Nitrogen form plays an important role in the growth of moso bamboo (Phyllostachys edulis) seedlings

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ABSTRACT

Background. This study aimed to gain an understanding of the growth response of Phyllostachys edulis (moso bamboo) seedlings to nitrogen (N) and potassium (K) to benefit nutrient management practices and the design of proper fertilizer in nursery cultivation.

Methods. An orthogonal array L₈(4 × 2⁴) was used to study the effects of N forms (NH₄⁺, NO₃⁻), N concentrations (8, 32 mmol/L), and K⁺ concentrations (0, 0.5, 1.5, 3 mmol/L) on seedling height, leaf number, chlorophyll content (SPAD value), biomass, root systems, and N content of P. edulis seedlings. Plants were grown in vermiculite under controlled greenhouse conditions.

Results. Our study showed that N form played a significant role in the overall performance of P. edulis seedlings, followed by the effect of N and K⁺ concentrations. Among the N forms, NH₄⁺ significantly improved the growth of P. edulis seedlings compared with NO₃⁻. Seedling height, leaf number, chlorophyll SPAD value, biomass, and root system architecture (root length, root surface area, root volume, and root tips) were greater with 8 mmol/L of NH₄⁺ treatments than with 32 mmol/L of NH₄⁺ treatments, whereas root diameter and N content of P. edulis seedlings were higher with 32 mmol/L of NH₄⁺ than with 8 mmol/L of NH₄⁺. K displayed inconsistent effects on the growth of P. edulis seedlings. Specifically, seedling height, leaf number, biomass and root volume increased when the K⁺ concentration was increased from 0 to 0.5 mmol/L, followed by a decrease when the K⁺ concentration was further increased from 0.5 to 3 mmol/L. Root average diameter of the seedlings was the highest with a K⁺ concentration of 1.5 mmol/L, and K had some inhibitory effects on the chlorophyll SPAD value of the seedlings. P. edulis seedlings performed well with 8 mmol/L NH₄⁺ and further tolerated a higher concentration of both NH₄⁺ and NO₃⁻, although excessive N could inhibit seedling growth. A lower concentration of K (≤ 0.5 mmol/L) promoted seedling growth and increasing K⁺ concentration in the nutrient solution did not alleviate the inhibitory effect of high N on the growth of P. edulis seedlings. Therefore, NH₄⁺ nitrogen as the main form of N fertilizer, together with a low concentration of K⁺, should be supplied in the cultivation and nutrient management practices of moso bamboo.

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INTRODUCTION

Moso bamboo (Phyllostachys edulis) is a species of large monopodial bamboo that is native to China and extensively cultivated throughout China, Japan, Korea, and Vietnam (Rao, Rao & Williams, 1998). Moso bamboo, which covers more than 3.87 hectares, representing 70% of the Chinese bamboo forest areas and 80% of the global distribution of moso bamboo, is the most important bamboo species in China (Song et al., 2016b). Moso bamboo can be planted by transplanting mother bamboo or planting seedling; the former method has generally been used for afforestation throughout China because vegetative propagation with mother bamboo typically takes only 5 years for it to form a grove compared with approximately 10 years by planting seedlings (Banik, 1980; Fu, 2000). However, both removal and replanting of the mother bamboo are labor intensive, high cost with low efficiency. In addition, human consumption of young edible shoots and environmental disturbance restrict the naturally rapid expansion of moso bamboo into a grove (Cai et al., 2008). In contrast, planting seedlings has many advantages, including easy handling, transporting, strong suitability and high survival rate with low cost (Fu, 2000). This provides a new approach for the regeneration and introduction of flowering bamboo forests. In fact, planting seedlings has been successfully applied in Guangxi Province, China to establish new bamboo groves (Qin, 2009; Chen, 2019). However, few studies have been conducted on the nutrient requirements of moso bamboo seedlings during cultivation and breeding. In addition, many bamboo forests are facing abandonment, with the forests potentially reverted to an unmanaged stand associated with a decline in soil organic matter accumulation and soil fertility (Christanty, Kimins & Mailly, 1997; Nakshima, 2001; Chen, Wang & Wang, 2016).

Nitrogen (N) is often an important factor for plant growth and productivity in terrestrial ecosystems. Under natural conditions, ammonium (NH$_4^+$) and nitrate (NO$_3^-$) are the two primary forms of inorganic N available to plants in soil (Xu, Fan & Miller, 2012). In well-aerated agricultural soils and disturbed or early-successional natural ecosystems, NO$_3^-$ is the major N source, whereas in flooded environments or acidic mature forests, NH$_4^+$ is the dominant N source (Glass et al., 2002; Britto & Kronzucker, 2006). Due to differences in environmental conditions, plant species, and the nutritional characteristics of N sources, plants have adapted to different N forms during long-term evolution and may show optimized growth with specific N forms (Britto & Kronzucker, 2013). For example, many conifers, ericaceous species and rice show improved growth with available NH$_4^+$, whereas some crops and early-successional pioneer species prefer NO$_3^-$ (Kronzucker, Siddiqi & Glass, 1997; Britto & Kronzucker, 2013). A previous study conducted by Li et al. (2014b) showed that the growth of P. edulis seedlings are slightly improved with available NO$_3^-$. However, Song et al. (2013) found that P. edulis tends to absorb NH$_4^+$ under natural conditions. Gu et al. (2016) indicated that the N form preferred by P. edulis is related to the
N concentration. Our study showed that under the low N concentrations (0.1, 0.4 mmol/L), there is no apparent N form preference, but the growth of bamboo seedlings especially the aboveground parts is improved with elevated NH$_4^+$ available from 2 to 40 mmol/L (Gu et al., 2016; Zou et al., 2020). Although NH$_4^+$ can be used as a sole N source and an essential intermediate, it can also result in toxicity symptoms in many plant species, especially when high NH$_4^+$ concentrations are provided as a sole N source or in combination with low levels of potassium (K) (Ten Hoopen et al., 2010). A previous study showed that shoot and root growth is significantly suppressed in cucumber grown with 10 mmol/L of NH$_4^+$ (Roosta & Schjoerring, 2008). Similar results have also been found in Arabidopsis thaliana, barley, tomato, and beans after high NH$_4^+$ treatments (Britto & Kronzucker, 2002; Britto & Kronzucker, 2006; Li et al., 2014a). In terms of moso bamboo, Li et al. (2014b) found that when the proportion of NH$_4^+$ exceeds 50% of the total N provided (40 mg/L), the growth of P. edulis seedlings is inhibited, and all of the seedlings eventually are died when NH$_4^+$ is supplied as a sole N source. However, Zou et al. (2020) showed that higher NH$_4^+$ concentrations (16∼40 mmol/L) are beneficial for the growth of aboveground organs, although root growth is suppressed to some extent with NH$_4^+$ levels ranging from 24 to 40 mmol/L. The contradictions in these studies indicate that N form preference and NH$_4^+$ tolerance of moso bamboo seedlings require further study.

K is another major plant nutrient that affects plant growth and metabolism (Wang et al., 2013). K plays a vital role in defending against biotic and abiotic stresses, and its role in the alleviation of NH$_4^+$ toxicity has been widely reported (Britto & Kronzucker, 2002; Szczersba et al., 2007; Wang et al., 2013). With similar hydration diameter charge and effects on membrane potential, K$^+$ and NH$_4^+$ compete each other for the limited ion channel proteins on the cell membrane (Ten Hoopen et al., 2010; Kong et al., 2014; Coskun, Britto & Kronzucker, 2017). K$^+$ can reduce the transport and accumulation of NH$_4^+$ by direct competition during uptake and can also alleviate the rapid NH$_4^+$ cycling at the plasma membrane, thus reducing NH$_4^+$ toxicity. On the other hand, K$^+$ can enhance NH$_4^+$ utilization by activating the enzymes of NH$_4^+$ assimilation and amino acid transport in plant cells (Wang, Siddiqi & Glass, 1996), which promotes NH$_4^+$ metabolism to reduce NH$_4^+$ toxicity (Ten Hoopen et al., 2010; Zhang et al., 2010).

Orthogonal arrays (often referred to as Taguchi methods) can be used to examine large numbers of factors in a much smaller number of experiments, allowing for the exploration of a unique subset of factor combinations. Therefore, it is a sophisticated time- and cost-efficient testing strategy (Lin, 1987; Qiao et al., 2013). Orthogonal experiments have been used to test the optimization of liquid fertilizer formulation, callus induction, plant regeneration medium in tissue culture and other hydroponic nutrient solution protocols for Sorghum bicolor (Gutiérrez-Miceli et al., 2008), Dendrocalamus latiflorus (Qiao et al., 2013), and Ipomoea spp. (Zhou & Lu, 2013). Using orthogonal arrays, we have studied the effects of different N and K$^+$ concentrations and N form on the growth response and N acquisition of P. edulis seedlings. We have also investigated the extent to which the growth indices of P. edulis seedlings are related to each other. The overall aims of this study were to (1) further clarify the N form preference of P. edulis seedlings and (2) determine the effects of K on the response of moso bamboo to different N forms. This study
Table 1  \( L_8 (4 \times 2^4) \) orthogonal array design of nutrient solution composition with the concentrations and forms of N and K.

| Orthogonal combination | K concentration (mmol/L) | N form | N concentration (mmol/L) |
|------------------------|---------------------------|--------|--------------------------|
| 1                      | 0                         | \( \text{NH}_4^+ \) | 8                         |
| 2                      | 0                         | \( \text{NO}_3^- \) | 32                        |
| 3                      | 0.5                       | \( \text{NH}_4^+ \) | 32                        |
| 4                      | 0.5                       | \( \text{NO}_2^- \) | 8                         |
| 5                      | 1.5                       | \( \text{NH}_4^+ \) | 32                        |
| 6                      | 1.5                       | \( \text{NO}_3^- \) | 8                         |
| 7                      | 3                         | \( \text{NH}_4^+ \) | 8                         |
| 8                      | 3                         | \( \text{NO}_2^- \) | 32                        |

presents the appropriate fertilization requirements of moso bamboo cultivation based on the experimental data.

**MATERIALS AND METHODS**

**Plant materials and growth conditions**

Seeds of *P. edulis* were collected from GuanYang City, Guangxi Province, China and stored at 4 °C before sowing. The moso bamboo seeds were soaked overnight in water at 40 °C, shelled, sterilized by soaking in 20% NaClO for 20 min, rinsed in sterile water at least five times, and then germinated in plastic pots (diameter of 150 mm, height of 130 mm) filled with vermiculite. One month later, approximately five cm tall, three foliate seedlings were selected for different treatments.

**N and K treatments**

\( L_8 (4 \times 2^4) \) orthogonal arrays were employed to study \( K^+ \) concentrations (0, 0.5, 1.5, 3 mmol/L), N forms (\( \text{NH}_4^+, \text{NO}_3^- \)), and N concentrations (8, 32 mmol/L) on seedling growth and N uptake (Table 1). The nutrient solution modified from *Norisada & Kojima (2005)* contains 2.5 mmol/L Ca\(_2^+\) as CaCl\(_2\) \cdot 2\text{H}_2\text{O}, 0.25 mmol/L MgSO\(_4\) \cdot 7\text{H}_2\text{O}, 0.6 mmol/L Na\(_2\text{HPO}_4\) \cdot 10\text{H}_2\text{O}, 0.01 mmol/L Fe-EDTA, 0.02 mmol/L H\(_3\text{BO}_3\), 2 \( \mu \text{mol/L MnCl}_2 \cdot 4\text{H}_2\text{O}, 2 \( \mu \text{mol/L ZnSO}_4 \cdot 7\text{H}_2\text{O}, 2 \( \mu \text{mol/L CuSO}_4 \cdot 5\text{H}_2\text{O}, 0.5 \( \mu \text{mol/L Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}, and 0.5 \( \mu \text{mol/L CoCl}_2 \cdot 6\text{H}_2\text{O}. The \( \text{K}^+, \text{NH}_4^+, \text{and NO}_3^- \) were supplied by using KCl, (\( \text{NH}_4\))\(_2\text{SO}_4\), and NaNO\(_3\), respectively. A nitrification inhibitor C\(_2\text{H}_4\text{N}_4\) (7 \( \mu \text{M} \)) was added to all treatments to prevent nitrification. One replicate consisted of six pots with two seedlings planted per pot, there were three replicates per treatment. Plants were grown in a greenhouse at 25/18 °C ± 3 °C day/night temperature, 65~70% relative humidity, and 14/10 h day/night photoperiod. Pots were rotated every week to eliminate location effects. After treatment for 2 months, aboveground growth traits, root systems, and N content were analyzed.

**Growth analysis and root morphology**

Height was measured with a ruler. The number of unfurled leaves above the cotyledonary node were counted. The chlorophyll content (SPAD value) of the leaves was determined...
with a chlorophyll meter (SPAD-502, Minolta). Root morphology, including root total length (RL), average diameter (AD), root surface area (RS), root volume (RV) and root tips were determined using an automatic scanning apparatus (EPSON color image scanner LA1600+, Toronto, Canada) equipped with WinRHIZO 2012 software (Regent Instruments, Quebec, Canada).

**Measurement of dry weight and N content**

When the above experimental treatment was completed, plants were dried at 105 °C for 30 min, and then dried to a constant weight at 70 °C for biomass determination. The samples were digested with H$_2$SO$_4$-H$_2$O$_2$, and the total N content was determined according to the Kjeldahl method (Chen et al., 2013).

**Statistical analysis**

The data were analyzed to determine the range (R and R’) of orthogonal tests using DPS7.05 statistical software (http://www.statforum.com). Using the same software, an analysis of variance (ANOVA) was conducted and post hoc comparisons were conducted using Duncan’s multiple comparison test with differences considered significant at $p < 0.05$.

**RESULTS**

**Effects of N and K on the growth of moso bamboo seedlings**

The effects of N and K on bamboo seedlings are listed in Table 2. The range analysis (R’ values) of the orthogonal array indicated that N form had a significant impact on the seedling height, biomass, leaf number, and SPAD value of *P. edulis*. Impacts to a lesser extent were observed for N concentration, while K$^+$ concentration had the least effect (R’$_N$ form > R’$_N$ concentration > R’$_K^+$ concentration, Table 3), suggesting that N form is more important than nitrogen and potassium concentration on growth of moso bamboo seedling. After two months of treatment with the different N forms (Table 1), the seedlings of moso bamboo performed better in the treatments of NH$_4^+$ (treatments 1, 3, 5, 7) than the treatments of NO$_3^−$ (treatments 2, 4, 6, 8), and seedlings showed greener, heathier leaves and less necrosis in the NH$_4^+$ treatments than in the NO$_3^−$ treatments (Fig. S1). The growth index of seedling height, biomass, leaf number and SPAD value increased 9.98%, 100%, 27.86%, and 257.14%, respectively, by comparing average value of these parameters between the treatments of 1, 3, 5, 7 and the treatments of 2, 4, 6, 8 (Table 2, Fig. 1). For the N concentrations (Table 1), the results showed that seedling height, biomass, leaf number and SPAD value increased by 8.27%, 33.33%, 11.03%, and 22.96%, respectively (Fig. 1), with the nutrition solutions of 8 mmol/L of nitrogen (treatments 1, 4, 6, 7) compared to 32 mmol/L of nitrogen (treatments 2, 3, 5, 8; Table 2).

The analysis of variance (Table 4) showed that the N form and N concentration had significant effects on seedling height, biomass, leaf number and SPAD value. The K$^+$ concentration also had significant effects on the leaf number of *P. edulis* seedlings. Therefore, moso bamboo seedlings treated with a normal nutrient solution supplemented with 8 mmol/L NH$_4^+$ and 0.5 mmol/L K$^+$ would show overall better growth indices.
Table 2  N and K on growth, N content, and root architecture of moso bamboo seedlings. Analytical results are means ± SE (n = 36). Mean values followed by the same letter (a, b, c, d, e, or f) are not significantly different within the same column according to a Duncan’s multiple comparison test at \( P < 0.05 \).

| Treatment | Mortality rate/(%) | Height (cm) | Leaf number | SPAD | Biomass (g) | N content (%) | Root length (cm) | Root surface area (cm²) | Root volume (cm³) | Average diameter (cm) | Root tips |
|-----------|--------------------|-------------|-------------|------|-------------|---------------|------------------|------------------------|-------------------|----------------------|-----------|
| 1         | 8.00a              | 16.63a      | 11.44a      | 27.55a | 0.22a       | 0.92ab        | 292.86a          | 30.80a                 | 0.26a             | 0.34ab               | 1438.72a |
| 2         | 0.00a              | 13.13b      | 7.75f       | 6.81d | 0.08de      | 0.84bc        | 117.98cd         | 12.34cd                | 0.10c             | 0.33ab               | 612.75cd |
| 3         | 0.00a              | 15.70ab     | 11.0ab      | 20.52c | 0.20a       | 0.72bc        | 209.02b          | 23.81b                 | 0.22ab            | 0.37ab               | 915.33b  |
| 4         | 0.00a              | 14.37ab     | 9.50de      | 6.49d | 0.12cd      | 0.39d         | 186.95b          | 18.58bc                | 0.15bc            | 0.32b                | 1019.58b |
| 5         | 0.00a              | 13.73b      | 10.17cd     | 21.51bc | 0.14bc     | 1.14a        | 99.45d           | 11.59cd                | 0.11c             | 0.37a                | 393.25d  |
| 6         | 0.00a              | 14.60ab     | 9.00e       | 7.59d | 0.11cde    | 0.60cd       | 176.63bc         | 18.13bc                | 0.15bc            | 0.32ab               | 812.17bc |
| 7         | 0.00a              | 15.67ab     | 10.33bc     | 25.42ab | 0.18ab     | 0.45d       | 196.28b          | 21.25b                 | 0.18b             | 0.34ab               | 802.50bc |
| 8         | 8.00a              | 14.03ab     | 7.36f       | 5.70d | 0.07e       | 0.39d        | 77.79d           | 8.43d                  | 0.07c             | 0.34ab               | 366.22d  |
Table 3  Range analysis of the concentrations and forms of N and K on the growth indices of the seedlings. R is the range of each factor level (R = X_{max} − X_{min}); R’ is the adjusted range.

| Range and adjusted range of growth indices | K concentration | N form | N concentration |
|-------------------------------------------|----------------|--------|----------------|
| Seedling height R                          | 0.87           | 1.40   | 1.17           |
| Seedling height R’                         | 0.55           | 1.99   | 1.66           |
| Biomass R                                 | 0.04           | 0.09   | 0.03           |
| Biomass R’                                | 0.02           | 0.13   | 0.05           |
| Leaf number R                             | 1.40           | 2.33   | 1              |
| Leaf number R’                            | 0.89           | 3.11   | 1.42           |
| SPAD value R                              | 3.68           | 17.10  | 3.13           |
| SPAD value R’                             | 2.34           | 24.28  | 4.44           |
| Root length R                             | 68.39          | 59.57  | 87.12          |
| Root length R’                            | 43.52          | 84.58  | 123.71         |
| Root surface area R                       | 6.73           | 7.50   | 8.15           |
| Root surface area R’                      | 4.28           | 10.65  | 11.57          |
| Root volume R                             | 0.06           | 0.07   | 0.06           |
| Root volume R’                            | 0.04           | 0.10   | 0.08           |
| Average diameter R                        | 0.01           | 0.03   | 0.02           |
| Average diameter R’                       | 0.004          | 0.043  | 0.028          |
| Root tips R                               | 441.38         | 184.77 | 446.35         |
| Root tips R’                              | 280.89         | 262.37 | 633.82         |
| Nitrogen content R                        | 0.75           | 0.46   | 0.06           |
| Nitrogen content R’                       | 0.26           | 0.29   | 0.09           |

Table 4  Variance analysis of the concentrations and forms of N and K on the growth indices of the seedlings.

| Source of variation | The test statistic F |
|---------------------|----------------------|
|                     | K concentration | N form | N concentration |
| Height              | 0.53             | 6.94   | 4.82            |
| Leaf number         | 8.65***          | 143.16**| 26.29**         |
| SPAD value          | 2.58             | 307.62**| 10.29*          |
| Biomass             | 2.70             | 61.25**| 8.26            |
| Root length/(cm)    | 7.14**           | 18.34**| 39.24**         |
| Root surface area/(cm²) | 5.71**      | 22.50**| 26.59*          |
| Root volume/(cm³)   | 3.94*            | 22.39**| 14.22**         |
| Average diameter/(cm) | 0.16           | 7.72   | 3.42            |
| Root tips           | 16.26**          | 10.13**| 59.13**         |
| Nitrogen content    | 18.13***         | 21.62**| 11.48**         |

Notes.

*indicates the significance level at p < 0.05.
**indicates the significance level at P < 0.01.
Effects of different N and K on root system architecture of moso bamboo seedlings

According to the resultant R’ values of root architecture parameters (Table 3), N form and N concentration (Table 1) played a significant role on the root growth of *P. edulis*, while K⁺ concentration showed the least effect. Root architecture parameters were improved with NH₄⁺ compared to NO₃⁻, and root traits were improved under the 8 mmol/L nitrogen (treatments 1, 4, 6 and 7; Tables 1 and 2) compared with the 32 mmol/L nitrogen (treatments 2, 3, 5 and 8; Tables 1 and 2), although the higher N concentration resulted in a greater increase in the root diameter of *P. edulis* (Table 2, Fig. 2). K had inconsistent effects on the root architecture of *P. edulis*. The total root length, root surface area and the root tips smaller with the increasing K⁺ concentrations. In contrast, the root volume and root diameter showed improved growth at a K⁺ concentration of 0.5 and 1.5 mmol/L, respectively (Table 2; Fig. 2).

The analysis of variance (Table 4) showed that K⁺ concentration, N form and N concentration had significant effects on most of the root system parameters. However, there was no evident influence of K⁺ and N concentrations on average root diameter. In general, *P. edulis* seedlings treated with 8 mmol/L NH₄⁺ (treatment 1) showed significantly better root architecture parameters than seedlings cultured in the other combinations/treatments (Table 2).
Effects of different N and K\(^+\) concentrations on the N content of moso bamboo seedlings

According to the resultant R’ values, the N form and K\(^+\) concentration (Table 1) played a more significant role than N concentration on the N content of moso bamboo seedlings (R’\(_{N \text{ form}}\) > R’\(_{K^+ \text{ concentration}}\) > R’\(_{N \text{ concentration}}\), Table 3). NH\(_4^+\) was a better N form than the NO\(_3^-\) for the N content of the seedlings, increasing 44.64\% by comparing the average value of treatments 1, 3, 5, and 7 and treatments 2, 4, 6, and 8 (Tables 1 and 2, Fig. 3). The N content decreased when the K\(^+\) concentration increased from 0 to 0.5 mmol/L, whereas the N content increased when the K\(^+\) concentration further increased from 0.5 to 1.5 mmol/L. The optimal K\(^+\) concentration for the N content of moso bamboo seedlings was 0.5 to 1.5 mmol/L. The N content of the seedlings increased by 32.2\% when the N concentration (Table 1) increased from 8 mmol/L (treatments 1, 4, 6 and 7) to 32 mmol/L (treatments 2, 3, 5 and 8; Tables 1 and 2, Fig. 3).

The analysis of variance showed that the effects of N form, N concentration, and K\(^+\) concentration (Table 1) on the N content of the seedlings were very significant (Table 4).
Furthermore, the results indicate that moso bamboo seedlings had higher N content in treatment 5 (32 mmol/L NH$_4^+$ + 1.5 mmol/L K$^+$) compared with the other treatments, except for treatment 1 (32 mmol/L NH$_4^+$ + 0 mmol/L K$^+$) (Table 2).

**Correlation analysis of the growth indices of moso bamboo seedlings**

The growth indices of seedling height, leaf number, SPAD value and biomass were positively correlated with each other, and the correlations were also positive for root system architecture parameters such as root length, root surface area, root volume and root tips (Table 5). Growth and biomass accumulation were positively correlated with the main root system architecture parameters, but negatively or not correlated with N content. There were no correlations between root system architecture and the N content of the seedlings (Table 5).

**DISCUSSION**

**Moso bamboo seedlings showed improved growth under the NH$_4^+$ nitrogen form**

All of the tested growth parameters and total N content were significantly increased with the NH$_4^+$ treatments compared to the NO$_3^-$ treatments, suggesting that the seedlings of moso bamboo prefer NH$_4^+$ (Figs. 1–3). Our results are consistent with those presented by Gu et al. (2016) and Zou et al. (2020) who showed that the growth of bamboo seedlings displayed a strong NH$_4^+$ preference. Similarly, Song et al. (2013) showed that N uptake in the field is mainly in the form of NH$_4^+$, accounting for 93.6% of the total inorganic N in bamboo-dominated forests. In addition, Ueda (1960) reported that the application of ammonium sulfate ((NH$_4$)$_2$SO$_4$) strongly promotes the production of new culms in the first year after fertilization of *P. edulis* and *P. reticulata* groves. However, such results contradict the findings presented by Li et al. (2014b) that moso bamboo shows limited preference for NO$_3^-$. Considering the cultivation medium, pH and nutrient supply are different among
Table 5  Correlation analysis of the growth indices of the seedlings.

| Correlations       | Height | Leaf number | SPAD     | Biomass | N content | Root length | Root surface | Root volume | Average diameter | Root tips |
|--------------------|--------|-------------|----------|---------|-----------|-------------|--------------|-------------|------------------|-----------|
| n = 8              |        |             |          |         |           |             |              |             |                  |           |
| Height             | 1      | –           | –        | –       | –         | –           | –            | –           | –                | –         |
| Leaf number        | 0.804  | 1           | –        | –       | –         | –           | –            | –           | –                | –         |
| SPAD               | 0.727  | 0.926**     | 1        | –       | –         | –           | –            | –           | –                | –         |
| Biomass            | 0.890**| 0.975**     | 0.903**  | 1       | –         | –           | –            | –           | –                | –         |
| N content          | 0.553  | 0.395       | 0.459    | 0.296   | 1         | 0.042       | 1            | –           | –                | –         |
| Root length        | 0.789  | 0.787       | 0.582    | 0.827   | 0.042     | 1           | –            | –           | –                | –         |
| Root surface       | 0.917**| 0.832**     | 0.653    | 0.884** | 0.093     | 0.992**     | 1            | –           | –                | –         |
| Root volume        | 0.923**| 0.880**     | 0.709    | 0.928** | 0.157     | 0.966**     | 0.990**      | 1           | –                | –         |
| Average diameter   | 0.155  | 0.5         | 0.588    | 0.448   | 0.554     | –0.094      | 0.023        | 0.155       | 1                | –         |
| Root tips          | 0.785  | 0.696       | 0.418    | 0.701   | 0.002     | 0.970**     | 0.939**      | 0.893**     | −0.239           | 1         |

Notes.
*Correlation is significant at the 0.05 level (2-tailed).
**Correlation is significant at the 0.01 level (2-tailed).

In aboveground organs, N is a structural element of chlorophyll, which affects the formation of chloroplasts and the accumulation of chlorophyll (Tucker, 2004). A previous study has reported that the chlorophyll content is closely linked to the leaf N content, and N deficiency leads to loss of the green color in the leaves, a reduced leaf area and photosynthetic intensity (Bojović & Marković, 2009). In the present work, the green color in the leaves and leaf area of the seedlings treated with NO$_3^-$ are decreased compared to those treated with NH$_4^+$ (Fig. S1), which may be associated with the leaf N content.
However, there was no correlation between the total N content and chlorophyll content (SPAD value) or other growth characters (Table 5). One reason for this may be that we measured the N content of the whole plant instead of determining the N content of the root, stem, and leaf separately, so the total N content is inconsistent with the leaf N content. Alternatively, the correlation between the total N content and other growth characters may have not been established during our short-term experiment. Different forms of N have a large effect on leaf growth because N increases the leaf area of plants, chlorophyll content of leaf blades and photosynthetic rate, influencing photosynthesis (Li, Wang & Stewart, 2013). Therefore, the greater growth of moso bamboo seedlings with NH$_4^+$ than with NO$_3^-$ might be also associated with the increased photosynthesis of the aboveground organs.

Size and architecture of the root system are important factors of nutrient acquisition efficiency as they ensure the total volume of soil explored by the plant, and the total surface of exchange between roots and the soil solution (Nacry, Bouguyon & Gojon, 2013). Root system architecture is highly plastic, strongly modulated by N availability. A change in the root system can greatly impact nutrient acquisition from soil. In the present study, N form has a dramatic impact on the root system architecture of moso bamboo seedlings. Accordingly, the overall plant height, leaf number and biomass could be improved with the greater root length, root surface area and root volume in the bamboo seedlings treated with NH$_4^+$ (Figs. 1 and 2), due to positive correlations between most parameters of root morphology and aboveground indices (Table 5).

**Moso bamboo suffers from the toxicity due to excessive NH$_4^+$**

Although NH$_4^+$ is a preferred N source, excessive NH$_4^+$ such as 32 mmol/L (treatments 3 and 5), suppress aboveground growth, biomass accumulation and root system, while the total N content is increased by 35.77% compared to treatments with 8 mmol/L NH$_4^+$ (treatments 1 and 7) (Table 2), suggesting that the *P. edulis* seedlings suffer from the toxicity due to excessive NH$_4^+$.

The uptake of NH$_4^+$ and NO$_3^-$ by the root system is determined by different affinities. In the low concentration range, the uptake is mediated by high-affinity transport systems (HATS), while under high concentrations (typically >0.5–1.0 mmol/L), the activity of low affinity transport systems (LATS) takes over from HATS (Nacry, Bouguyon & Gojon, 2013). Unlike HATS, the NH$_4^+$ uptake mediated by the LATS is not saturated and poorly regulated. It generally shows a linear increase with the increase of the external concentration, when NH$_4^+$ absorbed by roots far exceeds the amount of assimilation, it will cause excessive levels of NH$_4^+$ and thus plant NH$_4^+$ toxicity (Givan, 1979; Glass et al., 2002; Nacry, Bouguyon & Gojon, 2013). On the other hand, with the high similarity in the charge, size and hydration energy between NH$_4^+$ and K$^+$, the K$^+$ ion transporters and channels do not discriminate between NH$_4^+$ and K$^+$. The NH$_4^+$ can be transported through K$^+$ transporters and channels as well as nonselective cation channels (NSCC), which may also contribute to NH$_4^+$ toxicity, especially at low K$^+$ levels (Ten Hoopen et al., 2010). Although moso bamboo showed growth suppression with the high NH$_4^+$ treatments, there was no difference in the survive rate compared to the 8 mmol/L NH$_4^+$ treatments (Table 2), indicating moso bamboo seedlings were tolerant to high concentrations of ammonium.
Balkos, Britto & Kronzucker (2010) and Li et al. (2012) have underscored the central importance of NH$_4^+$/K$^+$ ratios to determine NH$_4^+$ toxicity and tolerance in plants. However, the alleviation of NH$_4^+$ toxicity by K$^+$ addition is not obvious for *P. edulis* seedlings. Under high NH$_4^+$ conditions, elevated exogenous K$^+$ from 0.5 mmol/L to 1.5 mmol/L (treatments 3 and 5) caused a further inhibition of plant growth and increased NH$_4^+$ accumulation in moso bamboo seedlings. The external K$^+$ may not be high enough to alleviate NH$_4^+$ toxicity, as described previously for *Arabidopsis* treated with 5~20 mmol/L KNO$_3$ (Zou et al., 2012). Alternately, moso bamboo may have evolved mechanisms of vacuolar nitrogen storage and downward transport of nitrogen from aerial parts to roots, allowing the plant to maintain nitrogen homeostasis, by which they may survive exposure to potentially toxic NH$_4^+$ concentrations, similar to the findings from other studies (Britto et al., 2001; Kronzucker et al., 2001; Britto & Kronzucker, 2002). Further studies need to be conducted to determine whether there are other mechanisms and the exact mode of actions of K$^+$ and NH$_4^+$ relevant to the toxicity and tolerance.

**Growth traits of moso bamboo are not improved by elevated K$^+$**

A previous study indicated that K$^+$ is the major osmotically active cation contributing to the maintenance of root cell turgor and expansion, therefore plant roots are usually poorly developed in the absence of K$^+$ (Dolan & Davies, 2004; Tsay et al., 2011). However, in the present study, K$^+$ did not seem to play an important role in the root development, as most of the measured root growth traits are not improved by the increased K$^+$ or are even better without K$^+$ (Fig. 2). Gao (2010) has also reported that there was a negative linear correlation between the available potassium storage in the soil layer and the average height of *P. edulis*, yet there was no obvious correlation between the storage amount of available potassium and the diameter of *P. edulis*. When K$^+$ concentration was elevated from 0 to 3 mmol/L, the N content of *P. edulis* reduced by 104.44% from treatments 1 to 7 when treated with 8 mmol/L NH$_4^+$ (Table 2). Similar findings have also been observed in barley and rice, where NH$_4^+$ absorption of barley roots decreases by 60% when external K$^+$ increases from 0.1 mmol/L to 1.5 mmol/L (Szczerba et al., 2007; Balkos, Britto & Kronzucker, 2010). Therefore, we speculate that the inhibition of root growth due to the elevated exogenous K$^+$ may be related to the reduced NH$_4^+$ uptake in moso bamboo.

In contrast to the competitive uptake between NH$_4^+$ and K$^+$, the acquisition of K$^+$ and NO$_3^-$ is usually cooperative (Tsay et al., 2011; Coskun, Britto & Kronzucker, 2017). The Dijkshoorn-Benzioni hypothesis has provided an intriguing example of the cooperative action of K$^+$ and NO$_3^-$ in plants, namely, that NO$_3^-$ is transported from roots to shoots in the xylem, using K$^+$ as a counter ion (Benzioni, Vaadia & Lips, 1971; Coskun, Britto & Kronzucker, 2014; Dijkshoorn, 1958). It may be due to the charge balance of K$^+$ and NO$_3^-$, which could be substituted by other cations, in particular Mg$^{2+}$ (Hagin, Olsen & Shaviv, 1990; Coskun, Britto & Kronzucker, 2014), low supply of K$^+$ can activate NO$_3^-$ assimilation, reinforcing the NO$_3^-$ reduction in roots (Balkos, Britto & Kronzucker, 2010; Zhang et al., 2010). In this study, increasing K$^+$ from 0.5 mmol/L to 1.5 mmol/L (treatments 4 and 6) with 8 mmol/L NO$_3^-$ treatment enhances the N content of moso bamboo seedlings by 53.85% (Table 2) in spite of a slightly growth inhibition of NH$_4^+$-preferring moso bamboo.
(Table 2), which might be related to the inhibited nitrate reductase activity due to the high endogenous NO$_3^-$ concentrations (Reddy & Menary, 1990). However, further studies are needed to determine the role of K in the response of moso bamboo to different N forms and the molecular physiological mechanisms of the low potassium requirements.

CONCLUSIONS

N form and concentration play a significant role in the growth and N content of moso bamboo seedlings, while K$^+$ concentration has a limited effect. The results from our experiments demonstrate improved growth of moso bamboo seedlings when treated with 8 mmol/L NH$_4^+$. Under elevated N concentrations, overall growth is inhibited though N content of the seedlings increases. In addition, root growth is inhibited with increasing K$^+$ concentrations. Although moso bamboo seedlings display some level of NH$_4^+$-tolerance, high concentrations of NH$_4^+$ can inhibit their growth, and increasing K$^+$ concentration in the nutrient solution does not relieve the inhibitory effect of high NH$_4^+$ (32 mmol/L). Therefore, it is recommended that moso bamboo seedlings should be fed 8 mmol/L NH$_4^+$ fertilizer with a moderate K$^+$ concentration.

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ADDITIONAL INFORMATION AND DECLARATIONS

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Competing Interests

Tianchi Wang is employed by The New Zealand Institute for Plant & Food Research Limited, Auckland, New Zealand. The authors declare there are no competing interests.
Author Contributions

- Na Zou conceived and designed the experiments, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
- Ling Huang and Huijing Chen performed the experiments, prepared figures and/or tables, and approved the final draft.
- Xiaofeng Huang analyzed the data, prepared figures and/or tables, and approved the final draft.
- Qingni Song analyzed the data, authored or reviewed drafts of the paper, and approved the final draft.
- Qingpei Yang conceived and designed the experiments, authored or reviewed drafts of the paper, and approved the final draft.
- Tianchi Wang performed the experiments, authored or reviewed drafts of the paper, and approved the final draft.

Data Availability

The following information was supplied regarding data availability:

The raw measurements are available in the Supplementary Files.

Supplemental Information

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REFERENCES

Balkos KD, Britto DT, Kronzucker HJ. 2010. Optimization of ammonium acquisition and metabolism by potassium in rice (Oryza sativa L. cv. IR-72). Plant Cell and Environment 33:23–34.

Banik RL. 1980. Propagation of bamboos by clonal methods and by seed. In: Lessard G, Chouinard A, eds. Bamboo research in Asia. Proceedings of a workshop, Singapore, 28–30 May: IDRC, Ottawa, Canada, 139–150.

Benzioni A, Vaadia Y, Lips SH. 1971. Nitrate uptake by roots as regulated by nitrate reduction products of the shoot. Physiologia Plantarum 24:288–290 DOI 10.1111/j.1399-3054.1971.tb03493.x.

Bojović B, Marković A. 2009. Correlation between nitrogen and chlorophyll content in wheat (Triticum aestivum L.). Kragujevac Journal of Science 31:69–74.

Britto DT, Kronzucker HJ. 2002. NH₄⁺ toxicity in higher plants: a critical review. Journal of Plant Physiology 159(6):567–584 DOI 10.1078/0176-1617-0774.

Britto DT, Kronzucker HJ. 2006. Plant nitrogen transport and its regulation in changing soil environments. Journal of Crop Improvement 15(2):1–23.

Britto DT, Kronzucker HJ. 2013. Ecological significance and complexity of N-source preference in plants. Annals of Botany 112(6):957–963 DOI 10.1093/aob/mct157.

Britto DT, Siddiqi MY, Glass ADM, Kronzucker HJ. 2001. Futile transmembrane NH₄⁺ cycling: a cellular hypothesis to explain ammonium toxicity in plants. Proceedings of the National Academy of Sciences 98(7):4255–4258 DOI 10.1073/pnas.061034698.
Cai C, Peng Z, Gao J, Wang H, Liu F. 2008. Seed germination characteristics of Phyllostachys edulis. Chinese Agricultural Science Bulletin 24(12):163–167.

Chen E. 2019. A study of techniques for moso bamboo seedling propagation and their afforestation effect. World Bamboo and Rattan 17(02):36–39 (In Chinese).

Chen G, Guo S, Kronzucker HJ, Shi W. 2013. Nitrogen use efficiency (NUE) in rice links to NH$_4^+$ toxicity and futile NH$_4^+$ cycling in roots. Plant and Soil 369:351–363 DOI 10.1007/s11104-012-1575-y.

Chen TH, Wang DH, Wang S. 2016. The trend of growth characteristics of Moso bamboo (Phyllostachys pubescens) forests under an unmanaged condition in central Taiwan. Taiwan Journal of Forest Science 31(2):75–88.

Christanty L, Kimins JP, Mailly D. 1997. ‘Without bamboo, the land dies’: a conceptual model of the biogeochemical role of bamboo in an Indonesian agroforestry system. Forest Ecology and Management 91(1):83–91 DOI 10.1016/S0378-1127(96)03881-9.

Coskun D, Britto DT, Kronzucker HJ. 2014. The physiology of channel-mediated K$^+$ acquisition in roots of higher plants. Physiologia Plantarum 151(3):305–312 DOI 10.1111/ppl.12174.

Coskun D, Britto DT, Kronzucker HJ. 2017. The nitrogen-potassium intersection: membranes, metabolism, and mechanism. Plant Cell and Environment 40(10):2029–2041 DOI 10.1111/pce.12671.

Dijkshoorn W. 1958. Nitrate accumulation, nitrogen balance and cation-anion ratio during the regrowth of perennial ryegrass. Netherlands Journal of Agricultural Science 6:211–221.

Dolan L, Davies J. 2004. Cell expansion in roots. Current Opinion in Plant Biology 7(1):33–39 DOI 10.1016/j.pbi.2003.11.006.

Fu J. 2000. Chinese moso bamboo: its importance. Bamboo 22(5):5–7.

Gao Z. 2010. Study on the soil nutrients status of phosphorus and potassium in stands of Phyllostachys pubescens and its growth efficiency. Journal of Nanjing Forestry University (Natural Science Edition) 34(6):33–37 (In Chinese).

Givan CV. 1979. Metabolic detoxification of ammonia in tissues of higher plants. Phytochemistry 18(3):375–382 DOI 10.1016/S0031-9422(00)81870-1.

Glass ADM, Britto DT, Kaiser BN, Kinghorn JR, Kronzucker HJ, Kumar A, Okamoto M, Rawat S, Siddiqi MY, Unkles SE, Vidmar JJ. 2002. The regulation of nitrate and ammonium transport systems in plants. Journal of Experimental Botany 53:855–864 DOI 10.1093/jexbot/53.370.855.

Gu H, Deng G, Hunag L, Yang H, Yang Q, Zou N. 2016. Growth response of bamboo seedlings to nitrogen nutrition in Phyllostachys heterocyla cv. Pubescens. Journal of Bamboo Research 35:44–49.

Gutiérrez-Miceli FA, García-Gómez RC, Rosales RR, Abud-Archila M, Angela OL, Cruz MJ, Dendooven L. 2008. Formulation of a liquid fertilizer for sorghum (Sorghum bicolor (L.) Moench) using vermicompost leachate. Bioresource Technology 99(14):6174–6180 DOI 10.1016/j.biortech.2007.12.043.
Hagin J, Olsen SR, Shaviv A. 1990. Review of interaction of ammonium-nitrate and potassium nutrition of crops. Journal of Plant Nutrition 13(10):1211–1226 DOI 10.1080/01904169009364147.

Hou. 2020. Mechanism analysis the growth response of Phyllostachys edulis seedling to different N forms. Master Thesis, Nanchang: Jiangxi Agricultural University, 1–65 (In Chinese).

Kong LA, Sun MZ, Wang FH, Liu J, Feng B, Si JS, Zhang B, Li SD, Li HW. 2014. Effects of high NH$_4^+$ on K$^+$ uptake, culm mechanical strength and grain filling in wheat. Frontiers in Plant Science 5:703 DOI 10.3389/fpls.2014.00703.

Kronzucker HJ, Britto DT, Davenport RJ, Tester M. 2001. Ammonium toxicity and the real cost of transport. Trends in Plant Science 6:335–337 DOI 10.1016/S1360-1385(01)02022-2.

Kronzucker HJ, Siddiqi MY, Glass ADM. 1997. Conifer root discrimination against soil nitrate and the ecology of forest succession. Nature 385:59–61 DOI 10.1038/385059a0.

Li B, Li G, Kronzucker HJ, Baluška F, Shi W. 2014a. Ammonium stress in Arabidopsis: signaling, genetic loci, and physiological targets. Trends in Plant Science 19(2):107–114.

Li G, Dong G, Li B, Li Q, Kronzucker HJ, Shi W. 2012. Isolation and characterization of a novel ammonium overly sensitive mutant, amos2, in Arabidopsis thaliana. Planta 235(2):239–252 DOI 10.1007/s00425-011-1504-y.

Li G, Hu X, Mou M, Xia G, Sheng W. 2014b. Effects of different nitrogen forms and ratios on the growth and nutrients absorption of bamboo seedlings. Journal of Fujian Agriculture and Forestry University (Natural Science Edition) 43(02):151–155.

Li S, Wang Z, Stewart BA. 2013. Responses of crop plants to ammonium and nitrate N. Advances in Agronomy 118:205–397 DOI 10.1016/B978-0-12-405942-9.00005-0.

Li Z, Zhang L, Deng B, Liu Y, Kong F, Huang G, Zou Q, Liu Q, Guo X, Fu Y, Niu D, Siemann E. 2017. Effects of moso bamboo (Phyllostachys edulis) invasions on soil nitrogen cycles depend on invasion stage and warming. Environmental Science and Pollution Research 24:24989–24999 DOI 10.1007/s11356-017-0186-9.

Lin C. 1987. A statistical analysis tool for variation simulation modeling. Annual Conference on Computers 13(1):386–391.

Nacry P, Bouguyon E, Gojon A. 2013. Nitrogen acquisition by roots: physiological and developmental mechanisms ensuring plant adaptation to a fluctuating resource. Plant and Soil 370:1–29 DOI 10.1007/s11104-013-1645-9.

Nakashima A. 2001. The recent situation of the bamboo forests management in the suburbs: a case study of the bamboo shoot producing districts in the suburbs of Kyoto City. Applied Forest Science 10:1–7.

Norisada M, Kojima K. 2005. Nitrogen form preference of six dipterocarp species. Forest Ecology and Management 216(1–3):175–186 DOI 10.1016/j.foreco.2005.05.020.

Qiao G, Li H, Liu M, Jiang J, Yin Y, Zhang L, Zhuo R. 2013. Callus induction and plant regeneration from anthers of Dendrocalamus latiflorus Munro. In Vitro Cellular and Developmental Biology Plant 49(4):375–382 DOI 10.1007/s11627-013-9498-8.
Qin Z. 2009. The technology of moso bamboo seedling nursery and afforestation. *Journal of Agricultural Technology Services* 26(9):98–99, 125. (In Chinese).

Rao AN, Rao RV, Williams JT (eds.) 1998. *Priority species of bamboo and rattan*. Beijing: Serdang, Malaysia, IPGRI-APO, Serdang, Malaysia and INBAR.

Reddy KS, Menary RC. 1990. Nitrate reductase and nitrate accumulation in relation to nitrate toxicity in *Boronia megastigma*. *Physiologia Plantarum* 78(3):430–434. DOI 10.1111/j.1399-3054.1990.tb09059.x.

Roosta HR, Schjoerring JK. 2008. Effects of nitrate and potassium on ammonium toxicity in cucumber plants. *Journal of Plant Nutrition* 31(7):1270–1283. DOI 10.1080/01904160802135050.

Song Q, Ouyang M, Yang Q, Lu H, Yang G, Chen F, Shi J. 2016a. Degradation of litter quality and decline of soil nitrogen mineralization after moso bamboo (*Phyllostachys pubscens*) expansion to neighboring broadleaved forest in subtropical China. *Plant and Soil* 404(1–2):113–124. DOI 10.1007/s11104-016-2835-z.

Song Q, Yang Q, Liu J, Yu D, Fang K, Xu P, He Y, Ni S, Pei Y, Jun L. 2013. Effects of *Phyllostachys edulis* expansion on soil nitrogen mineralization and its availability in evergreen broadleaf forest. *Chinese Journal of Applied Ecology* 24(2):338–344.

Song X, Gu H, Wang M, Zhou G, Li Q. 2016b. Management practices regulate the response of Moso bamboo foliar stoichiometry to N deposition. *Scientific Reports* 6:24107. DOI 10.1038/srep24107.

Szczerba MW, Britto DT, Balkos KD, Kronzucker HJ. 2007. Alleviation of rapid, futile ammonium cycling at the plasma membrane by potassium reveals K+-sensitive and -insensitive components of NH$_4^+$ transport. *Journal of Experimental Botany* 59:303–313.

Ten Hooopen F, Cuin TA, Pedas P, Hegelund JN, Shabala S, Schjoerring JK, Jahn TP. 2010. Competition between uptake of ammonium and potassium in barley and Arabidopsis roots: molecular mechanisms and physiological consequences. *Journal of Experimental Botany* 61(9):2303–2315. DOI 10.1093/jxb/erq057.

Tsay Y, Ho C, Chen H, Lin S. 2011. Integration of nitrogen and potassium signaling. *Annual Review of Plant Biology* 62(1):207–226. DOI 10.1146/annurev-arplant-042811-103837.

Tucker M. 2004. Primary nutrients and plant growth. In: Scribd, ed. *Essential plant nutrients*. Raleigh: North Carolina Department of Agriculture.

Ueda K. 1960. Studies on the physiology of bamboo, with reference to practical application. *Bulletin of the Kyoto University Forests* 30:1–169.

Wang MY, Siddiqi MY, Glass ADM. 1996. Interactions between K$^+$ and NH$_4^+$: effects on ion uptake by rice roots. *Plant, Cell and Environment* 19(9):1037–1046. DOI 10.1111/j.1365-3040.1996.tb00210.x.

Wang M, Zheng Q, Shen Q, Guo S. 2013. The critical role of potassium in plant stress response. *International Journal of Molecular Sciences* 14:7370–7390. DOI 10.3390/ijms14047370.

Xu G, Fan X, Miller A. 2012. Plant nitrogen assimilation and use efficiency. *Annual Review of Plant Biology* 63:153–182. DOI 10.1146/annurev-arplant-042811-105532.
Zhang F, Niu J, Zhang W, Chen X, Li C, Yuan L, Xie J. 2010. Potassium nutrition of crops under varied regimes of nitrogen supply. *Plant and Soil* 335:21–34 DOI 10.1007/s11104-010-0323-4.

Zhou Y, Lu G. 2013. Preferred studies on the nutritional solutions of hydroponic ornamental sweet potato. *Chinese Agricultural Science Bulletin* 31:129–136.

Zou N, Li B, Dong G, Kronzucker HJ, Shi W. 2012. Ammonium-induced loss of root gravitropism is related to auxin distribution and TRH1 function, and is uncoupled from the inhibition of root elongation in *Arabidopsis*. *Journal of Experimental Botany* 63(10):3777–3788 DOI 10.1093/jxb/ers068.

Zou N, Shi W, Hou L, Kronzucker HJ, Huang L, Gu H, Yang Q, Deng G, Yang G. 2020. Superior growth, N uptake, and NH$_4^+$ tolerance in the giant bamboo *Phyllostachys edulis* over the broad-leaved tree *Castanopsis fargesii* at elevated NH$_4^+$ may underlie community succession and favor the expansion of bamboo. *Tree Physiology* Epub ahead of print 2020 7 July DOI 10.1093/treephys/tpaa086.