting (Initial), at the end of first and second growing seasons after planting. Error bars represent SE of increments at the end of first and second growing seasons, and of size prior to planting. Bars marked with different capital and lower case letters significantly differed for total height (diameter) and its increment according to Duncan's test (α = 0.05), respectively. Comparison letters for first-year height are not presented owing to an occurring interaction.

year height increment of later rate was minimum. There was no significant difference in second-year height among pre-hardening rates. The dominance of total height in the highest exponential fertilization vanished at end of the second growing season (Tab. 2, Fig. 3). Both first-year and second-year diameter increments were superior in the 40 mg N per seedling of pre-hardening fertilization treatment, with the greatest diameter occurring in the lowest pre-hardening fertilization rate at the end of second growing season (Tab. 2, Fig. 3).

Fall fertilization also did not affect survival of Chinese pine seedlings. Survival of the control, 12, 24 and 48 mg N per seedling of fall fertilization was 92%, 93%, 93%, and 87% in the first growing season, and was 81%, 74%, 77%, and 73% in the second growing season, respectively. Pre-planting height and diameter were similar between fall-unfertilized and fertilized seedlings. Compared to the control, significant height increments in 12 and 24 mg N per seedling of fall fertilization treatments were not observed until the second growing season.

Discussion

Effect of pre-hardening fertilization on nursery and field performance

Under luxury N consumption, seedling dry mass remains unchanged but tissue N content continuously increases (Timmer 1996, McAlister & Timmer 1998, Salifu & Timmer 2003a, Birge et al. 2006). As defined by the conceptual model (Timmer 1996), absence of effects of exponential fertilization on biomass indicated that 40 mg N already reflected luxury consumption for the studied seedlings. This result is similar to our previous study on Chinese pine seedlings (Wang et al. 2015). Deficiency, luxury consumption, and toxicity have been determined in species of *Quercus* and *Picea* by evaluating the nursery response to more than six exponential fertilization rates (Salifu & Timmer 2003b, Birge et al. 2006, Salifu & Jacobs 2006). For red pine, Timmer & Armstrong (1987) proposed that 9.75 mg N yielded luxury consumption. In another study of red pine, however, 25 mg N was not designated as luxury consumption because a significant increase in biomass was still detected at 75 mg N (Miller & Timmer 1994). These conflicting results for *Pinus* spp. suggest that a wider range of nutrient availability should be tested to more accurately quantify N luxury consumption under varying cultural systems.

In recent years, there has been a trend to determine nursery fertilization in light of field performance on a specific site condition as well as seedling biomass and nutrient reserves accumulated during nursery growth (Hawkins et al. 2005, Heiskanen et al. 2009, Oliet et al. 2009, Jonsdottir et al. 2013, Villar-Salvador et al. 2013). For example, in very dry Mediterranean environments some studies showed that seedlings with low nutrient concentration or content performed better (Trubat et al. 2008, 2010, Cortina et al. 2013), whereas other studies indicated that increased tissue nutrient reserves improves seedling field performance (Luis et al. 2009, Cuesta et al. 2010, Villar-Salvador et al. 2012, Oliet et al. 2013). These contrasting results suggest that insight into nursery fertilization and field outplanting should be further tested, in particular with relation to climatic planting conditions or soil characteristics. In the present study, greater plant N reserves induced by the highest exponential fertilization rate did not promote survival and growth performance in the following two growing seasons. In contrast, the lowest pre-hardening fertilization rate (40 mg) yielded desirable growth performance. These findings suggest that, under the relatively dry conditions of our study, rational reduction of nursery fertilization might be a choice to improve seedling outplanted performance once these nursery fertilization rates are within the luxury range.

Effect of fall fertilization on nursery and field performance

The benefit of fall fertilizer on enhancement of foliage N concentration was found in Chinese pine as well as other tree seedlings (Margolis & Waring 1986a, Sung et al. 1997, Irwin et al. 1998, Heiskanen et al. 2009, Jonsdottir et al. 2013). This result is consistent with the goal of fall fertilization to nutrient load by enhancing foliar N concentration (Dumroese 2003). Increased foliar N concentration in fall-fertilized Chinese pine seedlings did not facilitate foliar N content, in accordance with that reported for holm oak seedling (Oliet et al. 2011) but in contrast to results for Douglas-fir, loblolly pine, and Norway spruce seedlings (Margolis & Waring 1986a, Sung et al. 1997, Heiskanen et al. 2009). Lack of increased foliar N content could be attributed to the absence of increasing foliar biomass of fall-fertilized seedlings, as per other previous studies (Irwin et al. 1998, Oliet et al. 2011).

The present and earlier findings of fall fertilization indicated mixed survival and growth responses to increased foliar N concentration. Fall fertilization had no effect on survival of Chinese pine, corresponding to results for Norway spruce, Douglas-fir, and Lutz spruce (Van Den Driessche 1988, Heiskanen et al. 2009, Jonsdottir et al. 2013). However, reduced survival was reported in fall-fertilized longleaf pine (*Pinus palustris* Mill.) seedlings (Shoulders 1959). Pre-planting diameter and height of fall-fertilized seedlings was similar to that of non fall-fertilized seedlings for Chinese pine and several other species (Margolis & Waring 1986b, Irwin et al. 1998, Heiskanen et al. 2009, Oliet et al. 2011). These trends were maintained in first-year diameter for Chinese pine, which was contrary to that reported for slash pine seedlings (Irwin et al. 1998). For height, favorable effects of fall fertilization did not appear until the second growing season in our study, despite frequent reports of improved first-year height growth in fall fertilized seedlings (Margolis & Waring 1986b, Irwin et al. 1998, Heiskanen et al. 2009). Inconclusive growth performance across these studies again demonstrates that comprehensive consideration of both nursery and field performance determines rational N supply in the nursery for seedling production to meet a particular site condition. Overall, 12 and 24 mg N fall fertilization rates are more effective during hardening for Chinese pine seedling in terms of foliar N concentration prior to planting, and total height and height increment at the second year after outplanting.

Interaction between pre-hardening and fall fertilization on nursery seedling N storage and subsequent field performance

In previous studies investigating fall fertilization, seedlings generally received the same dose of pre-hardening fertilization and were subsequently fall-fertilized at multiple treatment rates (Van Den Driessche 1988, Irwin et al. 1998, Heiskanen et al. 2009, Jonsdottir et al. 2013). This precludes the ability to distinguish how pre-hardening fertilization affects fall fertilization; and the use of a single pre-hardening fertilization rate potentially contributes to the inconsistent results of fall fertilization among studies. For black spruce seedlings, interactions influenced both N concentration and content of the whole plant (Boivin et al. 2004). Interaction only occurred in root N concentration of Chinese cork oak seedlings (Li et al. 2014) and in N content of the whole plant for Chinese pine in our study. Additionally, fall fertilization had adverse effects on N content when seedlings were fertilized at the pre-hardening rate of 120 mg N, indicating that this rate may have the approximated optimal nutrient input whereas additional fertilization has proven to induce some toxicity (Jacobs & Timmer 2005).

First-year height after outplanting indicated that fall fertilization had a slightly advantageous effect under 40 and 80 mg N per seedling of pre-hardening fertilization regimes, whereas negative impacts were observed at the highest rate. This could be related to incipient toxicity for seedlings fertilized at 120 mg that were subsequently fertilized with additional N in the fall. This interactive effect of pre-hardening and fall fertilization was transitory and vanished at the second-year of outplanting.

Conclusions

Based on nursery seedling growth and N content during the pre-hardening period, the three pre-hardening fertilization rates (40, 80 and 120 mg N) induced luxury consumption of Chinese pine seedlings. Fall fertilization of seedlings that received 40-80 mg N per seedling during pre-hardening further N loaded Chinese pine. For the highest pre-hardening fertilization rate, however, fall fertilization decreased N content.

Maximum diameter was observed in the lowest nursery exponential fertilization rate at the second year after outplanting. Supplemental fertilization during fall with 12 and 24 mg N per seedling were more effective in enhancing both total height and height increment at the second year after outplanting. Considering nursery and field performance, fertilization cost and potential nitrate leaching risks, combining pre-hardening fertilization with fall fertilization may serve as a more effective means to promote N storage and outplanting growth as compared with pre-hardening fertilization alone. Further research is needed, however, to optimize application rates under this fertilization scheme for Chinese pine and other important reforestation species under varying cultural regimes.

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References

Andivia E, Fernández M, Vázquez-Piqué J (2011). Autumn fertilization of *Quercus ilex* ssp. *ballota* (Desf.) Samp. nursery seedlings: effects on morpho-physiology and field performance. Annals of Forest Science 68: 543-553. - doi: 10.1007/s13595-011-0048-4

Bao SD (2000). Soil and agricultural chemistry analysis. Chinese Agriculture Press, Beijing, China, pp. 522.

Birge ZKD, Salifu KF, Jacobs DF (2006). Modified exponential nitrogen loading to promote morphological quality and nutrient storage of bareroot-cultured *Quercus rubra* and *Quercus alba* seedlings. Scandinavian Journal of Forest Research 21: 306-316. - doi: 10.1080/02827580600761611

Boivin JR, Salifu KF, Timmer VR (2004). Late-season fertilization of *Picea mariana* seedlings: intensive loading and outplanting response on greenhouse bioassays. Annals of Forest Science 61: 737-745. - doi: 10.1051/forest:2004073

Bremner JM, Mulvaney CS (1982). Nitrogen-total. In: "Methods of Soil Analysis" (Page AL ed). American Society of Agronomy, Madison, WI, USA, pp. 595-624.

Close DC, Bail I, Hunter S, Beadle CL (2005). Effects of exponential nutrient-loading on morphological and nitrogen characteristics and on after-planting performance of *Eucalyptus globulus* seedlings. Forest Ecology and Management 205: 397-403. - doi: 10.1016/j.foreco.2004.10.041

Cortina J, Vilagrosa A, Trubat R (2013). The role of nutrients for improving seedling quality in drylands. New Forests 44: 719-732. - doi: 10.1007/s11056-013-9379-3

Cuesta B, Villar-Salvador P, Puértolas J, Jacobs DF, Rey-Benayas JM (2010). Why do large, nitrogen rich seedlings better resist stressful transplanting conditions? A physiological analysis in two functionally contrasting Mediterranean forest species. Forest Ecology and Management 260: 71-78. - doi: 10.1016/j.foreco.2010.04.002

Dumroese RK (2003). Hardening fertilization and nutrient loading of conifer seedlings. In: Proceedings of the "Forest and Conservation Nursery Associations – 2002" (Riley LE, Dumroese RK, Landis TD eds). Proceedings RMRS-P-28, Rocky Mountain Research Station, USDA Forest Service, Ogden, UT, USA, pp. 31-36. [online] URL: http://www.fs.fed.us/rm/pubs/rmrs_p028.pdf?#page=37

Dumroese RK, Page-Dumroese DS, Salifu KF, Jacobs DF (2005). Exponential fertilization of *Pinus monticola* seedlings: nutrient uptake efficiency, leaching fractions, and early outplanting performance. Canadian Journal of Forest Research 35: 2961-2967. - doi: 10.1139/X05-226

Folk RS, Timmer VR, Scarrat JB (1992). Evaluating peat as a growing medium for jack pine seedlings. 1. Conventional laboratory indices. Canadian Journal of Forest Research 22: 945-949. - doi: 10.1139/x92-127

Hawkins BJ, Burgess D, Mitchell AK (2005). Growth and nutrient dynamics of western hemlock with conventional or exponential greenhouse fertilization and planting in different fertility conditions. Canadian Journal of Forest Research 35: 1002-1016. - doi: 10.1139/X05-026

Heiskanen J, Lahti M, Luoranen J, Rikala R (2009). Nutrient loading has a transitory effect on the nitrogen status and growth of outplanted Norway spruce seedlings. Silva Fennica 43: 249-260. - doi: 10.14214/sf.210

Huang GY, Dong LF, Qi FM (2004). Fertilization effect and application of diagnosis and recommendation integrated system in container system seedling raising of *Pinus tabulaeformis*. Journal of West China Forestry Science 33 (3): 26-29.

Imo M, Timmer VR (1992). Nitrogen uptake of Mesquite seedlings at conventional and exponential fertilization regimes. Soil Science Society of American Journal 56: 927-934. - doi: 10.2136/sssaj1992.03615995005600030041x

Irwin KM, Duryea ML, Stone EL (1998). Fall-applied nitrogen improves performance of 1-0 slash pine nursery seedlings after outplanting. Southern Journal of Applied Forestry 22: 111-116. [online] URL: http://www.ingentaconnect.com/content/saf/sjaf/1998/00000022/00000002/art00010

Islam MA, Apostol KG, Jacobs DF, Dumroese RK (2009). Fall fertilization of *Pinus resinosa* seedlings: nutrient uptake, cold hardiness, and morphological development. Annals of Forest Science 66. - doi: 10.1051/forest/2009061

Jacobs DF, Timmer VR (2005). Fertilizer-induced changes in rhizosphere electrical conductivity: relation to forest tree seedling root system growth and function. New Forests 30: 147-166. - doi: 10.1007/s11056-005-6572-z

Jonsdottir RJ, Sigurdsson BD, Lindström A (2013). Effects of nutrient loading and fertilization at planting on growth and nutrient status of Lutz spruce (*Picea x lutzii*) seedlings during the first growing season in Iceland. Scandinavian Journal of Forest Research 28: 631-641. - doi: 10.1080/02827581.2013.824503

Li GL, Liu Y, Zhu Y, Li QM, Dumroese RK (2012). Effect of fall-applied nitrogen on growth, nitrogen storage, and frost hardiness of bareroot *Larix olgensis* seedlings. Silva Fennica 46: 345-354. - doi: 10.14214/sf.45

Li GL, Zhu Y, Liu Y, Wang JX, Liu JJ, Dumroese RK (2014). Combined effects of pre-hardening and fall fertilization on nitrogen translocation and storage in *Quercus variabilis* seedlings. European Journal of Forest Research 133: 983-992. - doi: 10.1007/s10342-014-0816-4

Luis VC, Puértolas J, Climent J, Peters J, González-Rodriguez A, Morales D, Jiménez M (2009). Nursery fertilization enhances survival and physiological status in Canary Island pine (*Pinus canariensis*) seedlings planted in a semiarid environment. European Journal of Forest Research 128: 221-229. - doi: 10.1007/s10342-009-0257-7

Margolis HA, Waring RH (1986a). Carbon and nitrogen allocation patterns of Douglas-fir seedlings fertilized with nitrogen in autumn. I. Overwinter metabolism. Canadian Journal of Forest Research 16: 897-902. - doi: 10.1139/x86-160

Margolis HA, Waring RH (1986b). Carbon and nitrogen allocation patterns of Douglas-fir seedlings fertilized with nitrogen in autumn. II. Field performance. Canadian Journal of Forest Research 16: 903-909. - doi: 10.1139/x86-161

McAlister JA, Timmer VR (1998). Nutrient enrichment of white spruce seedlings during nursery culture and initial plantation establishment. Tree Physiology 18: 195-202. - doi: 10.1093/treephys/18.3.195

Miller BD, Timmer VR (1994). Steady-state nutrition of *Pinus resinosa* seedlings: response to nutrient loading, irrigation and hardening regimes. Tree Physiology 14: 1327-38. - doi: 10.1093/treephys/14.12.1327

Oliet JA, Tejada M, Salifu KF, Collazos A, Jacobs DF (2009). Performance and nutrient dynamics of holm oak (*Quercus ilex* L.) seedlings in relation to nursery nutrient loading and post-transplant fertility. European Journal of Forest Research 128: 253-263. - doi: 10.1007/s10342-009-0261-y

Oliet JA, Salazar JM, Villar R, Robredo E, Valladares F (2011). Fall fertilization of Holm oak affects N and P dynamics, root growth potential, and post-planting phenology and growth. Annals of Forest Science 68: 647-656. - doi: 10.1007/s13595-011-0060-8

Oliet JA, Puértolas J, Planelles R, Jacobs DF (2013). Nutrient loading of forest tree seedlings to promote stress resistance and field performance: a Mediterranean perspective. New Forests 44: 449-469. - doi: 10.1007/s11056-013-9382-8

Qu LY, Ali M, Quoreshi AM, Koike T (2003). Root growth characteristics, biomass and nutrient dynamics of seedlings of two larch species raised under different fertilization regimes. Plant and Soil 255: 293-302. - doi: 10.1023/A:1026159709246

Quoreshi AM, Timmer VR (2000). Early outplanting performance of nutrient-loaded containerized black spruce seedlings inoculated with Laccaria bicolor: a bioassay study. Canadian Journal of Forest Research 30: 744-752. - doi: 10.1139/x00-003

Rikala R, Heiskanen J, Lahti M (2004). Autumn fertilization in the nursery affects growth of *Picea abies* container seedlings after transplanting. Scandinavian Journal of Forest Research 19: 409-414. - doi: 10.1080/02827580410030190

Salifu KF, Timmer VR (2001). Nutrient retranslocation response of *Picea mariana* seedlings to nitrogen supply. Soil Science Society of America Journal 65: 905-913. - doi: 10.2136/sssaj2001.653905x

Salifu KF, Timmer VR (2003a). Nitrogen retranslocation response of young *Picea mariana* to nitrogen-15 supply. Soil Science Society of America Journal 67: 309-317. - doi: 10.2136/sssaj2003.3090

Salifu KF, Timmer VR (2003b). Optimizing nitrogen loading of *Picea mariana* seedlings during nursery culture. Journal of Forest Research 33: 1287-1294. - doi: 10.1139/x03-057

Salifu KF, Jacobs DF (2006). Characterizing fertility targets and multi-element interactions in nursery culture of *Quercus rubra* seedlings. Annals of Forest Science 63: 231-237. - doi: 10.1051/forest:2006001

Salifu KF, Jacobs DF, Birge ZKD (2009). Nursery nitrogen loading improves field performance of bareroot oak seedlings planted on abandoned mine lands. Restoration Ecology 17: 339-349. - doi: 10.1111/j.1526-100X.2008.00373.x

Shoulders E (1959). Caution needed in fall application of nitrogen to nursery stock. Tree Planters Notes 38: 25-27.

Sung SS, Black CC, Kormanik TL, Zarnoch SJ, Kormanik PP, Counce PA (1997). Fall nitrogen fertilization and the biology of *Pinus taeda* seedling development. Canadian Journal of Forest Research 27: 1406-1412. - doi: 10.1139/x97-112

Timmer VR (1996). Exponential nutrient loading: a new fertilization technique to improve seedling performance on competitive sites. New Forests 13: 275-295. - doi: 10.1023/A:1006502830067

Timmer VR, Armstrong G (1987). Growth and nutrition of containerized *Pinus resinosa* at exponentially increasing nutrient additions. Canadian Journal of Forest Research 17: 644-647. - doi: 10.1139/x87-105

Trubat R, Cortina J, Vilagrosa A (2008). Short-term nitrogen deprivation increases field performance in nursery seedlings of Mediterranean woody species. Journal of Arid Environments 72: 879-890. - doi: 10.1016/j.jaridenv.2007.11.005

Trubat R, Cortina J, Vilagrosa A (2010). Nursery fertilization affects seedling traits but not field performance. Journal of Arid Environments 74: 491-497. - doi: 10.1016/j.jaridenv.2009.10.007

Valdecantos A, Cortina J, Ramón Vallejo V (2006). Nutrient status and field performance of tree seedlings planted in Mediterranean degraded areas. Annals of Forest Science 63: 249-256. - doi: 10.1051/forest:2006003

Van Den Driessche R (1985). Late season fertilization, mineral nutrient reserves, and retranslocation in planted Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) seedlings. Forest Science 31: 485-496. [online] URL: http://www.ingentaconnect.com/content/saf/fs/1985/00000031/00000002/art00029

Van Den Driessche R (1988). Nursery growth of conifer seedling using fertilizers of different solubilities and application time, and their forest growth. Canadian Journal of Forest Science 18: 172-180. - doi: 10.1139/x88-027

Villar-Salvador P, Puértolas J, Jacobs DF (2013). Nitrogen nutrition and drought hardening exert opposite effects on the stress tolerance of *Pinus pinea* L. seedlings. Tree Physiology 33: 221-232. - doi: 10.1093/treephys/tps133

Villar-Salvador P, Puértolas J, Cuesta B, Peñuelas JL, Uscola M, Heredia-Guerrero N, Rey Benayas JM (2012). Increase in size and nitrogen concentration enhances seedling survival in Mediterranean plantations. Insights from an ecophysiological conceptual model of plant survival. New Forests 43: 755-770. - doi: 10.1007/s11056-012-9328-6

Wang J, Li G, Pinto J, Liu J, Shi W, Liu Y (2015). Both nursery and field performance determine suitable nitrogen supply of nursery-grown, exponentially fertilized Chinese pine. Silva Fennica 49 (3): 1295. - doi: 10.14214/sf.1295

Wang XS (2014). Fertilization experiments on container seedlings of *Pinus tabulaeformis*. Protection Forest Science and Technology 6: 46-47.

Way DA, Seegobin SD, Sage RF (2007). The effect of carbon and nutrient loading during nursery culture on the growth of black spruce seedlings: a six-year field study. New Forests 34: 307-312. - doi: 10.1007/s11056-007-9053-8

Wu G, Feng ZW (1994). Study on the social characteristics and biomass of the *Pinus tabulaeformis* forest systems in China. Acta Ecologica Sinica 14: 415-422.

Xu HC, Sun ZF, Guo GR, Feng L (1981). Geographic distribution of *Pinus tabulaeformis* Carr. and classification of provenance regions. Scientia Silvae Sinicae 17: 258-270.

Zhu Y, Dumroese RK, Pinto JR, Li GL, Liu Y (2013). Fall fertilization enhanced nitrogen storage and translocation in *Larix olgensis* seedlings. New Forests 44: 849-861. - doi: 10.1007/s11056-013-9370-z

