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# Nitrogen uptake strategies of mature conifers in Northeastern China, illustrated by the $^{15}\text{N}$ natural abundance method

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**Abstract: Background:** Conifers partition different N forms from soil, including ammonium, nitrate, and dissolved organic N (DON), to sustain plant growth. Previous studies focused on inorganic N sources and specific amino acid forms using  $^{15}\text{N}$  labelling, but knowledge of the contribution of DON to mature conifers' N uptake is still scarce. Here, we quantified the contribution of different N forms (DON vs.  $\text{NH}_4^+$  vs.  $\text{NO}_3^-$ ) to total N uptake, based on  $^{15}\text{N}$  natural abundance of plant and soil available N, in four mature conifers (*Pinus koraiensis*, *Pinus sylvestris*, *Picea koraiensis*, and *Larix olgensis*).

**Results:** DON contributed 31%, 29%, 28%, and 24% to total N uptake by *Larix olgensis*, *Picea koraiensis*, *Pinus koraiensis*, and *Pinus sylvestris*, respectively, whereas nitrate contributed 42 to 52% and ammonium contributed 19 to 29% of total N uptake for these four coniferous species.

**Conclusions:** Our results suggested that all four conifers could take up a relatively large proportion of nitrate, while DON was also an important N source for the four conifers. Given that DON was the dominant N form in study soil, such uptake pattern of conifers could be an adaptive strategy for plants to compete for the limited available N sources from soil so as to promote conifer growth and maintain species coexistence.

**Keywords:** Nitrogen uptake preference, Organic nitrogen, Inorganic nitrogen, Coniferous plantation,  $^{15}\text{N}$  natural abundance, Isotopic mixing model

## Background

Conifers are the main afforestation species in Northeast China and play indispensable roles in ecological services such as timber production and regulating regional climate (Zhou et al. 2020). Conifers use different N forms from soil as their primary N sources, including ammonium, nitrate, and dissolved organic N (DON) (Näsholm et al. 2009; Orwin et al. 2011). Given the relatively lower soil available N concentrations in temperate climates (Zhu et al. 2019), conifers would partition limited

available N sources to sustain plant growth (Kronzucker et al. 1997; Gao et al. 2020), which is an important mechanism underlying species coexistence (McKane et al. 2002). However, it remains unclear how conifers partition limited N resource due to the complex interactions between plant N uptake and a variety of environmental conditions (Britto and Kronzucker 2002; Houlton et al. 2007).

Previous studies mainly investigated the inorganic N sources (ammonium and nitrate) for conifer N uptake using  $^{15}\text{N}$  labelling (Zhu et al. 2019; Gao et al. 2020), in which conifers took up more nitrate in in situ experiments and took up more ammonium in hydroponic experiments (Kronzucker et al. 1997; Boczulak et al. 2014; Li et al. 2015; Liu et al. 2017). However, it remains unclear to what extent conifers can utilize organic N.

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Previous experiments have studied the contribution of specific amino acid forms using  $^{15}\text{N}$ - and  $^{13}\text{C}$ -labelled glycine, arginine, or glutamine (Öhlund and Näsholm 2004; Persson et al. 2006; Zhu et al. 2019). Results from such work may not represent the uptake patterns of DON by mature conifers because these amino acids generally only account for a small fraction of organic N pools (Yu et al. 2002; Andersson and Berggren 2005). Although DON is widely accepted as a potentially important N source (Näsholm et al. 2009; Andersen et al. 2017), the knowledge of the contribution of DON to N uptake by mature conifers is still limited.

Natural abundance of  $^{15}\text{N}$  ( $\delta^{15}\text{N}$ ) in plants and soil can provide integrated information related to plant-soil physiological processes (Phillips and Gregg 2003; Houlton et al. 2007; Craine et al. 2009). Recently, the development of methods for determining the  $\delta^{15}\text{N}$  of ammonium and nitrate provided the possibility to comprehensively study the  $\delta^{15}\text{N}$  of plants and soil extractable N (Liu et al. 2014; Zhang et al. 2015). Combined with isotopic mixing models, the  $^{15}\text{N}$  natural abundance method has been used to quantify the contribution of different N forms (DON, ammonium, and nitrate) to total N uptake based on the  $\delta^{15}\text{N}$  in foliage and soil extractable N in several previous studies (Houlton et al. 2007; Takebayashi et al. 2010; Zhang et al. 2018). The analysis of  $\delta^{15}\text{N}$  in foliage and different N forms in soil could assess the contribution of DON to coniferous species in a relatively convenient and practical approach (Peri et al. 2012; Zhang et al. 2018).

In this study, we used  $^{15}\text{N}$  natural abundance method to study the N uptake strategy of the four main conifers used for afforestation in Northeastern China, specifically *Pinus koraiensis*, *Pinus sylvestris*, *Picea koraiensis*, and *Larix olgensis*. We compared the results with our previous in situ labelling studies of these four conifers (Zhou et al. 2020). Our major objectives were (1) to explore the contribution of different N forms to mature conifers in the field and (2) to test whether the N uptake strategies are similar if assessed by  $^{15}\text{N}$  natural abundance or by in situ labelling methods.

## Methods and Materials

### Study site

This study was conducted at the Mengjiagang Forest Farm in Huanan County, Heilongjiang Province in Northeast China (46°25'N, 130°42'E, elevation 170–575 m). The mean annual temperature was 2.7 °C, and annual precipitation averaged 550 mm. There are hundreds of shrub and herb species and more than 20 tree species in the study area. In this study, we selected coniferous plantations of three evergreen species, *Pinus koraiensis*, *Pinus sylvestris*, and *Picea koraiensis*, and one deciduous species, *Larix olgensis*. These conifers are the dominant

plantation types and indispensable for timber production. These four conifers are associated with ectomycorrhizal fungi (Guo et al. 2008). The selected trees were about 40 years old and almost 20 m high. Soils are mainly dark brown soil, with pH values ranging from 5.5 to 5.8 (Zhou et al. 2020). The total N concentrations of bulk soil and foliage ranged from 0.38 to 0.39% and from 1.4 to 2.1%, and total C concentrations of bulk soil and foliage ranged from 4.93 to 5.38% and from 46.4 to 49.3%, respectively (Zhou et al. 2020).

### Sample collection

In each plantation, we selected five conifers with similar diameter at breast height. To avoid root intermingling, the distance between each tree was greater than 10 m, and there was no canopy overlap between each other. In July 2019, 20–50 fully developed leaves were collected for each tree. Mineral soil samples within 1.5 m from the trunk of each tree were collected with a 6 cm inner diameter auger and divided into two layers (0–10 and 10–20 cm). The  $\text{O}_{a+e}$  layer was removed before mineral soil was collected and restored after completion. Samples from eight soil cores in each plot were mixed to one composite sample by different soil depth, and then passed through a 2 mm sieve to remove plant materials and rocks.

### Chemical analyses

Within 6 h after sampling, 10 g fresh soil was extracted with 2 M KCl solution in a soil/solution ratio of 1:4. The extraction was frozen at  $-36$  °C until concentration and isotopic analysis. Leaf samples were dried at 65 °C to constant weight, and another subsample of soil was air-dried at room temperature to measure the  $\delta^{15}\text{N}$  and C, N concentrations by elemental analyzer-isotope ratio mass spectrometry (Elementar Analysen Systeme GmbH, Germany; IsoPrime100, IsoPrime Limited, UK).

The concentrations of ammonium and nitrate in the extracts were quantified by colorimetric determination using a continuous chemical analyzer (SmartChem200, Rome, Italy). Total dissolved N (TDN) was converted to nitrate by alkaline persulfate digestion (Doyle et al. 2004; Knapp et al. 2005), and then measured by colorimetric determination using a continuous chemical analyzer. Extractable DON was estimated as the difference between the concentrations of TDN and DIN (Takebayashi et al. 2010; Zhang et al. 2018). The  $\delta^{15}\text{N}$  of nitrate was determined by “the denitrifier method,” in which  $\text{NO}_3^-$  is converted to  $\text{N}_2\text{O}$  by the cultured denitrifying bacteria that lack  $\text{N}_2\text{O}$  reductase activity (Liu et al. 2014). The  $\delta^{15}\text{N}$  of TDN was measured as nitrate by alkaline persulfate digestion. The  $\delta^{15}\text{N}$  of ammonium was measured using the micro-diffusion method followed by alkaline hypobromite

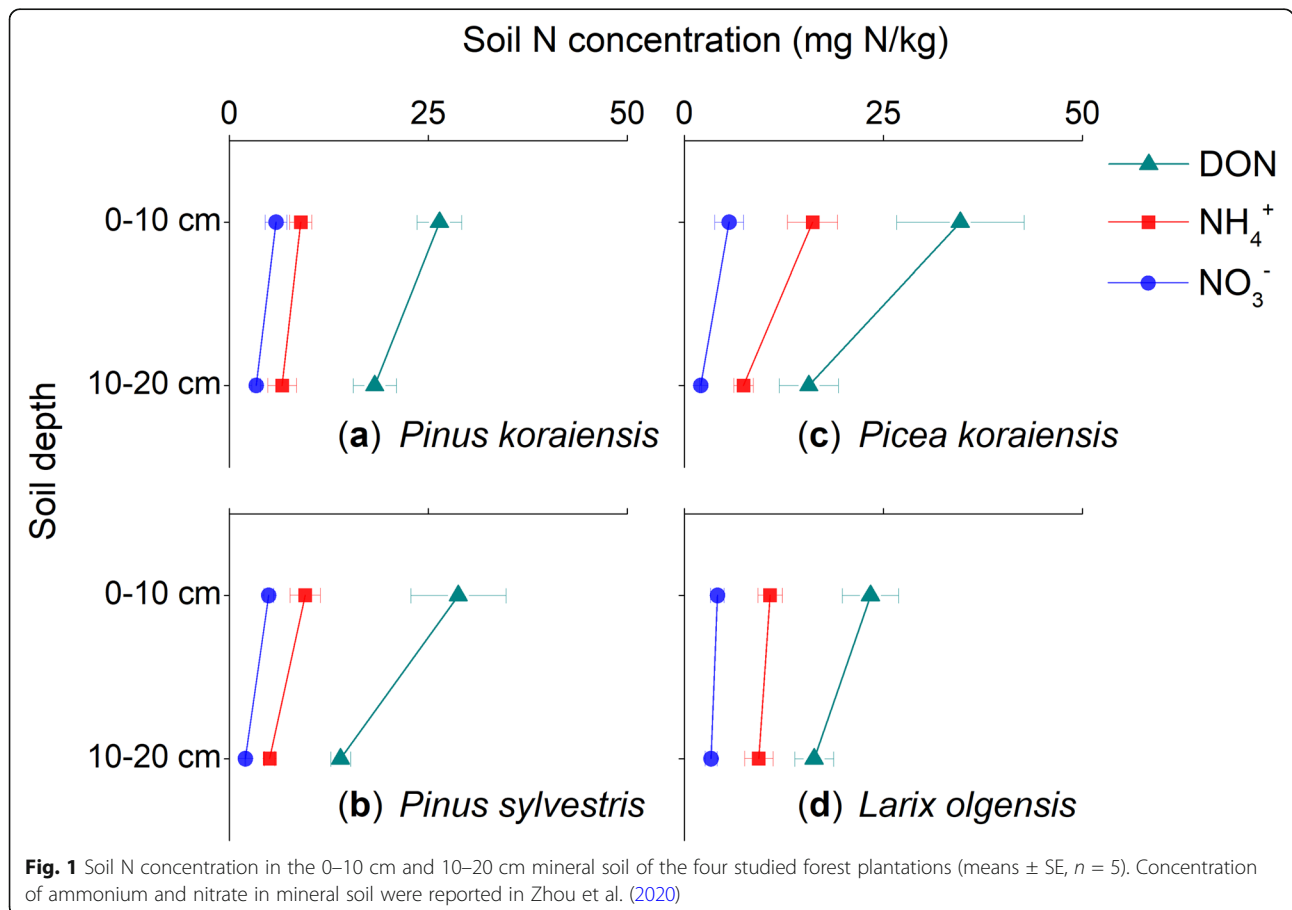
(BrO<sup>-</sup>) oxidation and hydroxylamine (NH<sub>2</sub>OH) reduction (Zhang et al. 2015). Stable N isotopic signatures of the produced N<sub>2</sub>O were analyzed using a continuous flow isotope ratio mass spectrometer (IsoPrime 100, IsoPrime Limited, UK) connected to a cryofocusing unit (Trace Gas Preconcentrator, IsoPrime Limited, UK). Several compounds were used as references: IAEA N3, USGS 32, USGS 34, and USGS 35 for nitrate and IAEA N1, USGS 25, and USGS 26 for ammonium. The analytical precision for δ<sup>15</sup>N was less than 0.3‰. The δ<sup>15</sup>N of DON was calculated using the following mass and isotopic balance equation:

$$\delta^{15}\text{N}_{\text{DON}} = (\delta^{15}\text{N}_{\text{TDN}} \times [\text{TDN}] - \delta^{15}\text{N}_{\text{NO}_3^-} \times [\text{NO}_3^-] - \delta^{15}\text{N}_{\text{NH}_4^+} \times [\text{NH}_4^+]) / [\text{DON}]$$

**Isotopic mixing model**

We used two types of isotopic mixing models, as described by Houlton et al. (2007), to estimate the proportional contribution (*f*) of different N forms to plant N uptake.

Considering the possible isotope (<sup>15</sup>N) fractionation during plant N uptake, previous studies had shown that it may cause approximately 2‰ of <sup>15</sup>N discrimination between root and shoots (Dawson et al. 2002; Houlton et al. 2007). In addition, relative to nonmycorrhizal plants, the ectomycorrhizal fungi may impart additional fractionation of 8‰ or less (Hobbie and Colpaert 2003; Craine et al. 2009; Zhu et al. 2019). Therefore, we assumed a combined isotopic effect of 10‰ in this study. We assumed that plants are characterized by a steady state between plant N uptake and losses through root decay below-ground and leaf fall above-ground (Houlton et al. 2007). If below- and above-ground N losses are equal, the δ<sup>15</sup>N of foliage will be 5‰ (half of the isotopic effect, 10‰) lower than the plant N sources (Houlton et al. 2007; Zhang et al. 2018). Moreover, to assess the uncertainty caused by isotope fractionation, we tested the sensitivity of the model if the isotopic effect varied from 4 to 12‰ (correction of 2–6‰). First, we assumed that plants could only utilize two N forms: ammonium and nitrate. The two end-member calculation takes on the following equations, and the \*δ<sup>15</sup>N<sub>foliage</sub> is the measured foliage corrected for the internal isotope effect:



$$1 = f_{\text{NO}_3^-} + f_{\text{NH}_4^+}$$

$$*\delta^{15}\text{N}_{\text{foliage}} = \delta^{15}\text{N}_{\text{foliage}} + 5\text{‰}$$

$$*\delta^{15}\text{N}_{\text{foliage}} = f_{\text{NO}_3^-} \times \delta^{15}\text{N}_{\text{NO}_3^-} + f_{\text{NH}_4^+} \times \delta^{15}\text{N}_{\text{NH}_4^+}$$

Second, another set of calculations included DON as a potential N source for plants in the following equations:

$$1 = f_{\text{NO}_3^-} + f_{\text{NH}_4^+} + f_{\text{DON}}$$

$$*\delta^{15}\text{N}_{\text{foliage}} = \delta^{15}\text{N}_{\text{foliage}} + 5\text{‰}$$

$$*\delta^{15}\text{N}_{\text{foliage}} = f_{\text{NO}_3^-} \times \delta^{15}\text{N}_{\text{NO}_3^-} + f_{\text{NH}_4^+} \times \delta^{15}\text{N}_{\text{NH}_4^+} + f_{\text{DON}} \times \delta^{15}\text{N}_{\text{DON}}$$

In addition to the above three end-member calculations, we used an alternative approach to estimate the contribution of DON to plant N uptake. First, we assumed that the relative contributions of ammonium and nitrate to total N uptake of these four conifers ( $f_{\text{NO}_3^-}$  and  $f_{\text{NH}_4^+}$ ) from the in situ labelling experiment were correct (Zhou et al. 2020). Second, we incorporated DON as a potential N source for plants and calculated the relative contribution of different N forms by three new end-member calculations according to the following equations:

$$1 = f_{\text{NO}_3^-} + f_{\text{NH}_4^+} + f_{\text{DON}}$$

$$f_{\text{NO}_3^-}/f_{\text{NH}_4^+} = 1.4 \text{ to } 3.3$$

$$*\delta^{15}\text{N}_{\text{foliage}} = f_{\text{NO}_3^-} \times \delta^{15}\text{N}_{\text{NO}_3^-} + f_{\text{NH}_4^+} \times \delta^{15}\text{N}_{\text{NH}_4^+} + f_{\text{DON}} \times \delta^{15}\text{N}_{\text{DON}}$$

In this study, the ‘‘Iso-Source’’ model was used to resolve this mathematically undetermined set of equations (Phillips and Gregg 2003; Houlton et al. 2007; Takebayashi et al. 2010; Zhang et al. 2018). This model iteratively generates source isotopic mixtures whose proportions ( $f$ ) sum to 1. The model compares each calculation against a known mixture of  $\delta^{15}\text{N}$  foliage and retaining only those mixtures that satisfy the known value (within some mass-balance tolerance) as defined by a dataset of feasible solutions. Although this model can only generate feasible solutions (presented here as the average probability, the distribution of feasible solutions can be found in the [supporting information](#)), it nevertheless provides a systematic method to constrain the attribution of N sources in an undetermined system. In our case, the calculated mixtures reflected combinations of the  $\delta^{15}\text{N}$  of DON, ammonium, nitrate, and foliage. We applied a mass-balance tolerance of 0.5‰ to our calculations.

## Statistical analysis

The differences in soil N concentrations and  $\delta^{15}\text{N}$  of different soil N forms among the four coniferous plantations were tested through one-way analysis of variance.  $P < 0.05$  was considered to indicate a significant difference among coniferous plantations. All statistical analyses were conducted using SPSS 19.0 (SPSS Inc., Chicago, IL, USA)

## Results

### Soil N concentrations

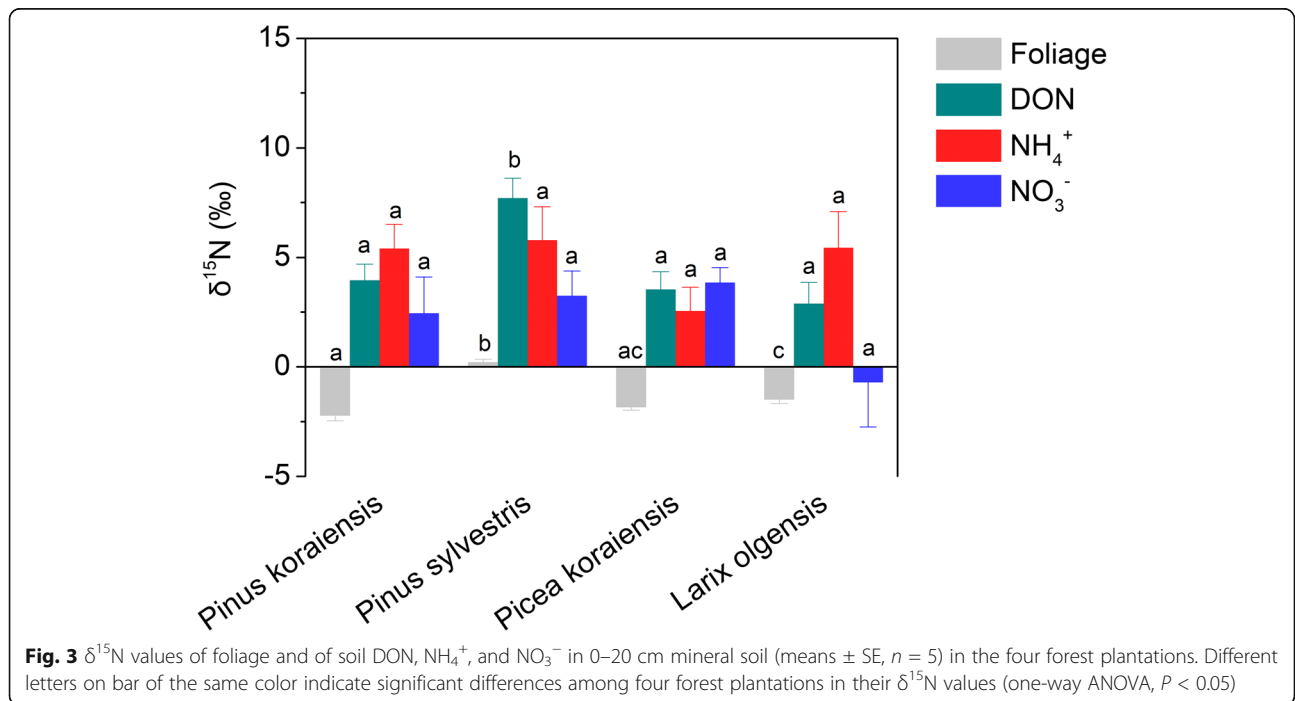
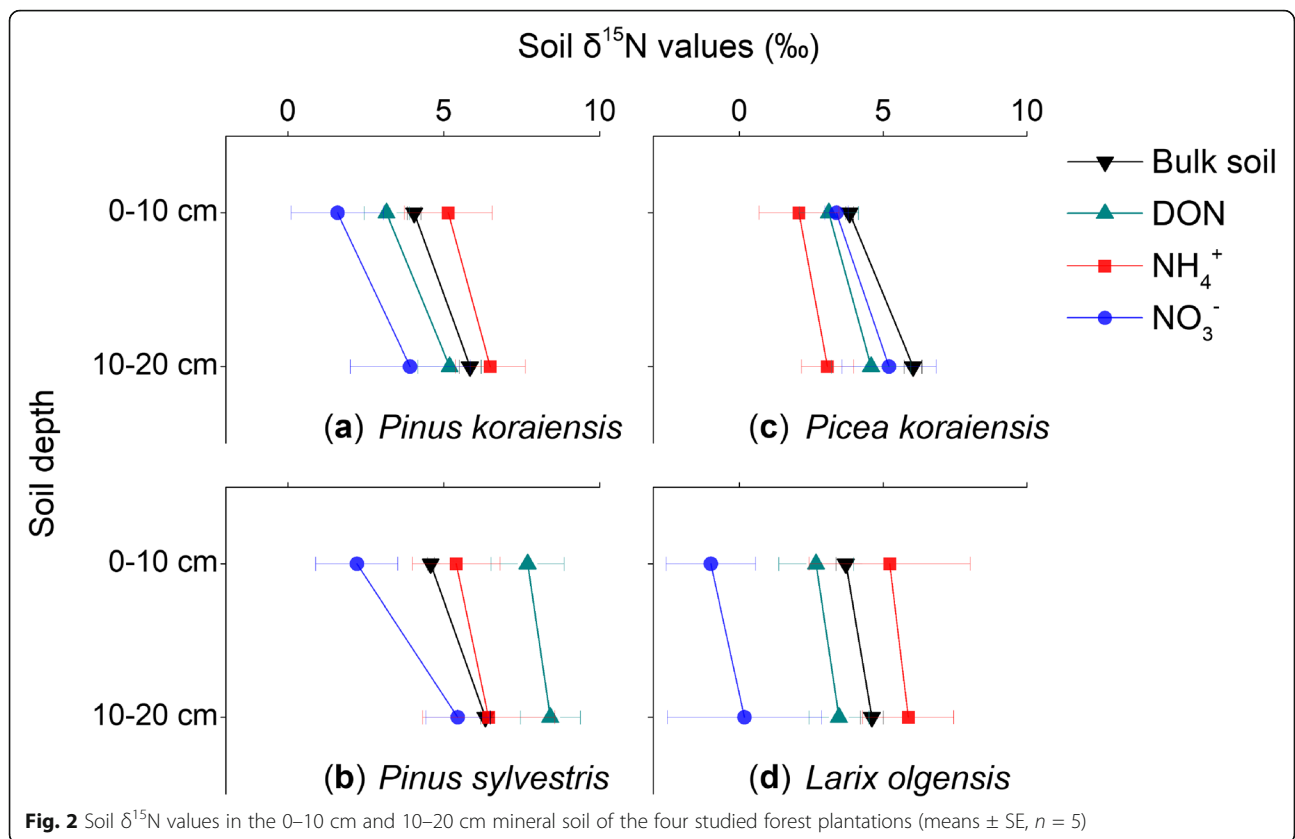
DON was the dominant dissolved N form in both 0–10 cm and 10–20 cm mineral soil of the four coniferous plantations, with concentrations ranged from 23.4 to 34.7 mg N kg<sup>-1</sup> in the 0–10 cm depth, and 14 to 18.3 mg N kg<sup>-1</sup> in the 10–20 cm depth (Fig. 1). Ammonium and nitrate concentrations ranged from 9 to 16.1 mg N kg<sup>-1</sup> and 4.2 to 5.9 mg N kg<sup>-1</sup> in the 0–10 cm depth, and 5.1 to 9.4 mg N kg<sup>-1</sup> and 2 to 3.4 mg N kg<sup>-1</sup> in the 10–20 cm depth (Fig. 1). Soil ammonium concentrations differed significantly among four plantations in the 0–10 cm depth, with the highest ammonium concentrations in *Picea koraiensis* plantation ( $P < 0.05$ ) (Fig. 1).

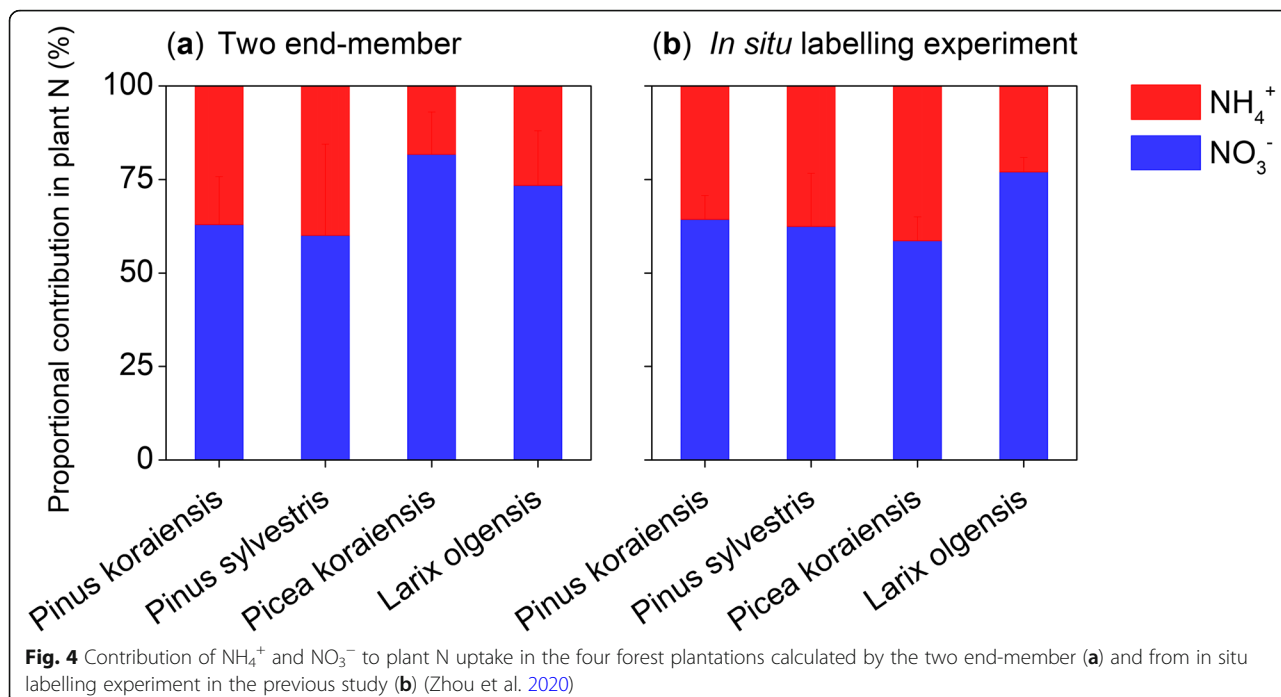
### $\delta^{15}\text{N}$ of different soil N forms and foliage

The  $\delta^{15}\text{N}$  of soil bulk N was 3.7 to 4.6‰ in the 0–10 cm depth, and 4.6 to 6.3‰ in the 10–20 cm depth (Fig. 2). After weighting by the N pool sizes for the two soil layers, the  $\delta^{15}\text{N}$  of soil DON ranged from 2.9 to 7.7‰ (Fig. 3). The  $\delta^{15}\text{N}$  of DON in *Pinus sylvestris* was significantly higher than the other three plantations ( $P < 0.05$ ). There were no significant differences in the  $\delta^{15}\text{N}$  of soil ammonium and nitrate among the four plantations ( $P > 0.05$ ), with ammonium from 2.5 to 5.8‰ and nitrate from -0.7 to 3.9‰ (Fig. 3). The  $\delta^{15}\text{N}$  of foliage ranged from -2.2 to 0.2‰ in the four plantations (Fig. 3), with the highest value for *Pinus sylvestris* and the lowest value for *Pinus koraiensis*.

### Soil N contribution for plant N uptake

Nitrate contributed 60 to 82% of total inorganic N uptake by four plantations based on the two end-member calculations (Fig. 4a). There was no significant difference of the nitrate contribution among the four plantations ( $P > 0.05$ ) (Fig. 4a). When DON was incorporated as an available N source in our calculation, DON contributed 31%, 29%, 28%, and 24% to total N uptake by *Larix olgensis*, *Picea koraiensis*, *Pinus koraiensis*, and *Pinus sylvestris*, respectively, based on the three end-member calculations, whereas nitrate contributed 42 to 52% and ammonium contributed 19 to 29% to total N uptake for these four conifers (Fig. 5a). According to the results of the in situ labelling experiment, DON contributed 18 to 52% of total





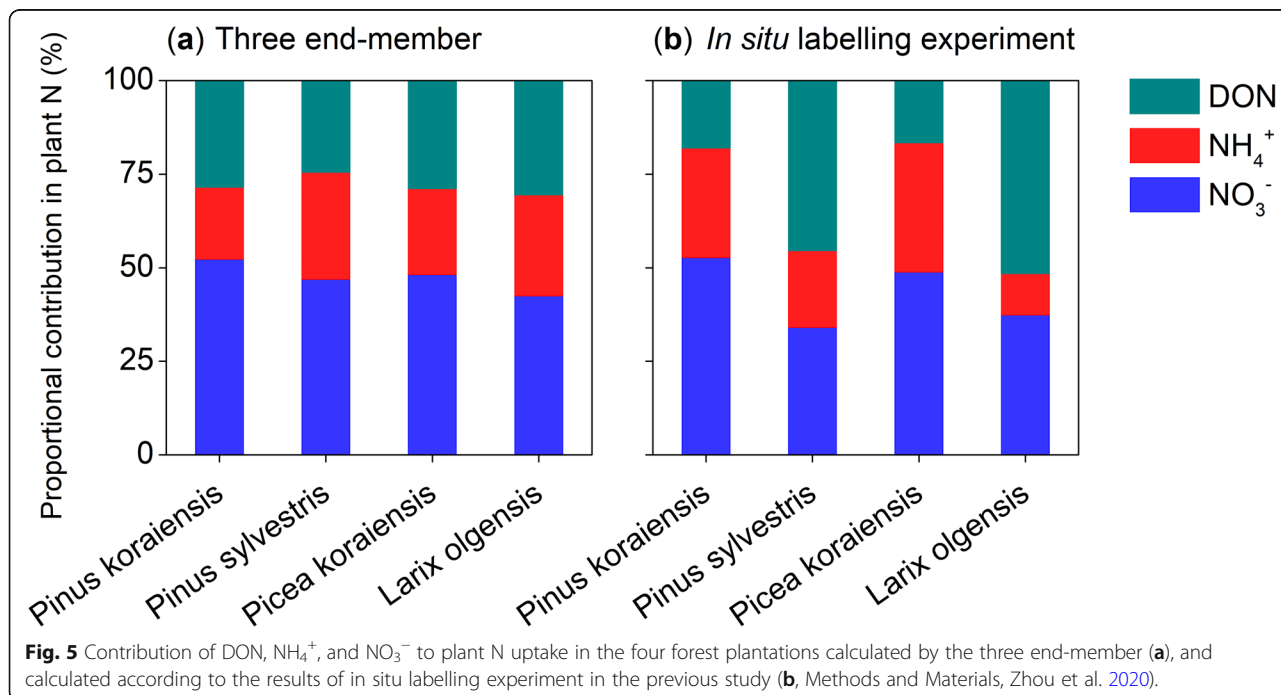
N uptake by the four conifers, nitrate contributed 34 to 53%, and ammonium contributed 11 to 35% (Fig. 5b).

**Discussion**

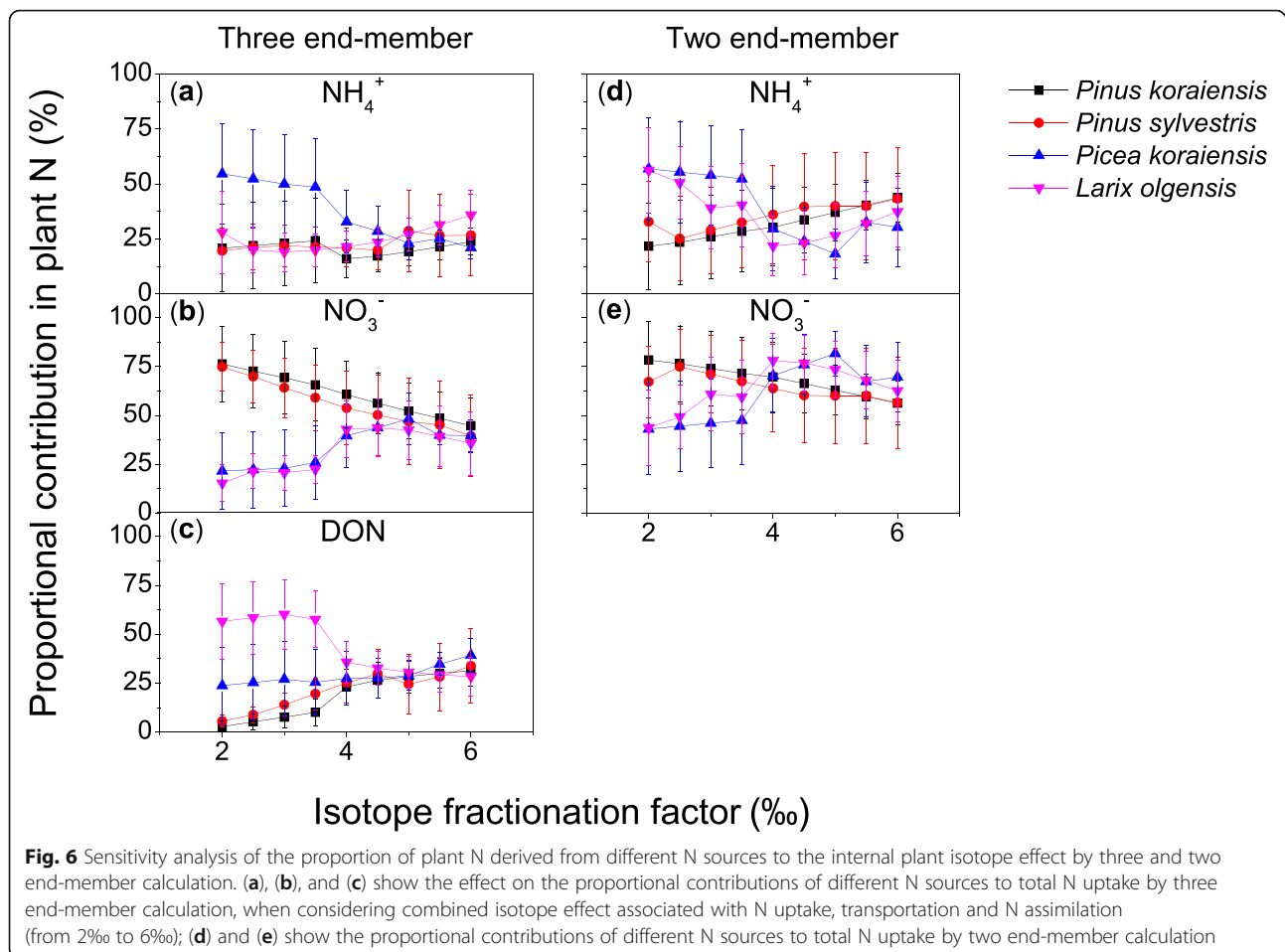
**Contribution of DON in comparison with DIN**

Our results suggested that all four mature conifers could assimilate DON efficiently, with DON contributing 24 to 31% to total N uptake in the three end-member

calculations and 18 to 52% in the in situ labelling experiment (Fig. 5). Consistent with previous studies using <sup>15</sup>N natural abundance (Table 1), organic N plays a key role in plant N acquisition (Takebayashi et al. 2010; Zhang et al. 2018). For example, the study carried out on the Tibetan Plateau suggested that DON contributed 23 to 39% of plant N uptake across 20- to 70-year-old *Picea asperata* plantations (Zhang et al. 2018). Organic N







**Fig. 6** Sensitivity analysis of the proportion of plant N derived from different N sources to the internal plant isotope effect by three and two end-member calculation. (a), (b), and (c) show the effect on the proportional contributions of different N sources to total N uptake by three end-member calculation, when considering combined isotope effect associated with N uptake, transportation and N assimilation (from 2‰ to 6‰); (d) and (e) show the proportional contributions of different N sources to total N uptake by two end-member calculation

uptake may allow conifers to circumvent microbial transformations to inorganic N. This effective competition with soil microbes for organic N will improve nutrient utilization efficiency of conifers (Harrison et al. 2007; Andersen et al. 2017), especially in relatively N-limited ecosystems.

Previous studies suggested that plant N uptake strategy may be related to the concentration of soil available N and that plants prefer to take up the most available N form in their rhizosphere (Russo et al. 2013; Mayor et al. 2014). Such uptake pattern could be an adaptive strategy for plants in nutrient competition (Andersen et al. 2017). In our study, DON was the dominant N form and accounted for almost 60% of dissolved soil N pools (Fig. 1). This might explain that mature conifers could take up a relatively large proportion of DON in this study, thereby reducing competition for the limited available N sources with microbes (Song et al. 2015). DON uptake by conifers can be energized by the proton gradient over the plasma membrane and facilitated by transport proteins (Näsholm et al. 2009; Svennerstam et al. 2011). These transporters can obtain amino acids from the soil solution, as well as from the roots (Jones et al. 2005). Previous studies have

demonstrated that transporters in conifer root epidermis have broad affinity for many kinds of amino acids, which could contribute to the uptake of DON by conifers (Uscola et al. 2017). Furthermore, N uptake strategy of ectomycorrhizal plants is largely mediated by mycorrhizal fungi (Hobbie et al. 2000; Hobbie and Högborg 2012; Wang et al. 2020). The four conifers in our study are associated with ectomycorrhizal fungi, which can mobilize and take up organic N (Chalot et al. 2002; Näsholm et al. 2009). Overall, based on the  $^{15}\text{N}$  natural abundance method, the contribution of soil DON to nutrient uptake of mature conifers in forests of Northeast China deserves further attention.

#### Nitrate contribution in N uptake in comparison with the $^{15}\text{N}$ labelling method

Nitrate was the important N source for the four conifers, with nitrate contributing 60 to 82% of total inorganic N uptake in the two end-member model (Fig. 4a) and 42 to 52% of total N uptake in the three end-member model (Fig. 5a). These results were consistent with the in situ labelling experiment that nitrate was preferentially taken up by conifers, accounting for 59 to 77% of

**Table 1** The proportional contribution of different N forms to plant N uptake in forest trees by using the  $^{15}\text{N}$  natural abundance method

Study sites	Ecosystem	MAT (°C)	Vegetations	Treatment	Proportional contribution (%)			References	
					$\text{NH}_4^+$	$\text{NO}_3^-$	DON		
Hawaii	Tropical forest	16	<i>Metrosideros polymorpha</i>	2200 mm (MAP)	15	80	5	Houlton et al. (2007)	
				<i>Cheirodendron trigynum</i>	2450 mm	17	78	5	Houlton et al. (2007)
				<i>Cibotium glaucum</i>	2750 mm	15	80	5	Houlton et al. (2007)
				<i>Melicope clusiifolia</i>	3350 mm	5	93	2	Houlton et al. (2007)
					4050 mm	93	5	2	Houlton et al. (2007)
Japan-Norikura	Forest	8.7	<i>Chamaecyparis obtusa</i>	Norikura-A	100	0		Takebayashi et al. (2010)	
				Norikura-B	95	5		Takebayashi et al. (2010)	
				Norikura-C	94	6		Takebayashi et al. (2010)	
				Norikura-A	48	38	14	Takebayashi et al. (2010)	
				Norikura-B	47	39	14	Takebayashi et al. (2010)	
Japan-Okutama	Forest	14.3	<i>Chamaecyparis obtusa</i>	Norikura-C	28	47	25	Takebayashi et al. (2010)	
				Okutama-A	100	0		Takebayashi et al. (2010)	
				Okutama-B	47	53		Takebayashi et al. (2010)	
				Okutama-A	46	40	14	Takebayashi et al. (2010)	
				Okutama-B	24	61	15	Takebayashi et al. (2010)	
Japan-Hachioji	Forest	14.8	<i>Chamaecyparis obtusa</i>	Hachioji-A	39	61		Takebayashi et al. (2010)	
				Hachioji-B	0	100		Takebayashi et al. (2010)	
				Hachioji-A	20	67	13	Takebayashi et al. (2010)	
				Hachioji-B	3	95	2	Takebayashi et al. (2010)	
Panama	Lowland tropical rainforest	27	<i>Alseis blackiana</i>		56	44		Mayor et al. (2014)	
				<i>Heisteria concinna</i>		43	57		Mayor et al. (2014)
				<i>Tetragastris panamensis</i>		70	30		Mayor et al. (2014)
				<i>Oenocarpus mapora</i>		30	70		Mayor et al. (2014)
Southwest China	Plantations	8.9	<i>Picea asperata</i>	20-year-old	27	73		Zhang et al. (2018)	
				30-year-old	42	58		Zhang et al. (2018)	
				40-year-old	100	0		Zhang et al. (2018)	
				50-year-old	93	7		Zhang et al. (2018)	
				70-year-old	100	0		Zhang et al. (2018)	
				20-year-old	19	43	38	Zhang et al. (2018)	
				30-year-old	31	46	23	Zhang et al. (2018)	
				40-year-old	34	30	36	Zhang et al. (2018)	
				50-year-old	33	33	34	Zhang et al. (2018)	
70-year-old	43	18	39	Zhang et al. (2018)					
Northeast China	Greenhouse study	22.4	<i>Pinus koraiensis</i>		33	24	43	Zhu et al. (2019)	
				<i>Larix kaempferi</i>		6	74	20	Zhu et al. (2019)
				<i>Quercus mongolica</i>		1	88	11	Zhu et al. (2019)
				<i>Juglans mandshurica</i>		11	68	21	Zhu et al. (2019)
Northeast China	Temperate forest	2.7	<i>Pinus koraiensis</i>		37	63		This study	
				<i>Picea koraiensis</i>		18	82		This study
				<i>Pinus sylvestris</i>		40	60		This study
				<i>Larix olgensis</i>		27	73		This study



**Table 1** The proportional contribution of different N forms to plant N uptake in forest trees by using the  $^{15}\text{N}$  natural abundance method (*Continued*)

Study sites	Ecosystem	MAT (°C)	Vegetations	Treatment	Proportional contribution (%)			References
					$\text{NH}_4^+$	$\text{NO}_3^-$	DON	
			<i>Pinus koraiensis</i>		19	53	28	This study
			<i>Picea koraiensis</i>		23	48	29	This study
			<i>Pinus sylvestris</i>		28	47	25	This study
			<i>Larix olgensis</i>		27	43	30	This study

total N uptake (Fig. 4b) (Zhou et al. 2020). Such uptake patterns differed from the results of hydroponic experiments in which conifers took up more ammonium than nitrate (Kronzucker et al. 1997; Socci and Templer 2011; Gruffman et al. 2014; Uscola et al. 2017), with ammonium contributing 55 to 98% of total N uptake (Zhou et al. 2020). However, the hydroponic method could not represent the uptake patterns of mature conifers in the field, since it ignores microbial competition for ammonium and adsorption of ammonium by soil minerals (Lavoie et al. 1992; Lucash et al. 2005; Fraterrigo et al. 2011). In addition, nitrate efflux is induced when plant roots are immersed directly in nitrate-labelled solutions (Socci and Templer 2011), resulting in underestimates of nitrate uptake by hydroponic methods (Wei et al. 2015).

The potential mechanisms for the efficient uptake and assimilation of nitrate are as follows: (1) Nitrate is usually more available for uptake in many ecosystems due to its high mobility (Johnson et al. 2000; Liu et al. 2017), while ammonium is preferentially assimilated by soil microbes and easily adsorbed by soil organic matter (Wang and Macko 2011; Epron et al. 2016). One study in a temperate grassland demonstrated that nitrate diffused through soil water more than one hundred times faster than ammonium (Owen and Jones 2001). Therefore, efficient assimilation of nitrate by conifers could avoid competition for ammonium with soil microbes and increase nutrient assimilation, despite the higher energy cost for nitrate assimilation than for ammonium assimilation in conifers (Kuzakov et al. 2013), especially under low concentrations of soil available N (Zhu et al. 2019). (2) Nitrate could promote uptake of other nutrients, such as  $\text{K}^+$ ,  $\text{Ca}^{2+}$ , and  $\text{Mg}^{2+}$ , and thereby contribute to plant growth (Hoffmann et al. 2007). In contrast, ammonium assimilation and the accompanying equimolar  $\text{H}^+$  production could acidify the rhizosphere and consequently repress cation uptake (Britto and Kronzucker 2002). (3) Different active nitrate uptake systems may promote nitrate uptake by plant roots (Liu et al. 2014). Previous studies suggested that conifers may have low-affinity transport systems (LATS) and high-affinity transport systems (HATS) (Behl et al. 1988), which can contribute to efficient nitrate uptake by conifers at low and high soil nitrate concentrations.

#### Uncertainties of the $^{15}\text{N}$ natural abundance method

In this study, we corrected the foliage  $\delta^{15}\text{N}$  by 5‰ in our calculation due to isotope fractionation during internal plant N allocation and ectomycorrhizal fungi transport (see “Methods and Materials”) (Hobbie and Colpaert 2003; Houlton et al. 2007; Zhang et al. 2018). However, we must admit that the isotopic fractionation effect may cause some uncertainties due to the different N status of different study coniferous plantations (Takebayashi et al. 2010; Zhang et al. 2018). To assess the uncertainties of conifer N uptake strategy, we tested the sensitivity of the two and three end-member calculations to a wide range of the isotope effect during plant N uptake from 4 to 12‰ (correction of 2–6‰ for the mixing model). We found that N uptake strategies of conifers were relatively sensitive to the isotope effect from 4 to 8‰ (i.e., correction of 2–4‰, Fig. 6). However, we calculated the  $^{15}\text{N}$  depletion of tree  $\delta^{15}\text{N}$  relative to soil inorganic N based on the previous in situ labelling experiment, with  $^{15}\text{N}$  depletion ranged from 4 to 6‰ (Zhou et al. 2020). Only marginal effects on the relative contribution of different N forms resulted when the isotope effect varied from 8 to 12‰ (correction of 4–6‰). Therefore, the relative contribution of different N forms calculated by the corrected isotope effect of 5‰ is robust in this study.

We note that DON is a complex mixture of different N-containing substances. The  $\delta^{15}\text{N}$  of soil DON varies across the components of this mixture, and the DON uptake by different conifers of these components may vary as well (Yu et al. 2002; Andersson and Berggren 2005). This could lead to a difference in  $\delta^{15}\text{N}$  between the DON in the soil and the DON taken up by conifers. Therefore, we selected five conifers in each plantation to minimize these impacts. Furthermore, compared to the  $^{15}\text{N}$  labelling method, the  $^{15}\text{N}$  natural abundance method would be affected by environmental factors, such as the seasonal changes of  $\delta^{15}\text{N}$  of different soil N forms (Liu et al. 2014). Thus, multiple sampling times should be tested in the future to examine whether seasonal differences exist in the N uptake strategies of conifers.

## Conclusions

Overall, efficient uptake of DON and nitrate by mature conifers in this study was consistent with the previous in situ experiment of these four conifers (Zhou et al. 2020). These results demonstrated that tree productivity and timber production in coniferous plantations should remain high, despite increasing proportions of nitrate in N deposition (Liu et al. 2013; Ackerman et al. 2018). However, the in situ labelling method was expensive to apply at field scales. Furthermore, it is difficult to study the uptake patterns of DON by mature conifers by the in situ labelling method. In contrast, the  $^{15}\text{N}$  natural abundance method was relatively convenient and practical (Zhang et al. 2018). Therefore, we suggest to use the  $^{15}\text{N}$  natural abundance method in the future to explore whether efficient utilization of nitrate is universal and generalizable across conifers.

## Abbreviations

DON: Dissolved organic N;  $\delta^{15}\text{N}$ : Natural abundance of  $^{15}\text{N}$ ; TDN: Total dissolved N; LATS: Low-affinity transport systems; HATS: High-affinity transport systems

## Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s13717-021-00306-4>.

**Additional file 1.** Supporting information

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## Authors' contributions

XZ, AW, YL, and YF conceived the study. XZ and AW performed field work. XZ, AW, YL, and YF performed analyses. All authors contributed to the writing and read and approved the final manuscript.

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## Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Declarations

### Ethics approval and consent to participate

Not applicable

### Consent for publication

Not applicable

### Competing interests

The authors declare that they have no competing interests

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