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ORIGINAL ARTICLE

Nitrogen uptake and allocation in *Populus simonii* in different seasons supplied with isotopically labeled ammonium or nitrate

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Abstract
Key message **The total uptake of $^{15}NO_3$-N was twofold higher than that of $^{15}NH_4$-N when supplied with ammonium and/or nitrate in different seasons; the seedlings fertilized with NO_3-N had good growth with high photosynthetic rate and total biomass.**

Abstract Appropriate fertilization is crucial for maximum plant growth and improving nitrogen use efficiency. Poplar is an important fast-growing tree species for biomass production, however, little is known about fertilizer management of poplar plantations growing on barren soil in different seasons. To understand nitrogen uptake and allocation of *Populus simonii* supplied with different forms of nitrogen in different seasons, we determined nitrogen uptake and allocation of *P. simonii* potted seedlings after a 4-day supply of $^{15}NH_4$-N, $^{15}NO_3$-N, $^{15}NH_4NO_3$, and $NH_4^{15}NO_3$ in May, July, and September. The total ^{15}N uptake was twofold higher when supplied with sole $^{15}NO_3$-N compared to sole $^{15}NH_4$-N in all the investigated seasons. In the presence of ammonium nitrate ($^{15}NH_4NO_3$ and $NH_4^{15}NO_3$), the total ^{15}N uptake was two times higher when supplied with $NH_4^{15}NO_3$ compared to $^{15}NH_4NO_3$. Per unit biomass, the ^{15}N-uptake ability of fine roots was higher in May and July compared to that in September. ^{15}N was present mainly in leaves in May and July, and was mainly stored in roots and stems in autumn. The effect of nitrogen on the growth of *P. simonii* seedlings was studied by fertilizing with NH_4-N, NO_3-N, and NH_4NO_3 for 8 weeks. The seedlings fertilized with NO_3-N had good growth with high photosynthetic rate and total biomass indicating that NO_3-N is crucial for *P. simonii* growth. These data contribute to understand the nitrogen uptake in different seasons in trees supplied with different forms of nitrogen. This provides important theoretical bases for fertilizer management of poplar plantations.

Keywords Nitrate · Ammonium · Nitrogen uptake and allocation · Seasonal variation · Growth characteristics · *Populus simonii*

Introduction

Nitrogen (N) is one of the main macronutrients for plant growth and development. Most crops grow on fertile soils, but forest trees are often planted on marginal lands where N availability is limited (Finzi et al. 2007). Thus, N fertilization is crucial to ensure high productivity and biomass of forest tree plantation. N is available in different chemical forms such as ammonium (NH_4-N), nitrate (NO_3-N), and organic N forms (McKane et al. 2002). Plants can assimilate both NH_4-N and NO_3-N, but some species perform better when fertilized with NH_4-N (Cruz et al. 1993; Britto and Kronzucker 2002; Bown et al. 2010; Metcalfe et al. 2011), while others show improved performance when grown with NO_3-N (Atkin and Cummins 1994) or a mixture of NO_3-N and NH_4-N (Öhlund and Näsholm 2001; Nicodemus 2007; Domenicano et al. 2011). The choice of

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nitrogen form for fertilization is very important for plant growth because plants will show best growth when supplied with their "favorite" nitrogen at appropriate times; therefore, appropriate fertilization plays a key role for maximizing plant growth and improving N use efficiency.

NH_4-N and NO_3-N have highly distinct consequences for plant growth (Haynes and Goh 1978; Britto and Kronzucker 2002; Domenicano et al. 2011). For example, NO_3-N treated hybrid poplar (*Populus maximowiczii* × *P. balsamifera*) plants show higher ratios of fine roots: coarse roots and higher specific root lengths compared to NH_4-N treated poplar plants (Domenicano et al. 2011). Plants might be able to use different forms of nitrogen in different seasons because they have different growth characteristics in spring, summer, and autumn (McKane et al. 2002). In spring, buds and new leaves appear. Plants have rapid growth in summer while they grow slowly in autumn with decreasing temperature and short photoperiod. There is a lot of evidence that available nitrogen compounds vary in relative proportions in different seasons (Cooke and Weih 2005; Contosta et al. 2011; Brereton et al. 2013; Gilson et al. 2014). However, little information is available about NH_4-N or NO_3-N uptake of fine roots and allocation in different tissues fertilized with NH_4-N and/or NO_3-N in different seasons (Socci and Templer 2011; Brereton et al. 2013).

Poplar is an important fast-growing and high-yielding tree species with a short-rotation coppice for bioenergy production or ecological purpose (Schweier and Becker 2013; Manzone et al. 2014). Especially, *Populus simonii* Carr. is an important ecological and commercial breeding species in northern China; it is tolerant to drought, salinity, cold, and heat with wide distribution from Qinghai to the east coast of China in longitude and from the Heilongjiang River to the Yangtze River in latitude (Weisgerber and Han 2001; Wei et al. 2012). *P. simonii* is considered as an important afforestation species in China due to its large distribution range, excellent stress tolerance, rapid growth, and regeneration ability. *P. simonii* often exhibits poor growth with short height in the salty, or water-limited, or barren sandy soil, while *P. simonii* can reach a height of 30 m and a diameter of 2.5 m in the fertile and moist soil (Lu 2002). The soil in the afforestation areas, particularly the Loess Plateau, typically suffers from nutrient deficiencies, thus, fertilization is needed for afforestation species, such as *P. simonii*, to improve growth. However, *P. simonii* plantations lack management; little is known about fertilizer management in different seasons of poplar plantations growing on barren soil.

Populus simonii plants often grow on saline and alkali soil, where most available nitrogen is NO_3-N (Weisgerber and Han 2001). We hypothesized that *P. simonii* seedlings would have higher N uptake when supplied with NO_3-N compared to NH_4-N irrespective of different N forms and seasons. To test this hypothesis, we investigated short-term nitrogen uptake dynamics in *P. simonii* supplied with different forms of nitrogen (NH_4-N or/and NO_3-N) in different seasons. The ^{15}N isotope-labeled NH_4-N and NO_3-N were applied to 2-year-old *P. simonii* potted seedlings in three seasons: spring, summer, and autumn. Our specific objectives were to: (1) investigate N uptake of fine roots supplied with different forms of nitrogen, (2) examine the impact of season of application on N uptake, (3) understand the nitrogen allocation to different tissues after a short-time supply of nitrogen at different times in the growing season. Meanwhile, we carried out another independent relative long-term fertilizer experiment to test the effect of nitrogen form on growth of *P. simonii* seedlings. Based on a previous study (Rennenberg et al. 2010), we hypothesized that NO_3-N would be more important compared to NH_4-N for the growth of *P. simonii* seedlings. This study is valuable because it improves our understanding of nitrogen form uptake in different seasons. Meanwhile, the different effects of nitrogen forms on *P. simonii* growth provide an important theoretical base for fertilizer management of poplar plantations.

Materials and methods

Plant cultivation

Two-year-old seedlings of *P. simonii* were collected from Wuqi county, Shaanxi province (36°58′57″N, 108°09′34″E) in early April. Each seedling was decapitated to a stem height of about 10 cm with three to five buds and then planted in a 3 l pot filled with a 1:1 ratio of sand and vermiculite. All shoots but one were removed once buds had extended to about 5 cm. The cuttings were cultivated in a glasshouse in the condition of natural light, day/night 25/20 °C, 75 % relative humidity for 5 weeks. After that, the heat in the glasshouse was turned off, a ventilation device was used to exchange air in the greenhouse to the outside, and therefore the temperature was similar to outside before isotope treatment. We watered the cuttings every 3 days and irrigated with 250 ml modified Hoagland nutrient solution once a week (Hoagland and Arnon 1950) containing EDTA·FeNa 10 μM, $MnSO_4 \cdot H_2O$ 5 μM, $ZnSO_4 \cdot 7H_2O$ 1 μM, $CuSO_4 \cdot 5H_2O$ 1 μM, H_3BO_3 30 μM, H_2MoO_4 0.5 μM, KH_2PO_4 1000 μM, $MgSO_4 \cdot 7H_2O$ 1000 μM, $CaCl_2$ 1000 μM, Na_2SO_4 1000 μM, and NH_4-NO_3 100 μM; pH 6.5. To make this study more comparable to the field condition, we cultivated *P. simonii* seedlings with relatively low N concentration because *P. simonii* often grows on N-limited sites. To increase nitrogen uptake, plants were irrigated with Hoagland nutrient solution free of nitrogen for 2 weeks before ^{15}N treatment.

^{15}N treatment and sampling

Plants were treated with 250 ml 99.09 atom% enriched to 0.1 mM ^{15}N isotope-labeled ammonium ($^{15}NH_4Cl$-N), 99.14 atom% enriched to 0.1 mM ^{15}N isotope-labeled nitrate ($K^{15}NO_3$-N), and ^{15}N isotope-labeled NH_4NO_3 (either $^{15}NH_4NO_3$ or $NH_4^{15}NO_3$) in three seasons: spring (28 May), summer (28 July), and autumn (28 Sep). Each treatment was applied to four seedlings. The total biomass of plants in May, July, and September was 3.03 ± 0.25, 5.31 ± 0.28, and 7.21 ± 0.44 g, respectively. Four seedlings served as controls. They did not receive any ^{15}N-labeled nitrogen and thus were used to determine the natural abundance of ^{15}N signature of plants (Templer and Dawson 2004). Plants were harvested 4 days following ^{15}N addition and partitioned into fine roots (<2 mm in diameter), roots (coarse root and main root), stems, and leaves. The experimental period was limited to 4 days, which was sufficient to detect enriched ^{15}N in different tissues of plants. The fine roots, roots, stems, and leaves of each seedling were dried at 65 °C for at least 48 h until the dry weight was unchanged, and then ground into powder for further analysis. The total nitrogen (N%) and ^{15}N abundance were determined using a Thermo Electron Flash EA 1112 elemental analyzer coupled to a Thermo Electron Delta V isotope ratio mass spectrometer (EA-IRMS). The ^{15}N abundance was expressed as a delta value ($\delta^{15}N$, per mil, ‰) relative to atmospheric N_2: $\delta^{15}N = [(R_{sample} - R_{standard})/R_{standard}] \times 1000$, where $R = {}^{15}N/{}^{14}N$ and $R_{standard}$ is atmospheric N_2, and atom% of ^{15}N is equal to $R \times 100/(1 + R)$. Reproducibility of the $\delta^{15}N$ measurements was better than 0.2 ‰.

We calculated the ^{15}N content of different tissues including fine root, root, stem, and leaf as: the total ^{15}N content = $DW_{tissue} \times N\%_{tissue} \times (atom\%_{tissue} - atom\%_{control})$.

The total ^{15}N uptake of the plant was equal to the total ^{15}N content of all tissues.

The ^{15}N uptake of fine roots was calculated as the total ^{15}N uptake of plant/dry weight of fine root reflecting the N-uptake ability of fine root per biomass.

The effect of nitrogen form on *P. simonii* plant growth

To know the effect of different nitrogen forms (NH_4-N, NH_4NO_3, NO_3-N) on plant growth, we carried out another independent experiment with 2-year-old *P. simonii* seedlings fertilized with NH_4Cl, or NH_4NO_3, or KNO_3 for 8 weeks. The *P. simonii* cuttings were also planted in the same glasshouse described above for 5 weeks and irrigated with 250 ml modified Hoagland nutrient solution once a week (Hoagland and Arnon 1950). The fertilized experiment was started on May 28 with similar height of *P. simonii* seedlings described above and the temperature was not controlled manually. There was a ventilation device to exchange air in the greenhouse to the outside; therefore, the temperature in the greenhouse was close to outside. To maximize the effect of nitrogen form on plant growth, we added a high concentration of nitrogen (250 ml modified Hoagland nutrient solution including 5 mM NH_4-N, NH_4NO_3, or NO_3-N) once a week in the fertilizer experiment compared to that described above. Each fertilizer treatment was applied to ten plants. Near the harvest date, the net photosynthetic rate was measured from 9:00 to 11:00 hours with a portable photosynthesis system (Li-Cor-6400; Li-Cor, Inc, Lincoln, NE) with an attached LED light source (500 μmol photon m^{-2} s^{-1}). The CO_2 concentration in the chambers was 400 μmol min^{-1}, and the air flow was 500 μmol s^{-1}. The chlorophyll content of each plant chosen for gas exchange was measured with a portable meter (Minolta SPAD 502 Meter). Simultaneously, the height of the main shoot of each plant was measured with a ruler. Plants were harvested 8 weeks after fertilization with different forms of nitrogen and partitioned into above-ground and below-ground parts. The above-ground and below-ground samples were dried at 65 °C to constant mass, and then dry weight was recorded.

Statistical analyses

N form and time effect on N uptake were analyzed by two-way analyses of variance (ANOVA). The effect of N form on the plant growth was assessed by one-way ANOVA to test the significant differences between treatments and plant parts. When significant, Fisher's least significant difference test (LSD) was used to identify differences between treatment means. The significance level was established at $p < 0.05$.

Results

Nitrogen uptake of *P. simonii* in different seasons

To understand the total N uptake by the whole *P. simonii* plants within 4 days, we determined the total ^{15}N uptake by summing the ^{15}N content of all the tissues. *P. simonii* plants had different total ^{15}N uptake when fertilized with different forms of nitrogen across the three investigated seasons (Fig. 1). The total ^{15}N uptake of plants was significantly affected by nitrogen forms ($^{15}NO_3$-N or $^{15}NH_4$-N) and the seasons ($p < 0.05$). The total N supplied by $^{15}NO_3$-N or $^{15}NH_4$-N was almost the same (0.375 mg/plant), yet

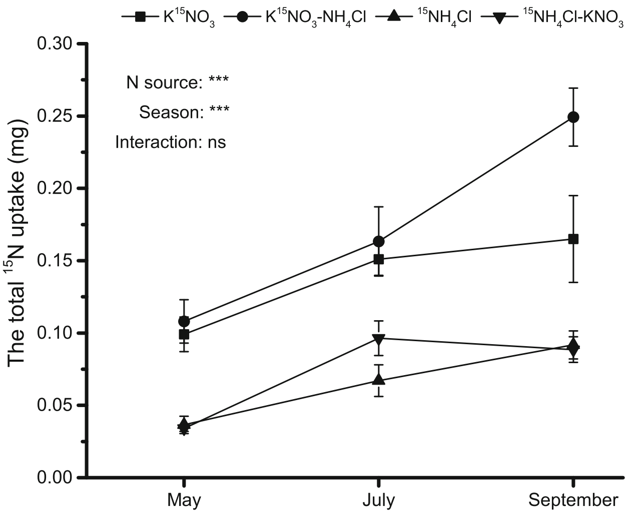

Fig. 1 The total ^{15}N uptake of *P. simonii* in three seasons treated with different ^{15}N forms. The data indicate the mean ± SD ($n = 4$). ***Significance at $p < 0.001$, *ns* no significance

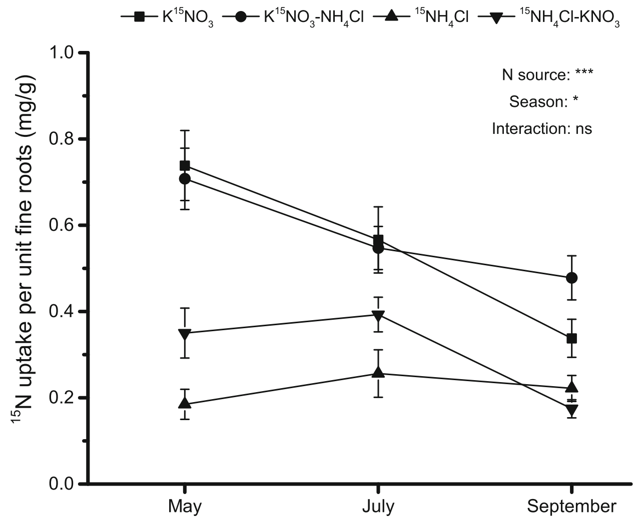

Fig. 2 ^{15}N uptake per unit fine roots in *P. simonii* in three seasons treated with different ^{15}N forms. The data indicate the mean ± SD ($n = 4$). ***Significance at $p < 0.001$, *significance at $p < 0.05$, *ns* no significance

different total ^{15}N uptake was observed in May, July, and September. The total uptake of ^{15}N when supplied with sole $^{15}NO_3$-N was two times higher than that of sole $^{15}NH_4$-N in all three investigated seasons. In the ammonium nitrate treatments, the total ^{15}N uptake was also two times higher when supplied with $NH_4^{15}NO_3$ compared to $^{15}NH_4NO_3$. Of the different nitrogen forms, the total ^{15}N uptake was the highest when supplied with $NH_4^{15}NO_3$. It was the lowest when supplied with sole NH_4^+. Across the three investigated seasons, the total ^{15}N uptake was highest in September and lowest in May.

Fine root is the most important organ for nitrogen acquisition in plants; therefore, we investigated N-uptake ability of fine roots defined as the total ^{15}N uptake of plant/dry weight of fine root. ^{15}N uptake of fine roots was significantly affected by the nitrogen forms and season of application (Fig. 2, $p < 0.05$). Per unit biomass, the ^{15}N-uptake ability of fine roots was higher in May and July compared to that in September supplied with a given nitrogen form (Fig. 2). Of the different nitrogen forms, the fine roots had the highest ^{15}N uptake when supplied with $^{15}NO_3$-N and the lowest ^{15}N uptake when supplied with $^{15}NH_4$-N. Per unit fine root biomass, the uptake of ^{15}N was significantly greater when supplied with NO_3-N compared to $^{15}NH_4$-N in *P. simonii* in the three investigated seasons, especially in May.

N allocation to different tissues

The relative ^{15}N distribution in different tissues is defined as ^{15}N content of a given tissue relative to the total uptake of ^{15}N by *P. simonii* plant in this study. The relative ^{15}N distribution varied in different tissues from May to September when supplied with ammonium and/or nitrate (Fig. 3). In May and July, most of the supplied ^{15}N was distributed in leaves, and the percent of ^{15}N was significantly influenced by the nitrogen forms ($p < 0.01$). In September, the proportion of ^{15}N allocation increased in stems or roots, while it largely decreased in leaves irrespective of the supplied nitrogen form suggesting that nitrogen assimilation was slowed in autumn.

The ^{15}N content of different tissues was significantly affected by N form (Fig. 4, $p < 0.05$). Generally, different tissues of plants (fine roots, main roots, stems, and leaves) had a higher ^{15}N content when supplied with $^{15}NO_3$-N or $NH_4^{15}NO_3$ compared to $^{15}NH_4$-N or $^{15}NH_4NO_3$ (Fig. 4). The supply of extra non-labeled NH_4-N ($NH_4^{15}NO_3$) increased the ^{15}N content compared to sole nitrate ($^{15}NO_3$–N) in all tissues except for the main roots. Leaves generally had the largest total ^{15}N content followed by fine roots and stems. The main roots had the lowest N content. The ^{15}N content of plants supplied with $^{15}NO_3$-N or $^{15}NH_4$-N varied from May to September in different tissues including fine roots, main roots, stems, and leaves (Fig. 4). All tissues generally had the lowest ^{15}N content in May among the three investigated seasons (May, July, and September) (Fig. 4). ^{15}N content of leaves decreased significantly from July to September in sole N-source treatments indicating that the translocation of N slowed down in September.

The percent of total N was also significantly affected by nitrogen forms in different tissues after a 4-day supply of different forms of nitrogen (Fig. 5). Of different nitrogen forms, nitrate could increase the total N concentration of the roots and stems compared to the other nitrogen forms (NH_4Cl or NH_4NO_3) in a given season. Leaves could have

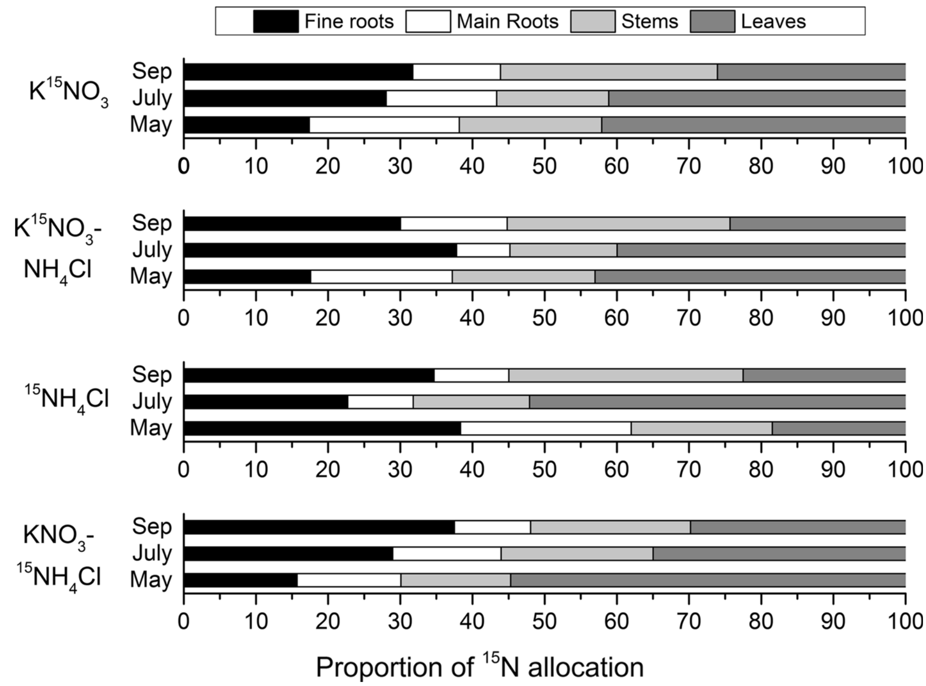

Fig. 3 Relative ^{15}N distribution in different tissues in three seasons (fine roots, main roots, stems, and leaves). The data indicate the mean of four replicates

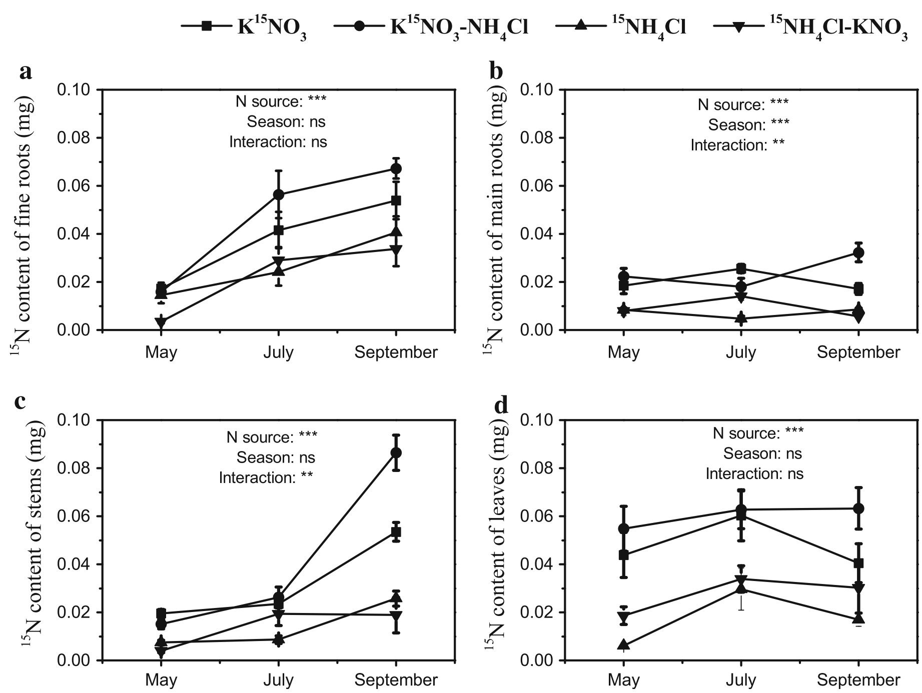

Fig. 4 The total ^{15}N content of different tissues in three seasons (**a** fine roots, **b** main roots, **c** stems, **d** leaves) after a 4-day supply of ^{15}N-labeled ammonium or nitrate. The data indicate the mean ± SD ($n = 4$). ***Significance at $p < 0.001$, **significance at $p < 0.01$, *ns* no significance

more total N concentration when supplied with sole ammonium compared to the other nitrogen forms (KNO_3 or NH_4NO_3). The total N concentration varied in different tissues in a given season. Generally, leaves had high total N concentration followed by fine roots and stems. The main roots had the lowest total N concentration. Across different seasons, the total N concentration in most of the tissues increased from May to September which was consistent with the total ^{15}N uptake indicating that plants could accumulate more nitrogen as they increased in size.

The effect of nitrogen forms on the growth of *Populus simonii*

The *P. simonii* seedlings showed slightly different growth characteristics when supplied with different forms of

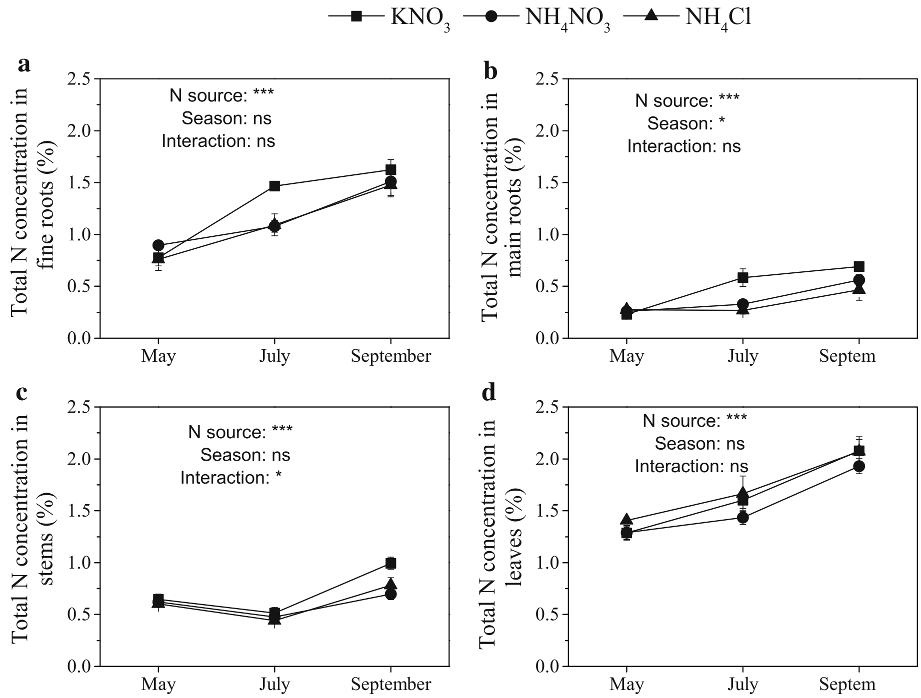

Fig. 5 The total N concentration of different tissues in three seasons (**a** fine roots, **b** main roots, **c** stems, **d** leaves) after a 4-day supply of ^{15}N-labeled ammonium or nitrate. The data indicate the mean ± SD ($n = 4$). ***Significance at $p < 0.001$, *significance at $p < 0.05$, *ns* no significance

Table 1 Height increment and photosynthesis of *P. simonii* seedlings fertilized with different forms of nitrogen for 8 weeks

N form	Height increment (cm)	Chlorophyll content (SPAD)	Net photosynthetic rate (mmol CO_2 m^{-2} s^{-1})
NO_3-N	40.38 ± 3.29a	36.71 ± 3.58a	15.36 ± 1.56a
NH_4-N	43.54 ± 3.36a	32.62 ± 3.56a	12.37 ± 1.58b
NH_4NO_3	44.99 ± 3.88a	36.95 ± 4.13a	14.64 ± 1.43a

Data indicate mean ± SD ($n = 10$). The *same letter* in a column indicates that the difference is not statistically significant, while *different letters* in the same column indicate significant difference at $p < 0.05$

Table 2 Biomass (dry weight) of *P. simonii* seedlings fertilized with different forms of nitrogen

N form	Total biomass (g)	Aboveground (g)	Belowground (g)	Belowground/aboveground
NO_3–N	9.01 ± 0.64a	5.85 ± 0.62a	3.66 ± 0.41a	0.63 ± 0.05a
NH_4–N	6.82 ± 0.76b	3.89 ± 0.54b	2.99 ± 0.29a	0.77 ± 0.09b
NH_4NO_3	8.65 ± 0.69ab	4.97 ± 0.43a	3.58 ± 0.31a	0.72 ± 0.08ab

Data indicate mean ± SD ($n = 10$). The *same letter* in a column indicates that the difference is not statistically significant, while *different letters* in the same column indicate significant difference at $p < 0.05$

nitrogen (NH_4-N, NO_3-N, and NH_4NO_3). The height increment and chlorophyll content were not significantly affected by the supplied nitrogen forms (Table 1). Plants supplied with NO_3-N or NH_4NO_3 had significantly higher net photosynthetic rate than those supplied with NH_4-N.

NH_4-N and NO_3-N also had different effects on the biomass of *P. simonii* seedlings. The *P. simonii* seedlings fertilized with NO_3-N had significantly higher total biomass and aboveground biomass (dry weight) compared to those fertilized with NH_4-N (Table 2). No significant difference was observed in the biomass of *P. simonii* seedlings supplied with NO_3-N and NH_4NO_3. Of the different nitrogen forms, NH_4NO_3 could slightly enhance the belowground biomass of plants. The ratio of belowground biomass to aboveground biomass in *P. simonii* seedlings was the highest when supplied with NH_4-N.

Discussion

N is one of the main macronutrients for plant growth, appropriate N addition is crucial to maximize the plant growth. Excess N fertilization is both an unnecessary cost to growers and a pollution risk to groundwater, while N deficiency often results in poor growth. Thus, appropriate N fertilization is necessary to improve plant growth and avoid N pollution in the soil. N is available in different forms, such as NH_4^+, NO_3^-, and organic N, and plants often show maximum growth with their preferred N form. It is crucial to determine the effect of different nitrogen forms on plant growth for a given species. Meanwhile, plants may need different forms of nitrogen in different seasons due to variable growth characteristics (Brockley 1995). It is therefore necessary for forest managers to understand nitrogen uptake of forest trees supplied with different forms of nitrogen in different seasons (Lea and Azevedo 2006).

N uptake of *P. simonii* with different N forms in different seasons

Fast-growing tree species such as *Populus* spp. are thought to be adapted to a high N supply, mainly NO_3^-, in the moving water table of the floodplain forests they inhabit (Rewald et al. 2014); Rennenberg et al. (2010) proposed that nitrate is much more important for nutrition of poplar species than for many other tree species. In the present study, the studied species *P. simonii* often grows on barren and N-deficient soils, thus we supplied low N content to make this study more close to the field conditions. Actually, under low nutrient supply net uptake of NO_3^- has been shown to be higher in *Populus tremuloides* than in *Pinus contorta* or *Pseudotsuga menziesii* (Min et al. 1999). In the present study, higher ^{15}N uptake was observed in *P. simonii* plants when supplied with $^{15}NO_3$-N compared to $^{15}NH_4$-N in different seasons. This result supported our initial hypothesis that *P. simonii* seedlings had higher N uptake when supplied with NO_3-N compared to NH_4-N irrespective of seasons. This might be due to higher concentration of nitrate when supplied with ammonium nitrate compared to sole ammonium/nitrate. A previous study reported that the nitrate reductase enzyme was active in *P. tremuloides* roots (Min et al. 1998). Additionally, nitrate is much more mobile in soil than ammonium, so the greater ^{15}N uptake of plant supplied with NO_3-N could be due to its greater mobility (Vitousek et al. 1982). The high NO_3-N uptake observed in this study may be an adaptation of *P. simonii* to its native habitat, which is on saline and alkali soil, where, the most available nitrogen is NO_3-N (Weisgerber and Han 2001).

The different amounts of nitrogen taken up in spring, summer, and autumn suggested that plants might require different amounts of nitrogen during different seasons. The different amounts of N taken up in different seasons could relate to the size of the root system. As the plants grew, their root systems grew, and therefore, could take up more nitrogen. The amount of N might be affected by the biomass of *P. simonii* seedlings which were different in spring, summer, and autumn. The uptake of ^{15}N increased with increasing plant biomass from May to September which suggests that the requirement of nitrogen increased as the plants increased in size (Li et al. 2009). The *P. simonii* seedlings still require nitrogen to maintain growth in autumn. Therefore, the seasonal rates of N addition should be considered to avoid N deficiency or N pollution in different seasons.

N allocation of *P. simonii* with different N forms during different seasons

For trees, N is variable in roots, stems, and leaves in different seasons (Weatherall et al. 2006; Brereton et al. 2013). In summer, most of the N was distributed in leaves because leaves constitute the dominant N sink during the summer. However, in autumn, N concentration is reduced in leaves and increased in roots and stems. This suggests that roots or stems are the main storage organs for N in autumn. High N in stems by autumn was also observed in willow (Brereton et al. 2013). Different N allocation in different tissues reflected N recycling across different seasons (Dohleman et al. 2012). This suggests that the stem is the major N storing tissue during winter (von Fircks et al. 2001).

The effect of nitrogen form on growth of *P. simonii*

In the present study, we studied the effect of inorganic N, NH_4-N, and/or NO_3-N, on *P. simonii* plant growth. *P. simonii* seedlings had a higher total biomass and above-ground biomass (dry weight) fertilized with NO_3-N or NH_4NO_3 compared to NH_4-N (Table 2). The results of this study support our initial hypothesis that NO_3-N would be more important compared to NH_4-N for the growth of *P. simonii* seedlings. This agreed with Rennenberg et al. (2010) who stated that most poplar species prefer NO_3-N. It is also consistent with a previous report that 50 % NO_3-N fertilization could improve biomass productivity of poplar and willow clones (Domenicano et al. 2011). Similarly, N fertilization with 80 % NO_3-N for *Populus deltoides* used for short-rotation coppice optimized whole-plant growth (Woolfolk and Friend 2003). Nitrogen forms also affect poplar root structure (Domenicano et al. 2011), and hence, the root biomass might also be affected by the nitrogen form.

In conclusion, NO_3-N is crucial for *P. simonii* plant growth. The amount of nitrogen required for *P. simonii* plants varied in different seasons; thus, the dose of nitrogen should be carefully considered for fertilization management in different seasons.

Author contribution statement Conceived and designed the experiments: Chunxia Zhang, and Zhong Zhao. Performed the experiments: Chunxia Zhang, Sen Meng, Yiming Li, and Li Su. Analyzed the data: Chunxia Zhang, and Sen Meng. Wrote the paper: Chunxia Zhang, and Zhong Zhao.

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Compliance with ethical standards

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

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