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Growth versus storage: response of *Pinus tabuliformis* and *Quercus mongolica* seedlings to variation in nutrient supply and its associated effect on field performance

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Abstract

Carbohydrate and nitrogen storage in seedlings can improve field growth and survival. However, carbohydrate and nitrogen reserves may compete. For instance, carbohydrate reserves can be accumulated due to sink limitation on growth triggered by nutrient deficiency. Few studies have addressed the effects of nutrient supply on carbohydrate and nutrient storage and subsequent field performance of *Quercus* and *Pinus* species with different shoot growth strategies at the early growing stage. We exposed seedlings of *Pinus tabuliformis* Carr and *Quercus mongolica* Fisch to three exponential fertilizer treatments (10, 50, 100 mg N) in the nursery for 18 weeks to assess the combined response of growth and storage of carbohydrate and nitrogen in seedlings during their first year of growth in the nursery, as well as their subsequent field performance over the next 2 years. At the end of nursery stage, high nutrient supply increased nitrogen storage in both species, increased carbohydrate concentration in *Q. mongolica* but decreased that in *P. tabuliformis* seedlings. *P. tabuliformis* showed higher winter mortality than *Q. mongolica* but field mortality of *P. tabuliformis* decreased with an increase in fertilizer dosage. Our findings suggest that *P. tabuliformis* seedlings exposed to 100 mg N per plant can mitigate the field winter mortality although it could be still under deficiency status. Given lower winter mortality in *Q. mongolica* seedlings, being exposed to at least 100 mg N per plant in the nursery can produce nutrient-loaded seedlings, and therefore can be considered for restoration programs in areas affected by low winter temperature.

Keywords Fertilization · Nitrogen storage · Carbohydrate storage · Plant quality · Field performance · *Pinus tabuliformis* · *Quercus mongolica*

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Introduction

Application of fertilizers during the nursery culture can effectively increase seedling size/biomass and nutrient storage in forest plantations. Initial seedling size or biomass is associated with the ability to outcompete weeds (Cuesta et al. 2010) and produce new roots (Grossnickle 2005). Consequently, larger seedlings show higher post-planting survival (Andivia et al. 2021; Villar-Salvador et al. 2015) and growth due to a large surface area for photosynthesis (Villar-Salvador et al. 2012).

Nitrogen and carbohydrate reserves are key requirements for plant growth and survival. Deciduous trees rely on the remobilization of N and carbohydrate stored in the previous growing season to support budburst and new growth (Hoch et al. 2003; Millard and Grelet 2010; Wang et al. 2019).Evergreen species rely on remobilization of N storage (> 74%) for new fine-root growth in early spring (Uscola et al. 2015). The amount of N remobilized depends on the amount of N stored (Millard and Grelet 2010) if no environmental factor inhibits the growth of sink organs. Although the driver of remobilization of C reserves is not fully understood (Brüggemann et al. 2011), some observations emphasized that carbohydrate reserves impact field growth and survival. For instance, fast growth leads to low carbohydrate reserves due to the growth competes with carbohydrate storage (Wiley et al. 2013), leading to a higher winter mortality (Luo et al. 2021); or high carbohydrate storage competes with growth, leading to lower field growth (Landhausser et al. 2012). Thus, the balance between growth and carbohydrate reserves could play an important role in field performance. In addition, N and carbohydrate reserves frequently affect plant cold and drought tolerance (O'Brien et al. 2014; Villar-Salvador et al. 2015). Carbohydrate reserves also act as important carbon sources for respiration during periods of resource shortage (Mooney 1972; Myers and Kitajima 2007; Weber et al. 2019). Consequently, N and carbohydrate reserves strongly affect the growth rate and survival after planting in the field (Landhausser et al. 2012; Luo et al. 2021; Oliet et al. 2009; Villar-Salvador et al. 2013; Villar-Salvador et al. 2004), as well as the seedling's capacity to recover foliage after disturbances (Millard et al. 2001; Palacio et al. 2020). However, the relationship among growth, N and carbohydrate storage are rarely investigated together, especially the effect on field performance.

Carbohydrates are well known as an indicator of sink limitation on growth under stressful conditions (Hoch 2015; Körner 2003). Nutrient deficiency can induce sink limitation on growth (Birk and Matson 1986), under which carbohydrate reserves can be accumulated due to low demand of photosynthates for growth, accompanied by decreased N reserves and growth. Nutrient loading (involving both nutrient sufficiency and luxury uptake described in (Salifu and Jacobs 2006) can lead to less plasticity in growth responding to nutrient supply, that is, seedlings with nutrient loading respond less in biomass to nutrient supply. This switch between two nutritional states stipulated by Timmer (1997), whose model defines *nutrient deficiency* as when plants grow, i.e., when the nutritional status of the plant increases with increased fertilization; and *nutrient loading* as when the internal nutrient concentration increases without the plant's total biomass changing (Salifu and Jacobs 2006; Timmer 1997). Nutrient loading involves fertilization in excess of the demand required for growth in nursery culture, leading to "luxury uptake" of nutrients. Thus, we expect nutrient loading increases storage of both N and carbohydrates due to the reduced need of both to sustain. The relationship among growth, N and carbohydrate reserves seems to be complex. Few studies have addressed how a switch from nutrient deficiency to nutrient loading may trigger an integrated response involving seedling biomass and storage of carbohydrate and N. How these three dimensions interact responding to nutrient supply and further effect on field performance needs to be elucidated.

Understanding this integrated response may be particularly important for the success of restoration programs in areas affected by high winter mortality, which frequently occurred in Northern China (Luo et al. 2021, 2022; Wang et al. 2015). *Quercus* and *Pinus* species are widely used in restoration programs across the world, from Mediterranean to certain temperate regions, since they are dominant in many temperate and Mediterranean regions of the Northern Hemisphere and, consequently, play a basic role in ecosystem functioning (Rogers 2009; Torres et al. 2021). The difference between the two species in seed size and shoot growth strategy indicates different initial nutrient supply to seedlings and different nutrient demands along the growing season. How these two species respond to different nutrient supply at early nursery stage helps to better understand the integrated response among growth, N and carbohydrate reserves. With small seeds, soil nutrient supply plays important role in seedling growth for *Pinus* species with determinate shoot growth strategy (Pallardy 2008), which grow mostly in early nursery stage. Conversely, although the resources from big acorns are almost sufficient during early growing stage (at least 4 weeks after emergency) for *Quercus* species (Milberg and Lamont 1997; Shi et al. 2018b; Villar-Salvador et al. 2010; Yi and Ju 2020) with semi-determinate shoot growth strategy (Hanson et al. 1986), which shows recurrent shoot growth during growing seasons, soil nutrient supply may also matter during later growing season (Oliet et al. 2009; Villar-Salvador et al. 2013; Villar-Salvador et al. 2004).

These considerations suggest that species with different shoot growth strategies and soil nutrient requirements at the early nursery stage may respond differently to nutrient supply, which can lead to later differences in field performance. Therefore, our study aimed to assess how changes in nutrient supply affect growth (in terms of seedling biomass) and storage (carbohydrate and N reserves). These questions were addressed using seedlings of two temperate species, *Pinus tabuliformis* Carr and *Quercus mongolica* Fisch. All seedlings were fertilized with doses of nitrogen-phosphorus-potassium (NPK) fertilizer based on an exponential regime (10, 50, and 100 mg) during the first year of growth in the nursery, and field performance was measured during the 2 years after outplanting. We tested the following hypotheses (1) lower dose nutrient supply can lead to an increase in carbohydrate concentration due to sink limitation on growth and higher dose nutrient supply can cause a decrease in carbohydrate concentration due to eased sink limitation on growth by nutrient supply. (2) Species with different shoot growth strategies and soil nutrient requirements at the early nursery stage may respond differently in carbohydrates to nutrient supply. Carbohydrate concentration in *Quercus* species responds to both nutrient deprivation and nutrient excess, due to sink limitation and low plasticity, respectively, that stimulate in both cases reserves building. (3) An increase in seedling size, N and carbohydrate storage would increase field growth and survival of both species.

Materials and methods

Seedling production

On February 23, 2017, seeds of *Q. mongolica* were collected from the National Seed Orchard for *Quercus mongolica* (Qigou Forest Farm, 41° 00′ N and 118° 27′ E, 526 m a.s.l.) and planted in hard plastic container cells (D60, Stuewe & Sons, Inc., Oregon, USA).

The cells with a diameter of 6.4 cm, depth of 36 cm, and volume of 983 ml were arranged in 48 trays with 20 cells, resulting in a density of 127 cells m^{-2}. The growing medium was a 3:1 mixture of peat (pH 6.0, screening 0–6 mm; Pindstrup Seeding, Ryomgaard, Denmark) and perlite (diameter 5 mm; Xinyang Jinhualan Mining, Henan, China). A total of 960 seedlings were cultivated in 48 trays, and these trays were placed on raised benches inside a ventilated polyethylene greenhouse under natural photoperiod conditions at the Chinese Academy of Forestry Sciences in Beijing (40° 40′ N, 116° 14′ E).

On March 4, 2017, seeds of *P. tabuliformis* were collected from the National Seed Orchard for Chinese Pine (Qigou Forest Farm, 41° 00′ N and 118° 27′ E, 526 m.a.s.l.) and planted in hard plastic container cells (SC10 Super Ray Leach "Cone-tainer" ™ Single Cell System, Stuewe & Sons Inc., Oregon, USA). The cells with a depth of 21 cm and volume of 164 ml were arranged within trays (Ray Leach "Cone-tainers"™ RL98c, Stuewe & Sons Inc., Oregon, USA) at 49 cells per tray, resulting in a density of 528 cells m^{-2}. The growing medium was the same 3:1 mixture of peat and perlite as for *Q. mongolica*. A total of 588 seedlings were cultivated in 12 trays, and these trays were placed on raised benches inside a ventilated polyethylene greenhouse under natural photoperiod conditions at the Chinese Academy of Forestry Sciences in Beijing (40° 40′ N, 116° 14′ E). Details on the seed traits of both species are listed in Table 1.

Fertilizer treatments

The fertilizer treatments began after seedling emergence on March 30, 2017. Seedlings were fertilized once a week based on an exponential fertilization regime (Timmer 1997), which was described in detail by Wang (2015). A solution was prepared by dissolving a 20N-20P-20K fertilizer that contained micronutrients (Peters Professional, ICL, Summerville, SC, USA) in tap water, and each seedling was supplied with 20 ml doses. By the end of the nursery cultivation period (July 27, 2017), each plant had received a total of 18 fertilizer applications, with doses corresponding to 10 mg (low), 50 mg (moderate), and 100 mg (high) nitrogen per seedling. For each species, all seeds were sown in the trays, and each tray was randomly arranged in each block for *Pinus tabuliformis*, and 4 trays were

Table 1 Effect of fertilizer treatments on morphological features of *Pinus tabuliformis* and *Quercus mongolica* seedlings

Species	Morphological feature	Fertilization dose			*p*
		Low	Moderate	High	
Pinus tabuliformis	Needle mass	0.16 ± 0.01^{b}	0.36 ± 0.04^{a}	0.40 ± 0.04^{a}	< 0.001
	Stem mass	0.08 ± 0.004^{c}	0.12 ± 0.01^{b}	0.14 ± 0.01^{a}	< 0.001
	Root mass	0.28 ± 0.01^{b}	0.30 ± 0.02^{ab}	0.35 ± 0.01^{a}	0.001
	Plant mass	0.52 ± 0.02^{b}	0.78 ± 0.05^{a}	0.90 ± 0.05^{a}	< 0.001
	Root mass fraction	0.53 ± 0.01^{a}	0.40 ± 0.02^{b}	0.41 ± 0.02^{b}	< 0.001
Quercus mongolica	Stem mass	0.21 ± 0.02	0.22 ± 0.02	0.21 ± 0.02	0.922
	Root mass	2.93 ± 0.28	3.29 ± 0.26	3.02 ± 0.34	0.635
	Plant mass	3.14 ± 0.28	3.51 ± 0.26	3.23 ± 0.35	0.637
	Root mass fraction	0.92 ± 0.01	0.94 ± 0.03	0.92 ± 0.01	0.795

Values are expressed as mean ± SE. Lowercase letters indicate significant differences among fertilizer treatments based on *lmer* function in lmerTest package and the Tukey post-hoc test randomly arranged in each block for *Quercus mongolica*. The specific fertilization schedule is depicted in Fig. 5.

Seedlings were watered to container capacity approximately twice a week, with one watering event coinciding with fertilization. The average greenhouse temperature was 25 °C during the day and 20 °C at night; temperature measurements were made at 30-min intervals using a JL-18 thermometer (Huayan Instrument and Equipment, Shanghai, China). The seedlings were moved outdoors to harden between late October and late November 2017, during which the average day temperature was 9 °C and average night temperature was 6 °C. Between December 1, 2017, and late February 2018, the seedlings were stored outdoors in a manually dug hole to avoid freezing damage and mortality over the winter.

Field experiment

The outplanting of seedlings was carried out at the Beijing Forestry University Northern Experimental Base at Pingquan in Hebei province (41° 13′ N, 118° 40′ E, 765 m.a.s.l.). The field site was in an area that experiences a temperate continental monsoon climate, characterized by seasonal drought that is presented as a dry winter, dry spring, and wet summer. The long-term mean annual temperature at this site is 7.3 °C and the total annual rainfall is 540 mm, 70% of which occurs during summer (June–September).

Precipitation and temperature data were recorded using an on-site weather station between January 2018 and December 2019. The annual precipitation varied from 398 in 2018 to 452 mm in 2019, while the corresponding mean annual temperature varied from 8.1 in 2018 to 8.4 °C in 2019. In the winter of 2018 (December-February), the air temperature dropped below -20 °C during 15 days. The plot for outplanting had a slope $<2\%$ and a soil depth of 45–60 cm. The soil texture in the surface layer (0–20 cm) comprised sand (73%), silt (11%), and clay (16%), while the soil pH was 6.2 and the organic carbon concentration was 0.7%. The soil in this plot was considered moderately fertile, with a total N concentration of 629 mg kg^{-1}, an available P concentration of 139 mg kg^{-1}, and an available K concentration of 113 mg kg^{-1} (Shi et al. 2018a).

P. tabuliformis seedlings were outplanted on April 15, 2018, and *Q. mongolica* seedlings on May 15, 2018. Seedlings were planted in manually dug holes ($0.4 \times 0.4 \times 0.4$ m) that were spaced 1 m apart. We used a randomized complete block design with four blocks, with all of treatments in each block randomly distributed. In the case of *Q. mongolica,* 60 seedlings were planted in each block, resulting in 240 seedlings per treatment. In the case of *P. tabuliformis,* 35 seedlings were planted in each block, resulting in 140 seedlings per treatment. Manual weeding was performed as required.

Plant sampling and measurements

To determine nutrient status in seeds, we randomly selected 25 acorns of *Q. mongolica* and 200 seeds of *P. tabuliformis* and assigned them to five random groups per species. On March 13, 2018, 1 month before outplanting, we randomly sampled five seedlings in each of the four trays per treatment from each species ($n = 20$ seedlings per fertilization treatment and species). After separating the seedlings into needles, stems, and roots, the growing medium was washed gently from the roots. Plant parts and seeds were then oven-dried at 70 °C for at least 48 h. We measured the mass of the stems, needles, dry roots, and seeds, and the root mass fraction was calculated as the root mass/total plant mass × 100.

Dried samples were analyzed to determine concentrations of nutrients and total non-structural carbohydrates (TNCs), which comprised soluble sugars (glucose, fructose and sucrose) and starch. Composite samples of each organ were made by pooling together the five samples of seedlings from the same tray for *P. tabuliformis*, and individual seedling organs were collected to determine nutrient and TNC concentrations for *Q. mongolica*. After grinding, plant material and seeds were passed through a 0.25-mm screen sieve and wet-digested using the H_2SO_4–H_2O_2 method. Then we used the standard Kjeldahl digestion method with water distillation (UDK-159, VelpScientifica, Italy) to determine total N concentration and content (Bremner 1996).

To determine TNC concentration and content, samples were ground to a powder in a ball mill. To extract soluble sugars, approximately 100 mg of finely ground plant material was boiled in distilled water (10 ml) for 30 min. The rest of the plant materials were used to determine the content of starch, which was extracted with perchloric acid for 10–15 min, followed by centrifugation and decantation. TNC concentration was determined using the anthrone method (Hansen and Moller 1975). Total glucose content was calculated photometrically at 630 nm in a UV–visible spectrophotometer (Agilent 8453, Shanghai, China). Absorbance values were converted to moles of glucose based on a standard curve derived from pure glucose (catalog no. S2974, Xilong, Guangdong, China), and these values were then converted to total glucose equivalents (Hansen and Moller 1975). TNC content was defined as the sum of the contents of soluble sugars and starch. TNC content for specific organs was calculated by multiplying TNC concentration by the mass of the organ.

We used vector diagnosis of nitrogen to detect whether seedlings reached sufficient status or still in deficient status. The methods of vector analysis were described in detail in the reference (Haase 1995).

We also recorded field mortality, stem height, and root collar diameter (RCD) 2 days after planting (T_0, spring 2018, only height and RCD recorded) and at the end of each growing season in late October 2018 (T_1) and 2019 (T_2), as well as at the end of the first winter in early spring 2019 ($T_{1\text{-AW}}$). Stem height was measured using a ruler, and RCD was measured using a Vernier caliper. These data were used to calculate stem volume (SV) based on the following equation:

$$\text{Stem volume (SV)} = \frac{1}{3}\pi\left(\frac{RCD}{2}\right)^2 Height \tag{1}$$

Seedling growth was calculated as the net increase in stem volume between T_0 and T_1 and between T_1 and T_2.

Statistical analysis

A mixed linear model with block as a random factor was used for each species to analyze the effect of fertilizer treatments on seedling morphology, nutrient content, and TNC concentration. A similar analysis was performed to assess seedling outplanting growth, but here we used field block as a random factor. Finally, a generalized mixed model for a binomial distribution and a logit link function was used to analyze the effects of the treatments on outplanting survival; once again, block was used as a random factor. The post hoc Tukey multiple comparison test was performed to detect the statistical differences. We used bartlett.test() and shapiro.test() in R to test normality and homogeneity of the residuals. Log or square root transformation of data was used to meet normality. The data were analyzed using the packages “lmerTest” and “multcomp” in R 3.6.3.

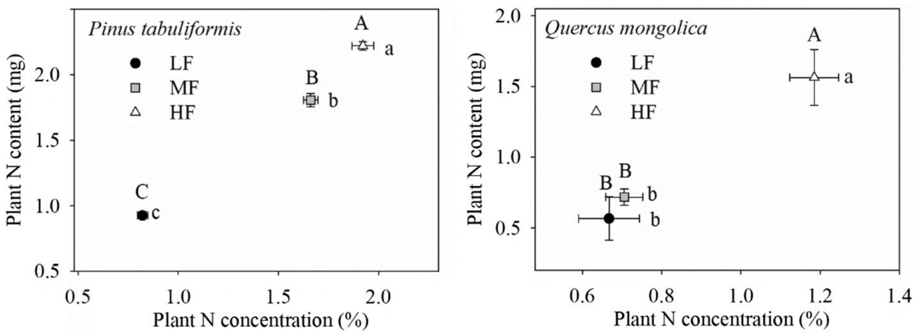

Fig. 1 Effect of fertilization on plant nitrogen concentration (x-axis) and content (y-axis) in *Pinus tabuliformis* and *Quercus mongolica* seedlings. Values are mean ± SE. Different lowercase and uppercase letters indicate significant differences ($p < 0.05$) among treatments based on ANOVA and the Tukey post-hoc test. HF, high-dose fertilization; LF, low-dose fertilization; MF, moderate-dose fertilization; N, nitrogen

Table 2 Summary results of ANOVA to examine the effects of fertilization on the concentration and content of nitrogen, soluble sugars, starch, and total non-structural carbohydrates at the whole plant level and for each plant fraction in *Pinus tabuliformis* and *Quercus mongolica* seedlings

Parameter	*Pinus tabuliformis*				*Quercus mongolica*		
	Plant	Needle	Stem	Root	Plant	Stem	Root
Concentration							
Nitrogen	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
Soluble sugars	**0.023**	**< 0.001**	0.035	0.102	**< 0.001**	**0.024**	**0.001**
Starch	0.189	0.904	**0.021**	0.156	0.280	**0.026**	**0.037**
TNC	**< 0.001**	0.201	**< 0.001**	**< 0.001**	**< 0.001**	0.077	**< 0.001**
Content							
Nitrogen	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
Soluble sugars	**< 0.001**	**< 0.001**	**< 0.001**	**0.028**	**0.038**	0.129	**0.041**
Starch	**0.001**	**< 0.001**	**< 0.001**	**0.040**	**0.017**	0.649	**0.023**
TNC	**< 0.001**	**< 0.001**	**< 0.001**	0.191	**0.028**	0.333	**0.029**

Bold values indicate significant differences among fertilizer treatments based on *lmer* function in lmerTest package when $p<0.05$. TNC, non-structural carbohydrate

Results

Effect of fertilization on growth and nutrient status

Fertilization dose significantly increased plant mass in *P. tabuliformis* but not in *Q. mongolica* (Table 1). While low-dose fertilization increased root mass fraction in *P. tabuliformis*, none of the three treatments affected root mass fraction in *Q. mongolica*.

Fertilization increased N concentration in both species, at the level of the whole plant and in each plant fraction (Fig. 1, Tables 2, 4). Similarly. N content in *P. tabuliformis* increased with increasing fertilizer dosage, while in *Q. mongolica* only the highest dosage increased N content over the remaining fertilization treatments.

Fertilization had a significant effect on the concentration of plant soluble sugars and TNCs in both species, but no effect on starch concentrations. In *P. tabuliformis* seedlings, the concentration of plant soluble sugars and TNCs decreased with an increase in fertilizer dosage (Fig. 2, Table 4). For stem, the concentration of stem soluble sugars, starch and TNC significantly increased with an increase in fertilizer dosage, while the opposite trend was observed in needle soluble sugars and root TNCs. Conversely, fertilization significantly increased the concentration of soluble sugars and TNCs in *Q. mongolica* seedlings in both at the level of the whole plant and organ. While, stem starch decreased with an increase in fertilizer dosage. In *P. tabuliformis,* plant TNC content increased with an increase in fertilizer dosage, but the difference in TNC content of *P. tabuliformis* seedlings was not significant after application of moderate or high fertilizer doses (Table 2, Fig. 2). No significant effect on plant TNC content was observed in *Q. mongolica.*

Carbohydrate and nitrogen reserves ratio (N/C reserves) at the level of whole plant increased with an increase in fertilizer doses in both species, but difference was not significant after application low and moderate fertilizer doses in *Q. mongolica* (Table 4). The results of nitrogen diagnosis showed that 100 mg N supply can produce seedlings with luxury consumption for *Q. mongolica* but *P. tabuliformis* seedlings were still under deficiency status under 100 mg N supply (Fig. 2A).

Effect of fertilization on field performance

Seedling field mortality during each growing season was < 10%. However, during the first winter, we observed higher mortality in *P. tabuliformis* seedlings (27–36%) than in *Q. mongolica* seedlings (8–10%; Fig. 3). At the end of the second growing season (T_2), *P. tabuliformis* seedlings showed higher variation in accumulated mortality across treatments (39–58%) than *Q. mongolica* seedlings (19–22%). Furthermore, at T_2, there was a significant decrease in *P. tabuliformis* seedling mortality with an increase in fertilizer dosage ($\chi^2=9.9$, $p=0.007$; Fig. 3). In contrast, fertilization in the nursery had no effect on mortality in *Q. mongolica* seedlings (T_1: $\chi^2=0.24$, $p=0.89$; $T_{1\text{-AW}}$: $\chi^2=0.68$, $p=0.711$; T_2: $\chi^2=0.53$, $p=0.767$; Fig. 3).

An increase in fertilizer dosage led to an increase in stem volume in both species during the two growing seasons (Fig. 4). However, this effect was not significant in *P. tabuliformis* seedlings during the second growing season.

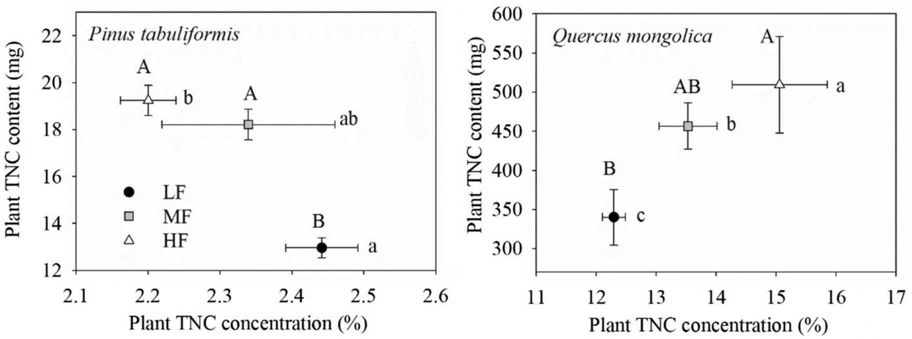

Fig. 2 Effects of fertilization on plant total non-structural carbohydrate (TNC) concentration (x-axis) and content (y-axis) in *Pinus tabuliformis* and *Quercus mongolica* seedlings. Values are mean ± SE. Different lowercase and uppercase letters indicate significant differences ($p < 0.05$) among treatments based on ANOVA and the Tukey post-hoc test. HF, high-dose fertilization; LF, low-dose fertilization; MF, moderate-dose fertilization; TNC, non-structural carbohydrate

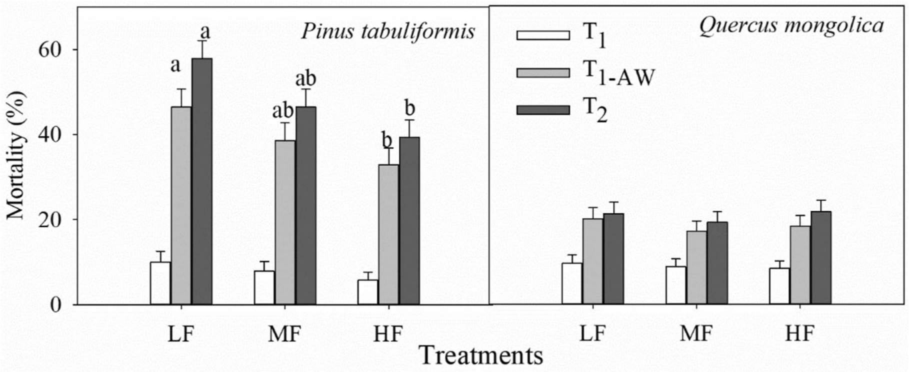

Fig. 3 Mortality of *Pinus tabuliformis* (left) and *Quercus mongolica* (right) seedlings at the end of the first (T_1) and second growing season (T_2), as well as after the first winter ($T_{1\text{-AW}}$), as a function of the fertilizer treatment applied in the nursery. Values are mean ± SE. Different lowercase and uppercase letters indicate significant differences ($p < 0.05$) among treatments based on ANOVA and the Tukey post-hoc test. HF, high-dose fertilization; LF, low-dose fertilization; MF, moderate-dose fertilization

There is a significant positive relationship between initial starch content and the field growth in first year ($SV_{1\text{-}0}$) in both species, while positive relationship between $SV_{1\text{-}0}$ and initial N content was only observed in *P. tabuliformis* (Table 5).

Discussion

Differences in carbohydrate allocation and seedling growth in response to nutrient supply

Our findings show that plant N concentration increased with an increase in N content in both species (Fig. 1), indicating that the seedlings were in the process of storing N for future use, as described in Fig. 3 of Sanz Pérez et al. (2007). We also found that plant biomass in *P. tabuliformis* seedlings increased with an increase in fertilizer dosage, while

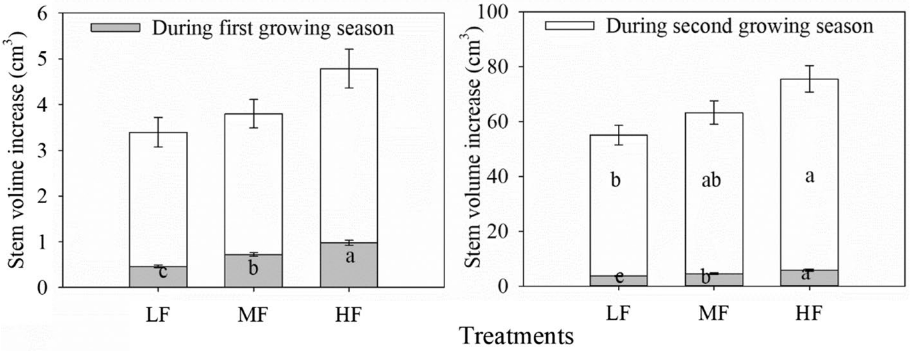

Fig. 4 Stem volume increase in *Pinus tabuliformis* (left) and *Quercus mongolica* (right) seedlings in the field during the first and second growing seasons. Values are mean ± SE. Different lowercase and uppercase letters indicate significant differences ($p < 0.05$) among treatments based on ANOVA and the Tukey post-hoc test. HF, high-dose fertilization; LF, low-dose fertilization; MF, moderate-dose fertilization

no such effect was observed in *Q. mongolica* seedlings (Table 1). We found that seedling growth in *P. tabuliformis* was N-limited, especially under the low-dose treatment. A supply of 100 mg N can produce nutrient-sufficient *P. tabuliformis* seedlings due to maximum growth obtained but not yet reach luxury consumption (Wang et al. 2015). Thus, *P. tabuliformis* seedlings can be considered nutrient-deficient (Salifu and Jacobs 2006) under 10 and 50 mg N treatments. This was also supported by the results of high N/C reserves under high fertilizer doses (Table A2) and the results of nutrient diagnosis (Fig. 2A). An increase in fertilization led simultaneously to seedling growth and nutrient storage in our *P. tabuliformis* seedlings when they receive a supply of 10–100 mg N.

In *P. tabuliformis* seedlings, plant carbohydrate concentration decreased with an increase in carbohydrate content, while the reverse was true for the *Q. mongolica* seedlings (Fig. 2). This indicates that the seedling growth was favored over carbohydrate storage in *P. tabuliformis* with an increase in fertilizer dose, especially within N-limited conditions (< 100 mg N). Our results correspond to those obtained for pine (Fife and Nambiar 1997), birch (McDonald et al. 1986) and other *Quercus* species (Sanz Pérez et al. 2007; Vizoso et al. 2008). Low nutrient supply can increase carbohydrate concentration due to sink limitation on growth (Körner 2003). With an increase in nutrient supply, sink limitation on growth caused by nutrients was partly alleviated and more photosynthates are allocated to growth instead of being allocated to carbohydrate storage. Thus, we observed opposite trends of carbohydrate and N concentration in *P. tabuliformis* in current study. Given determinate shoot growth strategy in most of *Pinus* species (Pallardy 2008), more growth demand during the early growing season may exacerbate the N limitation under low doses of fertilizer. Thus, this would lead to higher TNC reserves under low doses of fertilizer at the end of shoot growing season. Species with determinate shoot growth strategy would invest more photosynthates into TNC reserves once shoot ceased growth. For the current study, since trees were harvested at the end of hardening stage, we infer that the difference in TNC reserves at the end of shoot growing season keeps pace with that at the end of hardening stage though more photosynthates invested into TNC reserves.

In contrast, seedling growth in *Q. mongolica* was not limited by N supply even under the low-dose fertilizer treatment, that is, seedling size was not affected by the fertilizer treatments. Similar results were frequently reported in *Quercus* species (Kabeya and Sakai 2005; Sanz Pérez et al. 2007; Uscola et al. 2014; Yi and Wang 2016). Low response in biomass can be caused by two reasons. On the one hand, acorn reserves can cover seedling demands during the first year of growth (Kennedy et al. 2004; Milberg and Lamont 1997; Shi et al. 2018b).On the other hand, low plasticity to resources availability of *Quercus* species may be partly responsible for the constant seedling biomass under the three fertilizer treatments (Sanz Pérez et al. 2007).

In addition, we observed that both plant N concentration and content increased with an increase in dosage of fertilizer. Considering together with low plasticity in biomass, this indicates N storage of *Q. mongolica* seedlings was favored when they receive a supply of 10–100 mg N. In fact, acorn nutrient reserves can be remobilized completely to support seedling growth within 4 weeks after emergence (Shi et al. 2018b). Thus, given a semi-determinate shoot growth strategy in *Quercus* species, nutrient supply can meet nutrient demand during the later growing stage and further accelerate nutrient storage. Similar results were frequently reported in in evergreen *Quercus* species (Cuesta et al. 2010; Oliet et al. 2009; Villar-Salvador et al. 2004) and deciduous *Quercus* species (Salifu and Jacobs 2006; Shi et al. 2019). This implies that an external nutrient supply can accelerate the production of nutrient-loaded oak seedlings (Salifu and Jacobs 2006; Timmer 1997) due to low plasticity in growth. In this study, we infer that 50 mg N supply would produce nutrient-sufficient *Q. mongolica* seedlings and 100 mg N may accelerate seedlings to reach luxury consumption stages (Salifu and Jacobs 2006).

Q. mongolica seedlings favored carbohydrate storage over growth in response to an increase in nutrient supply. Our results are similar to those with rice (Guo et al. 2022) that leaf soluble sugars significantly increased under high doses of fertilizer and *Salix viminalis* that TNC amounts increased in perennial organs under high N supply when all leaves senesced. (Bollmark 1999). However, some opposite effects were observed in *Quercus* species (Kabeya and Sakai 2005; Sanz Pérez et al. 2007). The discrepancy may be caused by the difference in seedling phenological stages. In our study, we harvested seedlings at the end of dormant stages, which is comparable to the stage in *Salix viminalis* (Bollmark 1999). Thus, this implies that photosynthates increased by high N reserves (Evans and Clarke 2018; Niinemets 1999; Poorter et al. 2009) can be allocated more to carbohydrate storage instead of being used for growth (Hoch 2015), as evidenced by the lack of significant change in structural biomass. Furthermore, it seems that sink limitation on growth in *Q. mongolica* was caused by other factors other than nutrient (Birk and Matson 1986), such as low plasticity.

Taken together, we infer fertilization supply increases carbohydrate reserves when seedlings are at the stage of luxury consumption (Fig. 1 in (Salifu and Jacobs 2006)) since the growth will not be limited by nutrients and more photosynthates caused by high N reserves are allocated to carbohydrate storage instead of being used by growth.

High carbohydrate and N storage improve field performance

Studies have shown that nutrient and carbohydrate storage can improve the rate of subsequent growth (Landhausser et al. 2012; Uscola et al. 2015; Villar-Salvador et al. 2004; Wang et al. 2019), as well as the ability to resprout (Kabeya and Sakai 2005). When exposed to high doses of fertilizer in our study, both species showed increased field growth, perhaps because the high nutrient and carbohydrate storage facilitated remobilization after transplanting, increasing leaf area and subsequent C acquisition in a positive feedback loop (Pokharel and Chang 2016; Vizoso et al. 2008). This idea is also supported by that the positive relationship between plant N, starch and soluble sugars content and stem volume increase during the first growing season ($SV_{1\text{-}0}$) respectively *in P. tabuliformis*, and positive relationship between plant starch content and $SV_{1\text{-}0}$ *in Q. mongolica* (Table 5). It appears that starch content, known as the major carbohydrate storage, contributes more to the field growth. It seems that a minimum amount of fertilizer was needed to achieve this better growth, since low or moderate doses of fertilizer were ineffective in our experiments.

The overall winter mortality was lower in *Q. mongolica* seedlings than in *P. tabuliformis* seedlings. Mortality during the first growing season was comparable for the two species while most mortality difference occurred in winter, indicating that seedlings likely had been exposed to temperatures that exceeded their freezing tolerance. *P. tabuliformis* seedlings showed almost twice higher winter mortality in all three doses of fertilizer than that in *Q. mongolica*. This indicates *Q. mongolica* seedlings showed better winter stress resistance in the field than *P. tabuliformis* seedlings under the same amount of fertilizer treatment. This is supported by the difference in freezing tolerance for the two species that great damage would be caused at temperature −28.9 °C in *Q. mongolica* (PFAFdatabase) in USDA hardiness 5–8 zones) and at temperature −23.3 °C in *P. tabuliformis* (Bannister and Neuner 2001). In our study site, minimum temperature ranged between −20 and −26.3 °C for 15 days in the winter of 2018 (Table 6), which is beyond the minimum freezing temperature for *P. tabuliformis*. Moreover, precipitation during the winter was low (Table 6), which might have induced some water stress in the seedlings, thereby aggravating frost damage (Fernández-Pérez et al. 2018). Hence, the winter stress resistance in *Q. mongolica* may be attributed to much higher plant TNC concentration than that in *P. tabuliformis*.

However, within *P. tabuliformis*, the mortality of seedlings was lower when exposed to high doses of fertilizer, for which plant N and stem TNC reserves was higher. This indicates that higher N and TNC reserves led to less field mortality probably due to increased cold tolerance(Greer et al. 2000; Heredia-Guerrero et al. 2014; Toca et al. 2017). Since soluble sugars play important role in osmoregulation maintaining hydraulic integrity (Chen et al. 2020) and nitrogen is related to N-dependent cryoproteins (Griffith and Yaish 2004), high stem soluble sugar concentration (similar trend in starch and TNC) and N concentration can also partly explain the lower winter mortality under high fertilization treatment. Similar results were observed in another study conducted in the same site on *P. tabuliformis* where high winter mortality was attributed to low N and TNC reserves (Luo et al. 2021). In addition, exposed to high doses of fertilizer, plant N and TNC content was higher as well as stem volume increase during the first growing season was higher. This indicates that optimized growth and root mass led by higher N, TNC storage and initial seedling size under high doses of fertilizer can help to outcompete weeds and to exploit field resources (Luo et al. 2021). No difference in winter mortality was observed in *Q. mongolica*, which may be attributed to less morphological plasticity responding to fertilizer application and a higher intrinsic frost resistance.

Conclusions and implications for forest plantations

Although there was low morphological plasticity in the response of *Q. mongolica* to soil fertility, the dosage of fertilizer administered can accelerate the production of nutrient-loaded seedlings, which can in turn increase seedling storage of nutrients and carbohydrates as well as improve field growth. An increase in carbohydrate storage can also increase nutrient storage in nutrient-loaded seedlings such as *Q. mongolica*. Given that *P. tabuliformis* seedlings experience high winter mortality perhaps caused by exceeding freezing tolerance, we recommend producing *P. tabuliformis* seedlings applying a higher fertilizer rate than 100 mg N to promote nutrient and carbohydrate storage and help to mitigate the field mortality. We also recommend producing nutrient-loaded *Q. mongolica* seedlings by growing them in a nursery with fertilization of at least 100 mg N per plant. Such seedlings may be effective for restoration programs targeting areas affected by winter low temperature.

Appendix

See Figs. 5, 6 and Tables 3, 4, 5, 6.

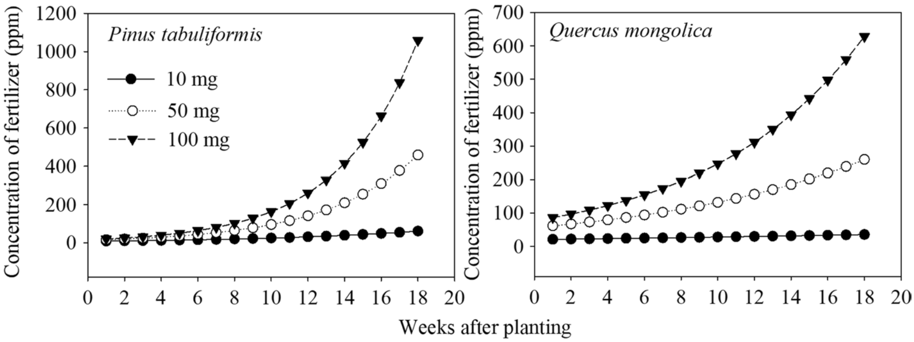

Fig. 5 Schedule of fertilizer treatments

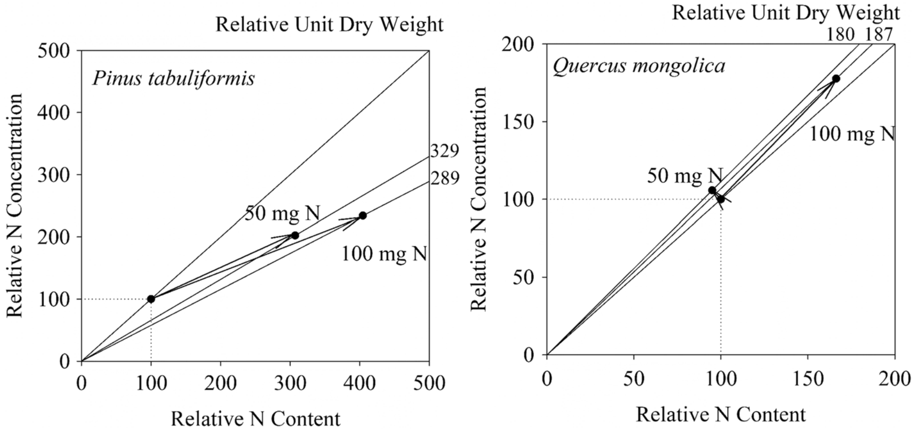

Fig. 6 Vector nomograms of relative changes in dry weight, nutrient content, and nutrient concentration occurring at the level of whole plant of seedling cultured under three fertilization regimes (10 mg, 50 mg and 100 mg N supply). Seedling status treated with 10 mg N is normalized to 100. Arrows reflect progressions in increase of fertilizer doses from 10 to 50 mg and 100 mg N supply, respectively. Note differences in axis scale between *Pinus tabuliformis* and *Quercus mongolica*

Table 3 Seed traits of *Pinus tabuliformis* and *Quercus mongolica*

Trait	*Pinus tabuliformis*	*Quercus mongolica*
Seed mass (mg)	36.21 ± 0.57	1382.8 ± 46.05
Nitrogen concentration (%)	4.16 ± 0.26	0.997 ± 0.03
Phosphorus concentration (%)	0.71 ± 0.04	0.21 ± 0.01
Potassium concentration (%)	0.31 ± 0.02	0.61 ± 0.02
Nitrogen content (mg)	1.51 ± 0.11	13.88 ± 0.91
Phosphorus content (mg)	0.26 ± 0.02	2.91 ± 0.15
Potassium content (mg)	0.11 ± 0.01	8.42 ± 0.5

Values are Mean ± SE

Table 4 Effect of fertilization on nitrogen and carbohydrate concentration in *Pinus tabuliformis* and *Quercus mongolica* seedlings cultivated in the nursery

	Species	*Pinus tabuliformis*				*Quercus mongolica*			
	Fertilizer dose	Low	Moderate	High	P	Low	Moderate	High	p
Organs	Nitrogen (%)	1.11 ± 0.06^{b}	1.85 ± 0.08^{a}	1.92 ± 0.04^{a}	**< 0.001**				
	ST (%)	0.90 ± 0.04	0.88 ± 0.03	1.02 ± 0.28	0.904				
Needle	SS (%)	2.21 ± 0.05a	1.75 ± 0.06^{b}	1.53 ± 0.05^{c}	**< 0.001**				
	TNC (%)	3.11 ± 0.07	2.64 ± 0.09	2.55 ± 0.28	0.201				
	Nitrogen (%)	0.75 ± 0.03^{c}	1.71 ± 0.05^{b}	2.01 ± 0.07^{a}	**< 0.001**	0.58 ± 0.06^{b}	0.69 ± 0.05^{b}	0.96 ± 0.03^{a}	**< 0.001**
	ST (%)	0.99 ± 0.09^{b}	0.99 ± 0.1ab	1.20 ± 0.06^{a}	**0.021**	1.44 ± 0.1^{a}	1.18 ± 0.09^{ab}	1.14 ± 0.07^{b}	**0.026**
Stem	SS (%)	1.77 ± 0.1^{ab}	1.68 ± 0.08^{b}	1.90 ± 0.04^{a}	**0.035**	5.82 ± 0.34^{b}	5.98 ± 0.28^{ab}	6.89 ± 0.27^{a}	**0.024**
	TNC (%)	2.76 ± 0.1^{b}	2.67 ± 0.1^{b}	3.10 ± 0.08^{a}	**< 0.001**	7.26 ± 0.33	7.17 ± 0.24	8.03 ± 0.29	0.077
	Nitrogen (%)	0.69 ± 0.03^{c}	1.57 ± 0.02^{b}	1.89 ± 0.07^{a}	**< 0.001**	0.69 ± 0.09^{b}	0.71 ± 0.05^{b}	1.20 ± 0.07^{a}	**< 0.001**
	ST (%)	0.85 ± 0.05	0.76 ± 0.03	0.75 ± 0.05	0.156	2.51 ± 0.1^{b}	2.72 ± 0.19^{ab}	3.15 ± 0.22^{a}	**0.037**
Root	SS (%)	1.10 ± 0.13	1.17 ± 0.16	0.97 ± 0.06	0.102	10.35 ± 0.23^{b}	11.18 ± 0.44^{ab}	12.17 ± 0.54^{a}	**0.001**
	TNC (%)	1.95 ± 0.08^{a}	1.93 ± 0.19^{ab}	1.72 ± 0.11^{b}	**< 0.001**	12.82 ± 0.23^{c}	14.01 ± 0.52^{b}	15.54 ± 0.78a	**< 0.001**
	Nitrogen (%)	0.82 ± 0.03^{c}	1.66 ± 0.04^{b}	1.92 ± 0.05^{a}	**< 0.001**	0.67 ± 0.08^{b}	0.71 ± 0.05^{b}	1.19 ± 0.06^{a}	**< 0.001**
	ST (%)	0.89 ± 0.04	0.82 ± 0.05	0.83 ± 0.04	0.189	2.39 ± 0.08	2.62 ± 0.18	2.84 ± 0.29	0.280
Plant	SS (%)	1.55 ± 0.07^{a}	1.52 ± 0.07^{ab}	1.37 ± 0.02^{b}	**0.023**	9.93 ± 0.21^{b}	10.82 ± 0.41^{ab}	11.83 ± 0.54^{a}	**< 0.001**
	TNC (%)	2.44 ± 0.05^{a}	2.34 ± 0.12^{ab}	2.20 ± 0.05^{b}	**< 0.001**	12.29 ± 0.19^{c}	13.53 ± 0.48^{b}	15.06 ± 0.79^{a}	**< 0.001**
	N/C reserves	0.35 ± 0.02^{c}	0.69 ± 0.03^{b}	0.83 ± 0.07^{a}	**< 0.001**	0.08 ± 0.02^{b}	0.09 ± 0.03^{b}	0.37 ± 0.17^{a}	**0.044**

SS, soluble sugars; ST, starch; TNC, total non-structural carbohydrates. Values are mean ± SE

Lowercase letters indicate significant differences among treatments based on lmer function in lmerTest package and the Tukey post-hoc test

Bold values indicate significant differences among fertilizer treatments based on lmer function in lmerTest package when $p<0.05$

Table 5 regression between plant nitrogen and TNC reserves with field growth and survival, respectively

		mortalityT_1	mortality$T_{1\text{-AW}}$	mortalityT_2	$SV_{1\text{-}0}$	$SV_{2\text{-}0}$
Pinus tabuliformis	Plant N content	0.049(−)	0.059(−)	0.052 (−)	0.530(+)	0.053(+)
		0.488	0.447	0.499	**0.007**	0.471
	Plant TNC content	0.043(−)	0.083(−)	0.113 (−)	0.531(+)	0.135(+)
		0.541	0.39	0.313	**0.011**	0.266
	Plant ST content	0.067(−)	0.101(−)	0.108(−)	0.583(+)	0.227(+)
		0.445	0.341	0.324	**0.006**	0.139
	Plant SS content	0.017(−)	0.049(+)	0.091(−)	0.363(+)	0.044(+)
		0.704	0.512	0.367	**0.049**	0.536
		mortalityT1	mortalityT1-AW	mortalityT2	SV1-0	SV2-0
Quercus mongolica	Plant N content	0.041(−)	0.022(+)	0.032(−)	0.048(+)	0.010(+)
		0.526	0.642	0.577	0.495	0.746
	Plant TNC content	0.162(−)	0.118(−)	0.028(−)	0.299(+)	0.240(+)
		0.194	0.274	0.601	***0.066***	0.106
	Plant ST content	0.071(−)	0.027(+)	0.001(−)	0.337(+)	0.161(+)
		0.403	0.608	0.91	**0.048**	0.197
	Plant SS content	0.054(−)	0.032(+)	0.066(−)	0.118(+)	0.092(+)
		0.469	0.576	0.419	0.274	0.337

Upper line is r^2 and lower line is *p*-value; + indicates positive relationship; − indicates negative relationship

Bold values indicate significantdifferences among fertilizer treatments based on regression when $p<0.05$

Bold and italicized values indicate significant difference among fertilizer treatments based on regression when $0.05 \leq p<0.1$

Table 6 Number of days with extreme freezing temperatures and precipitation at the outplanting site during the winter of 2018

Extreme freezing temperatures	No. of days (2018)	
−15 to −20 °C	36	
−20 to −25 °C	14	
−25 to −30 °C	1	
Month	First winter season (2018)	
	No. of days	Precipitation (mm)
November of 2018	1	2.3
December of 2018	1	0.2
January of 2019	0	0
February of 2019	3	1.4
March of 2019	3	8.5
April of 2019	2	5.6
May of 2019	6	68.4

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Declarations

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

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