Characteristics of NH$_4^+$ and NO$_3^-$ Fluxes in *Taxodium* Roots under Different Nitrogen Treatments

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Abstract: To understand the characteristics of net NH$_4^+$ and NO$_3^-$ fluxes and their relation with net H$^+$ fluxes in *Taxodium*, net fluxes of NH$_4^+$, NO$_3^-$ and H$^+$ were detected by a scanning ion-selective electrode technique under different forms of fixed nitrogen (N) and experimental conditions. The results showed that higher net NH$_4^+$ and NO$_3^-$ fluxes occurred at 2.1–3.0 mm from the root apex in *T. ascendens* and *T. distichum*. Compared to NH$_4^+$ or NO$_3^-$ alone, more stable net NH$_4^+$ and NO$_3^-$ fluxes were found under NH$_4$NO$_3$ supply conditions, of which net NH$_4^+$ flux was promoted at least 1.71 times by NO$_3^-$, whereas net NO$_3^-$ flux was reduced more than 81.66% by NH$_4^+$ in all plants, which indicated that NH$_4^+$ is preferred by *Taxodium* plants. *T. ascendens* and *T. mucronatum* had the largest net NH$_4^+$ and total N influxes when NH$_4^+$:NO$_3^-$ was 3:1. $^{15}$N Atom% and activities of N assimilation enzymes were improved by single N fertilization in the roots of *T. distichum*. In most cases, net H$^+$ fluxes were tightly correlated with net NH$_4^+$ and NO$_3^-$ fluxes. Thus, both N forms and proportions could affect N uptake of *Taxodium*. These findings could provide useful guidance for N management for better productivity of *Taxodium* plants.

Keywords: N management; net fluxes; productivity; proton; non-invasive micro-test technique

1. Introduction

Nitrogen (N) plays a significant role in plant growth and development since it is a crucial component of plants’ chlorophylls, nucleic acids, proteins, and secondary metabolites [1]. Ammonium (NH$_4^+$) and nitrate (NO$_3^-$) are two primary forms of inorganic N absorbed and used by plants, and their fluxes in roots are varied with the distance from the apex. Spatial variability in the fluxes of NH$_4^+$ and NO$_3^-$ has been explored along the roots in some herbaceous and woody plants [2–5]. For instance, the maximal net NH$_4^+$ influx happened at the root apex in *rice* (*Oryza sativa* L.) [2] and *Populus simonii* [5], whereas the highest net NH$_4^+$ influx appeared at 5 mm, 10 mm, and 5–20 mm from the root apex in *lodgepole pine* (*Pinus contorta*) [6], *Populus populalis* [7] and *Douglas-fir* (*Pseudotsuga menziesii*) [6], respectively. In the case of NO$_3^-$, previous studies observed that the highest net NO$_3^-$ flux occurred at 0–10 mm in *P. contorta* [6], and at 15 mm from the apex in *P. simonii* [5] and *P. populalis* [7]. In rice, net NO$_3^-$ influx increased to a maximum at 21 mm from the apex and then gradually declined [2]. Obviously, different plant species have distinct patterns of NH$_4^+$ and NO$_3^-$ flux rates along the fine roots.

Apart from the spatial variation along the roots, NH$_4^+$ and NO$_3^-$ fluxes are also affected by environmental factors such as N levels. A previous study in tea (*Camellia sinensis*) roots demonstrated increased net influxes of NH$_4^+$ and NO$_3^-$ when the solution concentration increased from 0.2 mM to 1.2 mM under KNO$_3$ and NH$_4$Cl [1]. However, research on *Picea glauca* revealed a converse result in most cases; the roots presented net NH$_4^+$ and NO$_3^-$ influxes in 50 µM with net effluxes in 1500 µM solutions [8]. When roots were treated in 10, 100, and 1000 µM NH$_4$NO$_3$ solutions, net NH$_4^+$ influxes increased...
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2. Materials and Methods

2.1. Plant Cultivation

Semi-lignified cuttings (10 cm in length, 0.3 cm in diameter) of T. ascendens, T. distichum, and T. mucronatum were selected. After being soaked in 3% 3-indoleacetic acid (IAA) solution for 2 min, they were repotted into a pot containing 1:1 volume of peat:perlite in a ventilated greenhouse at the Institute of Botany, Jiangsu Province and Chinese Academy of Sciences (35° 03′ N, 118° 49′ E), under normal growth conditions (approximately 25 °C) in a photoperiod of 14/10 h of light/dark. Two months later, cuttings with uniform size and development were selected for the NMT experiments.

Seeds were collected from a healthy T. distichum grown in the Institute of Botany, Jiangsu Province and Chinese Academy of Sciences. They were planted in black plastic pots (5 × 5 × 15 cm) filled with 1:1 volume of peat:perlite in a climate chamber (23–25 °C/15–18 °C, day/night; light per day, 14 h; photosynthetic photon flux, 160 μmol m−2 s−1; relative air humidity, 50–60%). After 19 weeks of growth, the plants were transferred into black plastic boxes (25 × 15 × 14.5 cm, 4 plants per box) containing 4 L of

gradually in P. popularis but decreased by degrees in Populus alba × Populus glandulosa [4]. In contrast, the hybrid presented higher net NO3− influxes than P. popularis in most cases [4]. This phenomenon revealed that the N concentrations in soils have prominent effects on the uptake of NH4+ and NO3−, and they are significantly related to the plant species. Additionally, previous studies revealed that interactions between NH4+ and NO3− exist on fluxes of both ions [7,9]. The interactions between NH4+ and NO3− are complicated among plants [10], and the underlying mechanisms remain unclear [7]. It is documented that the presence of NH4+ and NO3− negatively affect the uptake of each other, but NH4+ is preferred in C. sinensis [1]. However, net NH4+ influx was induced by the simultaneous provision of NO3−, and net NO3− influx was inhibited in the presence of NH4+ in roots of P. popularis and Populus asperata [7,11]. Moreover, a previous study on Douglas-fir and lodgepole pine showed that net NH4+ uptake remained unchanged in the presence or absence of NO3− [6]. Overall, interactions between NH4+ and NO3− and their preferences may result in changes of NH4+ and NO3− fluxes under different proportions of NH4+ and NO3− supply. Nonetheless, little information is available on the fluxes of NH4+ and NO3− in plant roots under fluctuating proportions of both inorganic N forms.

On the other hand, fluxes of NH4+ and NO3− are correlated with the plasma membrane PM-H+-ATPase activity that extrudes H+ from the cytosol to the outside at the expense of adenosine triphosphate (ATP) [12]. Previous research found that NO3− is transported across the plasma membrane via NO3−/H+ symporters with the involvement of PM-H+-ATPase [13]. The concentration of NH4+ can increase the activity of PM-H+-ATPase [14]. Furthermore, the expression of genes encoding PM-H+-ATPase was positively associated with fluxes of NH4+ and NO3− [8]. The significant correlations between NH4+, NO3− fluxes, and H+ uptake rate have been observed in many plants [12,15–17].

Taxodium species including T. ascendens, T. distichum, and T. mucronatum have been introduced from southeastern America to many countries owing to their economic and ecological benefits [18]. For instance, they can be used as woody bioenergy crops [19]. Taxodium oil showed adequate bioassay for insecticidal activity [20]. Compounds isolated from the bark can exhibit cytotoxic substances, thus treating against cancer cells [21]. Moreover, Taxodium plants have been selected as suitable species for afforestation in many challenging areas [22,23]. Although N is crucial for Taxodium growth and development, less information is available on the fluxes of NH4+ and NO3− as well as their correlation with H+ flux in fine roots. In this study, a non-invasive micro-electrodes technique was employed to investigate NH4+, NO3− and H+ fluxes in fine roots of T. ascendens, T. distichum, and T. mucronatum under different N forms and their proportions. Our objectives were (i) to determine the distance from the root apex of Taxodium plants where there are greater net NH4+ and NO3− fluxes; (ii) to illustrate the characteristics of NH4+, NO3− and H+ fluxes and their interactions under different N forms and proportions.
modified 1/4 Hoagland’s nutrient solution [24]. All nutrient solutions were continuously aerated with an air pump, and each solution was refreshed every other day. After 16 d, plants were used to explore $^{15}$N Atom% and enzymatic activities.

2.2. Experimental Design

To determine the positions along the root where the maximal influxes of $\text{NH}_4^+$ and $\text{NO}_3^-$ occur, a preliminary experiment was carried out at 14 positions, in turns, 0, 0.3, 0.6, 0.9, 1.2, 1.5, 1.8, 2.1, 2.5, 3.0, 5.0, 8.0, 15.0 and 30.0 mm away from the root apex. The measuring solution was 0.5 mM MES (2-(N-Morpholino) ethanesulfonic acid hydrate buffer), pH 6.0, to which either 1.0 mM $\text{NH}_4\text{Cl}$ for $\text{NH}_4^+$ or 1.0 mM $\text{KNO}_3$ for $\text{NO}_3^-$ was added. After that, the position where the greater net uptake of $\text{NH}_4^+$ and $\text{NO}_3^-$ occurred was detected to carry out the following experiments.

To investigate the net fluxes of $\text{NH}_4^+$ and $\text{NO}_3^-$ under different N forms and proportions, the measuring solutions were designed as (1) $\text{NH}_4^+$ ($\text{NH}_4\text{Cl}$): 0.1 and 1.0 mM, (2) $\text{NO}_3^-$ (KNO$_3$): 0.1 and 1.0 mM and (3) $\text{NH}_4^+:\text{NO}_3^-$: 1:3, 1:1, and 3:1 (total N = 2 mM), containing 0.5 mM MES, pH 6.0.

To explore the biomass, $^{15}$N Atom% and enzymatic activities under single N fertilization, 24 seedlings with similar performance (ca. 15 cm in height) were selected and divided into three groups (8 plants in each group). Three N treatments: 0 mM $\text{NH}_4\text{Cl}$ and K$^{15}\text{NO}_3$ (serving as control, CK), 1 mM $\text{NH}_4\text{Cl}$ and 1 mM K$^{15}\text{NO}_3$ in 1/4 modified Hoagland’s nutrient solution [24] were applied. Dicyandiamide (7 μM, C$_2$H$_5$N$_4$) was added into the nutrient solution to inhibit nitrification [19]. After 3 d, 4 plants from each treatment were harvested and used for measurements of $^{15}$N Atom%, and the remaining 4 plants in each treatment were used for enzymatic activities.

2.3. Measurement of $\text{NH}_4^+$, $\text{NO}_3^-$ and $\text{H}^+$ Fluxes

To understand the real-time $\text{NH}_4^+$, $\text{NO}_3^-$ and $\text{H}^+$ uptake by the fine roots under different treatments, ions flux alterations on the root surface were measured by using a non-invasive micro-test technology (NMT) system (youngerusa.com; xuyue.net) (Figure 1a).

![Figure 1](image-url)

**Figure 1.** Root tip in NMT experiment (a) and the vertical section in the root of *T. distichum* (b).

The measurement procedures were described by Zhao et al. [5]. Firstly, ion-selective microelectrodes designed with 2–4 μm apertures were manufactured and silanized. Secondly, for the $\text{NH}_4^+$ electrode, in sequence, 100 mM $\text{NH}_4\text{Cl}$ was used as a backfilling solution, followed by an $\text{NH}_4^+$ selective liquid ion exchange cocktail (#09879, Sigma, St. Louis, MI, USA)
USA). Similarly, for the NO$_3^-$ electrode, 10 mM KNO$_3$ was used as the backfilling solution, followed by a NO$_3^-$ selective liquid ion exchange cocktail (#72549, Sigma). For the H$^+$ electrode, 15 mM NaCl and 40 mM KH$_2$PO$_4$ were used as backfilling solution, followed by an H$^+$ selective liquid ion exchange cocktail (#95293, Sigma). Prior to the flux measurements, the microelectrodes were calibrated. For NH$_4^+$ calibration, 0.05/0.5 mM NH$_4$Cl in addition to other compounds (0.5 mM MES, pH 6.4/5.4) were used in the measuring solution; for NO$_3^-$ calibration, 0.05/0.5 mM KNO$_3$ in addition to the compounds (0.5 mM MES, pH 6.4/5.4) were used in the measuring solution; for H$^+$ calibration, pH 6.4/5.4 in addition to 0.5 mM MES were used in the measuring solution. Only electrodes with Nernstian slopes higher than 55 mV per tenfold concentration difference were used.

After that, fine white roots, 15–35 mm from the apex, were selected. They were fixed at the bottom of the petri dish filled with 10–20 mL measuring solutions for 20 min. After being equilibrated, the samples were transferred to another petri dish containing 5 mL fresh solution and then were put under the microscope. The tips of the microelectrodes were aligned and kept 30 µm away from the target point, which is a specific distance from the apex of the root. Net fluxes of NH$_4^+$/NO$_3^-$ were recorded at each measurement point for 5 min. Not only eight biological repetitions (eight fine roots from four plants) but also 50 measurement time points in each repetition were considered.

2.4. Determination of $^{15}$N Uptake and Enzyme Activities

The roots were harvested and rinsed three times in distilled water. The $^{15}$N Atom% ($^{15}$N AT%) and the amount of plant N derived from $^{15}$N-labeled fertilizer ($Ndff\%$) were detected [25].

Activities of nitrate reductase (NR, EC 1.7.99.4), nitrite reductase (NiR, EC 1.7.2.1), glutamine synthetase (GS, EC 6.3.1.2), glutamate synthetase (GOGAT, EC 1.4.7.1) and glutamate dehydrogenase (GDH, EC 1.4.1.2) in the roots were assayed [4].

2.5. Statistical Analysis

In order to determine the NH$_4^+$, NO$_3^-$ and H$^+$ fluxes along the root tip, imFluxes V2.0 (xuyue.net) was used to obtain the data at each measuring point. The positive values represent net influxes, and the net negative values represent net effluxes. To analyze data for the ion fluxes, biomass, $^{15}$N AT% and enzyme activities, one-way ANOVA (Duncan’s multiple range tests at 5% level) was performed with the SPSS 25.0 (Statistical Product and Service Solutions, IBM, New York, NY, USA). GraphPad Prism version 9.1 was used to draw figures.

3. Results

3.1. Net Fluxes of NH$_4^+$ and NO$_3^-$ along the Root Tip

Net fluxes of NH$_4^+$ and NO$_3^-$ were determined along the root tip up to 30.0 mm from the apex, and their fluxes were widely varied at different locations (Figure 2a,b). The NH$_4^+$ fluxes ranged from 17.02 (net influx) to 88.89 (net influx) pmol cm$^{-2}$ s$^{-1}$ in T. ascendens, and varied dramatically from −30.52 to 167.15 pmol cm$^{-2}$ s$^{-1}$ in T. distichum, and fluctuated between −90.94 and 46.31 pmol cm$^{-2}$ s$^{-1}$ in T. mucronatum when supplied as 1 mM NH$_4$Cl (Figure 2a). Intriguingly, both T. ascendens and T. distichum showed strong NH$_4^+$ uptake rates from 2.1 to 3.0 mm along the root tip (Figure 2a).

Net NO$_3^-$ fluxes ranged from −49.68 to 102.15 pmol cm$^{-2}$ s$^{-1}$ in T. ascendens, from −80.61 to 185.98 pmol cm$^{-2}$ s$^{-1}$ in T. distichum, and from −7.88 to 112.27 pmol cm$^{-2}$ s$^{-1}$ in T. mucronatum when fed with 1 mM KNO$_3$ (Figure 2b). The maximal net NO$_3^-$ influxes of T. ascendens, T. distichum, and T. mucronatum were detected at 15.0, 3.0, and 5.0 mm from the root apex, respectively (Figure 2b). As a result, the following experiments selected 2.5 mm from the root apex as the specific position to investigate the net fluxes of NH$_4^+$ and NO$_3^-$. Moreover, 2.5 mm from the apex belongs to the elongation zone of the root tip in Taxodium plants (Figure 1b).
3.2. Net Fluxes of NH$_4^+$ and NO$_3^-$ under Different N Forms

As NH$_4$Cl and KNO$_3$ were added separately, the NH$_4^+$ and NO$_3^-$ fluxes fluctuated widely for all tested plants at 2.5 mm from the root apex during a 5-min period (Figure 3). Both NH$_4^+$ and NO$_3^-$ fluxes of T. distichum and T. mucronatum showed a tendency towards net influx. T. ascendens, however, tended to show net efflux of NH$_4^+$ and net influx of NO$_3^-$ (Figure 3a,d,g). When supplied with mixed N (NH$_4$NO$_3$), stable fluxes of NH$_4^+$ and NO$_3^-$ were observed, and distinctly, NH$_4^+$ fluxes were much greater than NO$_3^-$ fluxes in all Taxodium plants (Figure 3b,e,h). Compared to 1 mM NH$_4$Cl, average net fluxes of NH$_4^+$ were stimulated by 688%, 171%, and 762% under 1 mM NH$_4$NO$_3$ in roots of T. ascendens, T. distichum, and T. mucronatum, respectively (Figure 3c,f,i). Thus, the increase of NH$_4^+$ fluxes was as follows: T. mucronatum > T. ascendens > T. distichum (Figure 3c,f,i). The same order was observed for the decreases in net NO$_3^-$ fluxes, which were decreased by 314%, 220%, and 81.66% under 1 mM NH$_4$NO$_3$ compared with that under 1 mM KNO$_3$, respectively (Figure 3c,f,i).
3.3. Net NH$_4^+$, NO$_3^−$ and H$^+$ Fluxes under Different N Concentrations

Except for $T. ascendens$ exposed to 1.0 mM NH$_4$Cl, all three species showed a tendency for net NH$_4^+$ influx when fed with 0.1 or 1.0 mM NH$_4$Cl (Figure 4a). Additionally, the net influx of NH$_4^+$ in $T. ascendens$ was significantly greater ($p < 0.05$) than those in the other two species under 0.1 mM NH$_4$Cl (Figure 4a). Compared to 0.1 mM NH$_4$Cl, net influx of NH$_4^+$ was promoted by 2.40 and 2.84 times under 1.0 mM NH$_4$Cl treatment in $T. distichum$ and $T. mucronatum$, respectively (Figure 4a). Apart from $T. ascendens$ and $T. distichum$ treated with 0.1 mM KNO$_3$, all the plants displayed a tendency for net NO$_3^−$ influx when supplied with 0.1 or 1.0 mM KNO$_3$ (Figure 4b). Moreover, the fluxes of NO$_3^−$ were significantly lower ($p < 0.05$) in 1.0 mM than in 0.1 mM KNO$_3$ in $T. mucronatum$ (Figure 4b).

At the same time, net H$^+$ fluxes were determined in this study (Figure 4c). Here, we found that H$^+$ presented net effluxes under all treatments except for $T. ascendens$ under 1.0 mM KNO$_3$, $T. distichum$ under 0.1 mM NH$_4$Cl, and $T. mucronatum$ under 1.0 mM NH$_4$Cl (Figure 4c). Other than $T. distichum$ exposed to 0.1 mM NH$_4$Cl, the change tendency of net H$^+$ fluxes was similar to the variations of net NH$_4^+$ and NO$_3^−$ fluxes when the solution concentration was increased from 0.1 mM to 1.0 mM (Figure 4c).
3.4. Net NH$_4^+$ and NO$_3^-$ Fluxes Under Different N Proportions

Under 2.0 mM TN (total nitrogen) consisting of various proportions of NH$_4$Cl and KNO$_3$ (1:3, 1:1, and 3:1), T. ascendens, T. distichum, and T. mucronatum showed great diversities in the fluxes of NH$_4^+$, NO$_3^-$ and TN (Figure 5). The net influx of NH$_4^+$ ranged from 88.00 to 1480.80 pmol cm$^{-2}$ s$^{-1}$ across the three tested species (Figure 5a). It was 0.96 and 10.15 times greater under NH$_4$: NO$_3$ at 1:1 and 3:1 than at 1:3 in T. ascendens, respectively (Figure 5a). Similarly, the net NH$_4^+$ influxes were 5.21 and 7.76 times higher than 1:3 when treated with 1:1 and 3:1, respectively, in T. mucronatum (Figure 5a). A decreasing trend of net NH$_4^+$ influx was observed for T. distichum with an increase in the NH$_4^+$ proportion (Figure 5a).

Compared with the net NH$_4^+$ influx, the net flux of NO$_3^-$ was much lower, ranging from -100.53 to 84.85 pmol cm$^{-2}$ s$^{-1}$ under different proportions of NH$_4$Cl and KNO$_3$ (Figure 5b). It is surprising that the net influx of NO$_3^-$ observed under the 1:3 solution was replaced by net efflux when the proportion changed to 1:1 and 3:1 in T. ascendens (Figure 5b). T. distichum, however, presented a totally converse trend whereby the net flux of NO$_3^-$ significantly ($p < 0.05$) increased by 1.68 times when the NH$_4^+$ proportion was raised from 1:1 to 3:1 (Figure 5b). In the case of T. mucronatum, there was net efflux under 1:3 and 1:1 and net influx under 3:1 (Figure 5b).

The trend of TN fluxes ranging from 114.23 to 1500.48 pmol cm$^{-2}$ s$^{-1}$ was similar to the fluxes of NH$_4^+$ (Figure 5c), and the highest net influx of TN was observed for T. mucronatum and T. ascendens when the proportion of NH$_4$Cl: KNO$_3$ was 3:1 (Figure 5c). Among the three species, T. distichum displayed the lowest net NH$_4^+$, NO$_3^-$ and TN fluxes in all measuring solutions (Figure 5c).

In addition, the net H$^+$ fluxes were determined in this study (Figure 5d). All the treatments showed net H$^+$ influx when the two forms of N were provided together (Figure 5d).
3.5. $^{15}$N AT%, Ndff% and Enzyme Activities in the Roots of T. distichum

Compared with CK, $^{15}$N AT% was elevated by 70.27% or 29.73% in 1 mM $^{15}$NH$_4^+$-treated or 1 mM $^{15}$NO$_3^−$-treated T.distichum roots (Table 1). Similar results were also observed in Ndff% (Table 1). Compared to CK, however, no significant difference was found in the root biomass of T.distichum supplied with 1 mM $^{15}$NH$_4^+$ or $^{15}$NO$_3^−$ during the 3 d experiment period (Table 1).

**Table 1.** Biomass, $^{15}$N AT% and Ndff% in the roots of T. distichum under three N treatments.

| Treatments | Root Biomass/g | $^{15}$N AT% | Ndff% |
|------------|----------------|--------------|-------|
| CK         | 0.30 ± 0.01 $^{bc}$ | 0.37 ± 0.00 $^{c}$ | /     |
| 1 mM $^{15}$NH$_4^+$ | 0.34 ± 0.02 $^{ab}$ | 0.63 ± 0.03 $^{a}$ | 2.65 ± 0.31 $^{a}$ |
| 1 mM $^{15}$NO$_3^−$ | 0.26 ± 0.01 $^{c}$ | 0.48 ± 0.04 $^{b}$ | 1.16 ± 0.38 $^{b}$ |

Different letters behind the values in the same column indicate significant differences among the treatments according to Duncan’s Multiple Range Test at 5% level. $^{15}$N AT%: $^{15}$N Atom; Ndff%: $^{15}$N from N source.

$^{15}$NH$_4^+$ or $^{15}$NO$_3^−$ fertilization also has positive impacts on the activities of N assimilation enzymes (Table 2). When compared with CK, NR, NiR, GS, GDH and GOGAT activities were enhanced by 50.63%, 33.74%, 39.40%, 48.25% and 59.36%, respectively in 1 mM $^{15}$NH$_4^+$-supplied T.distichum roots (Table 2). Similarly, the activities of NR and NiR were increased by 221.03% and 11.93%, respectively in 1 mM $^{15}$NO$_3^−$-fed T.distichum roots (Table 2).
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Table 2. Activities of N assimilation enzymes in the roots of *T. distichum* under three N treatments.

| Treatments | NR Activity µmol h⁻¹ mg⁻¹ Protein | NiR Activity µmol h⁻¹ mg⁻¹ Protein | GS Activity µmol h⁻¹ mg⁻¹ Protein | GDH Activity µmol h⁻¹ mg⁻¹ Protein | GOGAT Activity µmol h⁻¹ mg⁻¹ Protein |
|------------|-----------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|
| CK         | 0.58 ± 0.02 c                      | 2.43 ± 0.04 c                      | 0.33 ± 0.00 b                      | 1.04 ± 0.06 b                      | 0.78 ± 0.03 b                      |
| 1 mM ¹⁵NH₄⁺ | 0.87 ± 0.02 b                      | 3.25 ± 0.08 a                      | 0.46 ± 0.22 a                      | 1.54 ± 0.08 a                      | 1.24 ± 0.03 a                      |
| 1 mM ¹⁵NO₃⁻ | 1.92 ± 0.04 a                      | 2.72 ± 0.01 b                      | 0.34 ± 0.01 b                      | 1.01 ± 0.04 b                      | 0.74 ± 0.01 b                      |

Different letters behind the values in the same column indicate significant differences between the treatments according to Duncan’s Multiple Range Test at 5% level. NR: nitrate reductase; NiR: nitrite reductase; GS: glutamine synthetase; GDH: glutamate dehydrogenase; GOGAT: glutamate synthetase.

4. Discussion

4.1. Spatial Variability of Net NH₄⁺ and NO₃⁻ Fluxes along the Fine Roots

Fine roots consist of four distinct regions, including root cap, meristematic, elongation, and maturation zones, characterized by different anatomical and functional features [7]. These anatomical and functional diversities could bring about distinct absorbing abilities for NH₄⁺ and NO₃⁻ in different root zones [26–28]. Spatial variability of net NH₄⁺ and/or NO₃⁻ flux has been observed in fine roots of various plant species [29]. For example, maximal net NH₄⁺ influx occurred at the root apex in rice [2] and *P. simonii* [5], and at 5 mm, 10 mm, and 5–20 mm from the root apex in *P. contorta* [6] and *P. popularis* [7], and *Douglas-fir* [6] respectively. Such spatial variation of net NH₄⁺ and NO₃⁻ influxes along the root axis was also observed in our research. The largest net influxes of NH₄⁺ and NO₃⁻ were detected at m from the apex of *T. ascendens* and *T. distichum*, which belongs to the elongation zone. Such differences are possibly because of cytosolic concentrations of NH₄⁺ and NO₃⁻ in the elongation zone being lower than the thresholds needed for N assimilation to support the fast growth [30,31]. Similar results were observed in studies of *Arabidopsis*, where larger net NH₄⁺ fluxes were shown in the elongation zones [28,32]. Moreover, *Phyllostachys edulis* showed relatively higher net influxes of NH₄⁺ and NO₃⁻ at 2–5 mm from the root apex [30]. The net NH₄⁺ or NO₃⁻ fluxes were found to be higher in segment I (0–35 mm) than segment II (35–70 mm) in *Populus × canescens* [31]. In addition, we found that *T. distichum* had the greatest NH₄⁺ and NO₃⁻ uptake rates among the three *Taxodium* species.

4.2. Net NH₄⁺ and NO₃⁻ Fluxes under Single N Treatments

Generally, environmental N levels have a significant impact on the NH₄⁺ and NO₃⁻ fluxes of fine roots [4]. For instance, gradual increases in the fluxes of NH₄⁺ and/or NO₃⁻ were determined when supplied N was elevated in *P. populnea* and *P. alba × P. glandulosa* [4], and *C. sinensis* [1]. However, the opposite results were observed in *P. glauca* [8], wheat [24], and corn (*Zea mays* L.) [33]. In our study, except for *T. ascendens* under NH₄Cl and *T. mucronatum* under KNO₃ solutions, most outcomes showed elevated NH₄⁺ or NO₃⁻ uptake rates resulting from increasing NH₄Cl or KNO₃ supply. Consistently, higher ¹⁵N AT% and ¹⁵Niff% were induced by ¹⁵NH₄⁺ or ¹⁵NO₃⁻ treatment in the roots of *T. distichum*. Moreover, ¹⁵NH₄⁺ or ¹⁵NO₃⁻ fertilization also brought about higher activities of NR, NiR, GS, GDH and GOGAT in the roots of *T. distichum*. These results suggest that N fertilization could be applied to stimulate NH₄⁺ and NO₃⁻ absorption and assimilation capacities for *Taxodium* plants in practice.

Although an increasing N supply is likely to enhance N uptake in most cases, the provision of just NH₄⁺ could lead to soil acidification [34]. In most cases, to maintain ion homeostasis, roots release H⁺ while absorbing NH₄⁺, decreasing pH in the growth medium [35,36]. Eventually, this may lead to physiological and morphological disturbance of plants and then bring about toxicity and low production [37]. For example, acidification can significantly induce aluminum absorption, which is harmful to the development of plants [38]. In contrast, after absorption of NO₃⁻, OH⁻ could be released, contributing to
the increase of pH [39]. Thus, a balanced supply of NH$_4^+$ and NO$_3^-$ is expected to improve the N uptake of plants and the soil environment.

4.3. Net NH$_4^+$ and NO$_3^-$ Fluxes under Mixed N Treatments

Many studies had demonstrated that the uptake of NH$_4^+$ and NO$_3^-$ was affected by each other when both N forms were provided [1,7]. In this study, the presence of NO$_3^-$ stimulated the uptake of NH$_4^+$, whereas the net fluxes of NO$_3^-$ were inhibited by NH$_4^+$ in *Taxodium* plants. Similar results were found in the roots of corn, tea, wheat, rice and *Brassica campestris* [1,2,9,24,33], which indicated that NH$_4^+$ and NO$_3^-$ might interact with each other under coexistence N forms. These results might be related to cytosolic NH$_4^+/NO_3^-$ thresholds [30]. In detail, more NH$_4^+$ may be required for plant development when NO$_3^-$ was provided, while pre-existing NH$_4^+$ may reduce the thresholds of NO$_3^-$ in the plant [1]. Considering that a higher net NH$_4^+$ influx than NO$_3^-$ was observed, it can be concluded that *Taxodium* plants show a preference for NH$_4^+$. It is noted that when NH$_4$Cl or KNO$_3$ was solely supplied, the fluxes of NH$_4^+$ or NO$_3^-$ in the three species were erratic. However, stable net NH$_4^+$ and NO$_3^-$ fluxes were observed when NH$_4^+$ and NO$_3^-$ were both present in the solution, indicating a better balance in the mixed solution. In addition, this interesting phenomenon was reported in the study of *C. sinensis*, which might be the result of the competition between NH$_4^+$ and NO$_3^-$, and the underlying mechanism needs to be further studied [1].

Because of the greater N uptake in mixed treatments than in single N conditions, strong net uptake of NH$_4^+$ in fine roots of *Taxodium* species was expected to occur when NH$_4^+$ and NO$_3^-$ were supplied in different proportions [37]. In the case of tea, the maximum net NH$_4^+$ influx was observed when NH$_4^+:NO_3^-$ was 1:1, and the highest net NO$_3^-$ influx occurred when NH$_4^+:NO_3^-$ was 1:2:1 [1]. In blueberry (*Vaccinium corymbosum* L.), the mRNA levels of ammonium transporter 3 (VcAMT3) involved in NH$_4^+$ uptake as well as nitrate transporter 1.5 (VcNRT1.5) and VcNRT2 involved in NO$_3^-$ uptake was highest when the NH$_4^+:NO_3^-$ ratio was 2:1 [40]. The highest growth rate, which is positively correlated with N uptake, of *T. aestivum* L., *Brachiaria brizantha*, and *Pseudostellaria heterophylla* was found when the NH$_4^+$ and NO$_3^-$ were supplied equivalently [1,24,41,42]. In this study, the best uptake rates of N were found when NH$_4^+:NO_3^-$ was 3:1, 1:3, and 3:1 for *T. ascendens*, *T. distichum*, and *T. mucronatum*, respectively, which could provide an applicable proportion of NH$_4^+$ and NO$_3^-$ when producing special N fertilizer for the productivity of *Taxodium* plants. Additionally, the optimal equilibrium between NH$_4^+$ and NO$_3^-$ supply largely differed between the three *Taxodium* species, implying that the induction of N transport systems require distinct NH$_4^+$ and NO$_3^-$ ratios among these plants.

In the present study, we found that with the change in the proportion of NH$_4^+:$ NO$_3^-$ (total N concentration: 2 mM), the NH$_4^+$ influxes were improved more than NO$_3^-$. This observation indicates a preference for NH$_4^+$ over NO$_3^-$, which is in good agreement with our previous outcomes. In most plant species, NH$_4^+$ is first absorbed into cells and then directly converted to amino acids, whereas cytosolic NO$_3^-$ is assimilated at a higher energy cost. It is reduced to NO$_2^-$ with the help of nitrate reductase (NR) and is further converted into NH$_4^+$ in plastids by nitrite reductase (NiR), which requires more energy than NH$_4^+$ for both transportation and further reduction [43]. On the other hand, the flux discrepancies between NH$_4^+$ and NO$_3^-$ might result from the lower activity of NO$_3^-$ transport systems as NH$_4^+$, which reduces the expression of the NO$_3^-$-related genes [1]. In blueberry plants, the expression of AMTs and NRTs was largely affected by the different ratios of NH$_4^+:$ NO$_3^-$ [40]. A previous study has indicated that different AMTs determined the uptake of NH$_4^+$ to a certain extent, which was mediated by the external concentration [44]. Furthermore, various AMTs and NRTs have different substrate affinities appropriate to different N concentrations [5,8,10]. Therefore, the complicated fluxes of NH$_4^+$ and NO$_3^-$ when supplied at different proportions might be related to the distinct energetic and biochemical characteristics of uptake and assimilation pathway between NH$_4^+$ and NO$_3^-$ in plant roots [4,5,24].
4.4. Net $\text{NH}_4^+$ and $\text{NO}_3^-$ Fluxes Associated with $\text{H}^+$

In this study, the alteration of $\text{H}^+$ fluxes was tightly associated with the variation in $\text{NH}_4^+$ or $\text{NO}_3^-$. Previous studies revealed that $\text{H}^+$ fluxes might be correlated with the transport of $\text{NH}_4^+$ and $\text{NO}_3^-$, since $\text{NH}_4^+$ is transported into root cells through a symporter (co-transport with $\text{H}^+$) and/or a uniporter, and $\text{NO}_3^-$ is co-transported with $\text{H}^+$ via a symporter into the cytosol [12–14]. Additionally, by maintaining a proton gradient, plasma membrane PM-$\text{H}^+$-ATPase facilitates transport by pumping $\text{H}^+$ into the apoplast during the uptake of $\text{NH}_4^+$ or $\text{NO}_3^-$ in some parts of the roots [4,43,45]. The activities of PM-$\text{H}^+$-ATPase are determined by the transcript levels of corresponding mRNAs [4]. Although inconsistent results of $\text{H}^+$ fluxes under different N treatments were observed in the present study, $\text{H}^+$ still plays an essential role in plant uptake of $\text{NH}_4^+$ and $\text{NO}_3^-$. Similar to a previous study in fine roots of $P. \text{popul} \text{ar} \text{i} \text{s}$ [7], our data indicated a tendency for net $\text{H}^+$ uptake when two forms of N were supplied simultaneously. Intriguingly, fluxes of $\text{H}^+$ fluctuated under both single N sources. Through our findings, we suspect that there may be an interaction between net $\text{H}^+$ flux and net $\text{NH}_4^+$/NO$_3^-$ flux in roots of $\text{Taxodium}$ species. Similar results were observed by Garnett et al. [15]. The specifics of the proposed interaction remain unclear. It is challenging to find out the specific mechanism underlying the correlation between $\text{H}^+$ and $\text{NH}_4^+$/NO$_3^-$ in $\text{Taxodium}$ roots.

5. Conclusions

In summary, spatial variability of $\text{NH}_4^+$ and $\text{NO}_3^-$ fluxes was observed along fine roots of $\text{Taxodium}$ plants, and $T. \text{ascend} \text{ens}$ and $T. \text{d} \text{ist} \text{ich} \text{um}$ had higher fluxes of $\text{NH}_4^+$ and $\text{NO}_3^-$ at 2.1–3.0 mm from the root apex. In most cases, net fluxes of $\text{NH}_4^+$ and $\text{NO}_3^-$ increased with the elevated single N levels. $\text{NH}_4^+$ and $\text{NO}_3^-$ affected each other when they were both supplied, and $\text{Taxodium}$ plants preferred $\text{NH}_4^+$. Higher net N influxes were found when $\text{NH}_4^+$ and $\text{NO}_3^-$ were simultaneously supplied than sole N treatments, especially in $T. \text{ascend} \text{ens}$ and $T. \text{muc} \text{ron} \text{atum}$ at 3:1 of $\text{NH}_4^+$:$\text{NO}_3^-$. Additionally, $\text{H}^+$ fluxes were tightly correlated with net $\text{NH}_4^+$ and $\text{NO}_3^-$ fluxes. These findings are valuable for understanding the characteristics of $\text{NH}_4^+$ and $\text{NO}_3^-$ fluxes in the fine roots of $\text{Taxodium}$ plants in the context of single and various ratios of N supply, and could provide a scientific basis for N management for silvicultural practice and better productivity of $\text{Taxodium}$ plants.

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