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Higher response of terrestrial plant growth to ammonium than nitrate addition

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Running Title: Different preference of plants for N forms

Type of paper: Primary Research Article

Key Points:

- The deposition rates of nitrate and ammonium are spatially different on the globe.
- Terrestrial plant growth is more sensitive to ammonium than nitrate addition.
- Experimental findings with NH_4NO_3 or urea addition should be scaled up with caution.

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Abstract

Terrestrial plant growth and ecosystem productivity are strongly limited by availability of nitrogen (N). Atmospheric deposition of wet N as nitrate and ammonium has been rapidly increased since the industrial revolution, associated with a high spatial variation of changes in the ammonium- to nitrate-N ratio (i.e., NH_4^+-N/NO_3^--N). However, whether and how terrestrial plants respond differently to NH_4^+-N and NO_3^--N addition have never been quantitatively synthesized. Here, we first did a literature survey and analysis on the model projections of future changes in NH_4^+-N/NO_3^--N in atmospheric N deposition. Most models predicted an increase in the global average of NH_4^+-N/NO_3^--N ratio, but decreasing trends in western Europe and eastern China. Then, a meta-analysis was applied to compare the different growth responses of 402 plant species to NH_4^+-N and NO_3^--N addition from 217 N fertilization studies. In general, a greater response of plant growth to NH_4^+- N (+6.3% g^{-1} N) than NO_3^--N (+1.0% g^{-1} N) addition was detected across all species. The larger sensitivity of plant growth to NH_4^+- than NO_3^--N was found in all plant functional types except for grasses. In addition, the NO_3^--N addition promoted terrestrial plants to allocate more biomass to above-ground, whereas NH_4^+-N addition significantly enhanced below- but not above-ground growth. These results imply that the global accelerating N deposition could stimulate plant growth more in regions with increasing (e.g., North America) than decreasing (e.g., eastern China) NH_4^+-N/NO_3^--N ratio. The findings suggest future assessments and predictions on the vegetation response to atmospheric N enrichment could benefit from a better understanding of plant strategies for acquiring different forms of N.

Key words: ammonium, biomass, inorganic N, nitrate, nitrogen deposition, plant growth

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1. Introduction

Nitrogen (N) is the most abundant element in the atmosphere and an essential component for organisms on land and in the sea (Vitousek & Howarth, 1991). The deposition rate of atmospheric N to land has dramatically increased by about three folds since the industrial revolution, and is expected to be even faster in the future (Galloway et al., 2008; Kanakidou et al., 2016). The loading of N in wet deposition mainly has two different forms, including ammonium and nitrate. Recent observations have shown large spatial differences in the shifting trend of the composition in N deposition (i.e., ammonium vs. nitrate). For example, N deposition is shifting from nitrate- to ammonium-dominated in the United States (Du et al., 2014; Li et al., 2016), but a contrasting change is observed in China (Liu et al., 2013, 2016). The high N deposition rate has been acknowledged to enhance plant growth and primary productivity in most terrestrial ecosystems (LeBauer & Treseder, 2008; Xia & Wan, 2008; Fernandezmartinez et al., 2014). The N-enhanced plant growth has become critical in sustaining the major functions of terrestrial ecosystems, such as fueling all life on land (Zhu et al., 2002; Smil, 2004) and absorbing more atmospheric CO_2 to mitigate climate change (Schimel, 1995; De Vries et al., 2014; Maaroufi et al., 2015). Thus, the functions of terrestrial ecosystem is largely affected by both the changes in composition of the plant-available N and the different effects between ammonium and nitrate addition on plant growth. However, neither of these two impacts has so far been evaluated.

Anthropogenic activities are altering both total N loads and the dominant form of N deposition. Also, global fertilizer use has generally shifted from oxidized to reduced form of N, with urea use now >50% of global N fertilizer, surpassing nitrate as the most common N fertilizer worldwide (Glibert et al., 2006). In addition, the availability of ammonium (NH_4^+-N) and nitrate (NO_3^--N) in the soil could be differently affect by the changes of global environmental factors. For example, field experiments with adding N usually increase the concentration of soil NH_4^+-N less than NO_3^--N (Lu et al., 2011), whereas CO_2 enrichment stimulates soil NH_4^+-N to NO_3^--N ratio (NH_4^+-N/NO_3^--N) but not NH_4^+-N or NO_3^--N concentration (Liang et al., 2016). Thus, a survey on the future global changes of the NH_4^+-N/NO_3^--N ratio in the projected atmospheric N deposition as well as the impacts of N addition on soil NH_4^+-N/NO_3^--N are necessary.

Although plants utilize other forms of N such as nitrite and amino acids as well, they mainly uptake ammonium and nitrate under natural conditions (Olsson & Falkengren-Grerup,

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2000). Ammonium is mainly originated from agriculture (including human and animal excrement, and fertilizer volatilization), while nitrate mainly stems from fossil-fuel combustion by power plants and automobiles (Hosker & Lindberg, 1982). Although NH_4^+-N and NO_3^--N are considered equivalent in most N fertilization experiments (Stevens et al., 2004; Manning et al., 2006), a number of recent studies have reported differential N preference among plant species (ven den Berg et al., 2016; Glibert et al., 2016; Tho et al., 2017). For example, plant species that grows in calcareous or slightly acidic soils favor nitrate or a combination of nitrate and ammonium, whereas plants in acidic habitats prefer to uptake ammonium (Gigon & Rorison, 1972; De Graaf et al., 1998; Falkengren-Grerup & Schottelndreier, 2004; Sheppard et al., 2014). Plant species of different functional types vary in N use strategy and thus respond differently to N addition (Xia & Wan, 2008). However, it is unclear whether the preference of plant growth for N form also varies among plant function types. For some plant species, the addition of NH_4^+-N appears to be toxic for plant growth (ven den Berg et al., 2005; De Schrijver et al., 2008), while other studies have emphasized that the toxic effect might be limited to certain plant functional groups (e.g. bryophyte; Paulissen et al., 2005; Verhoeven et al., 2011) or depends on soil properties (ven den berg et al., 2005; Li et al., 2014). During the past a few decades, a tremendous amount of manipulative studies have conducted to study the response of plant species to N addition (Piwpuan et al., 2013; Maaroufi et al., 2015; ven Den Berg et al., 2016). Most of these studies have reported the form of added N and the functional type of plant species. These studies enable a quantitative synthesis on the different impacts between ammonium and nitrate additions on plant growth.

In this study, we applied a weighted meta-analysis of observed plant response to N addition from 217 manipulative experiments. The effect of different N forms (nitrate, ammonium, NH_4NO_3 and urea) on plant growth was calculated across the globe and compared among plant functional types. This major question of this study is whether and how the effects of nitrate on plant growth is different from that of ammonium. Because many ecological studies are using NH_4NO_3 or urea as the fertilizer, we also aim to compare their effects on plant growth with the nitrate and ammonium additions.

2. Materials and Methods

2.1. Data collection

2.1.1. Response of plant growth to N addition

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We searched ISI Web of Science, PubMed, Google Scholar, and JSTOR with the terms of "nitrogen fertilization (or N addition or N deposition)" and "plant biomass (or plant growth)". Then, papers meeting the following criteria were selected to do the further analysis:

(i) The study included both control and N treatments. If N was added together with other treatments (e.g. CO_2 enrichment), we took the effect of additional treatment (e.g. CO_2 enrichment) as the control, and their combined effect (e.g. CO_2 enrichment plus N addition) as the N treatment;
(ii) The biomass responses to N addition was reported at the species level. Means, sample sizes, and standard deviations under both the control and N addition treatments were provided;
(iii) The N forms, including nitrate (NO_3^--N), ammonium (NH_4^+-N), ammonium nitrate (NH_4NO_3) and urea, were clarified in the study, and also the N dose was measured as N per unit area (g N m^{-2});
(iv) Crop species were excluded from our analysis.

Overall, there were totally 198 papers, 402 species and 2709 data included in the data set 1 (Supporting information Notes S1). The data of means and variations in both control and N addition treatments were collected directly from original tables or extracted from the figure using SigmaScan (Systat Software Inc., San Jose, CA, USA). The global distribution of study sites was showed in the Figure 1. A list of the species and their original studies could be found in the supplementary Table S1.

2.1.2. Response of soil nitrogen availability to N addition

To evaluate the responses of soil NH_4^+-N/NO_3^--N ratio under future enhanced N addition, we collected updated the dataset of Lu et al. (2011) on the ISI Web of Science. We only used the results from 29 papers which reported the effect of N fertilization on soil NH_4^+-N or NO_3^--N pool. Only those studies with NH_4NO_3 and urea addition were included, because these two fertilizers are mostly widely used (Supporting information Notes S2). Meta-analyses were used to estimate the effects of N addition on soil NH_4^+-N, NO_3^--N and the ratio of NH_4^+-N/NO_3^--N. Other factors, e.g. the treatment of application, interactive climate variables, or the species information were not considered in this analysis.

2.1.3. Survey on historical and projected changes in atmospheric N deposition

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To estimate the trend of atmospheric N deposition, we did a literature survey on the modelled patterns of future N deposition (i.e., total deposited N, NH_x, NO_y, and the ratio of NH_x/NO_y). The projected trends of N deposition from different literatures were presented in the Table 1. The trend of global gridded (0.5°×0.5°) NH_x/NO_y over 2010-2100 were analyzed based on the environmental driver data sets for the Multi-scale Synthesis and Terrestrial Model Intercomparison Project (MsTMIP) project (Huntzinger et al., 2013; Wei et al., 2015) (https://daac.ornl.gov/cgi-bin/dsviewer.pl?ds_id=1220). The gridded time-varying N deposition was derived from the map in Dentener (2006), which provided the maps of global N deposition in 1860, 1993, and 2050. The ratio of NH_x/NO_y was calculated at each 0.5°×0.5° grid in each year from 2010 to 2100. The trend of NH_x/NO_y in each grid during 2010-2100 was extracted from the linear regression analysis.

2.2. Statistical analyses

2.2.1. Soil ammonium to nitrate ratio (NH_4^+-N/ NO_3^--N)

When both soil ammonium (NH_4^+-N) and nitrate (NO_3^--N) concentration with the same unit were provided in one study, the soil NH_4^+/NO_3^- ratio were calculated as below:

$$X = \frac{X_{NH_4^+}}{X_{NO_3^-}} \qquad (1)$$

where $X_{NH_4^+}$ and $X_{NO_3^-}$ are means of the soil and ammonium and nitrate concentration. Its standard deviation was estimated by:

$$S_c = \sqrt{\left(\frac{S_{NH_4^+}}{X_{NH_4^+}}\right)^2 + \left(\frac{S_{NO_3^-}}{X_{NO_3^-}}\right)^2} \qquad (2)$$

where $S_{NH_4^+}$ and $S_{NO_3^-}$ are the SD of soil nitrate and ammonium concentration, respectively.

2.2.2. The relative response of plant growth to N addition

The meta-analysis followed the techniques described in Hedges et al., (1999), Wan et al., (2001) and Xia & Wan (2008). In this study, N was added in various amounts (N_{amount}; g N m^{-2}) among studies, so we first normalized the plant growth and its variation of the N treatment as:

$$X_e' = X_c + \frac{X_e - X_c}{N_{amount}} \qquad (3)$$

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Biogeosciences Discussions

$$S_e' = S_c + \frac{S_e - S_c}{N_{amount}} \quad (4)$$

where X_c (or S_c) and X_e (or S_e) are the means (or standard deviation) of the biomass response in the control and N addition treatments, respectively. X_e' and S_e' represent the mean and standard deviation of plant growth under the treatment of per unit amount (g N m^{-2}) of N addition. Then, the relative response (*RR*) of plant biomass to per unit amount of N addition was calculated as the log-transformed ratio:

$$RR = \ln\left(\frac{X_e'}{X_c}\right) \quad (5)$$

with the variance as:

$$v_{RR} = \frac{(S_c)^2}{n_e(X_c)^2} + \frac{(S_e^{'})^2}{n_e(X_e^{'})^2} \quad (6)$$

where n_c and n_e represent the sample size for control and treatment, respectively. The reciprocal of its variance ($w = \frac{1}{v_{RR}}$) was considered as the weight of each RR. Then the mean response ratio (RR_{++}) and its standard error were calculated as:

$$RR_{++} = \frac{\sum_{i=1}^{m}\sum_{j=1}^{k_i} w_{ij}RR_{ij}}{\sum_{i=1}^{m}\sum_{j=1}^{k_i} w_{ij}} \quad (7)$$

$$S(RR_{++}) = \frac{1}{\sum_{i=1}^{m}\sum_{j=1}^{k_i} w_{ij}} \quad (8)$$

where *m* is the number of groups, and k_i is the number of comparisons in the ith group. The 95% confidence interval (95% CI) was calculated as $RR_{++} \pm 1.96\, S(RR_{++})$ by bootstrapping the data using Metawin 2.0. The percentage changes were presented in the figures as back transformed from the log response ratio (i.e., $[\exp(RR_{++}) - 1] \times 100\%$).

The effects of nitrogen addition on plant growth were evaluated as significant, if the 95% CI overlap zero. This meta-analysis also followed the theory of heterogeneity described by Gurevitch & Hedges (1993), in which total heterogeneity (Q_T) is divided into within-group (Q_w) and between-group (Q_b). If Q_b is larger than a critical value, there would be significant difference between categories. Statistical significance was tested at the $P < 0.05$ level.

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For meta-analysis, some researchers have advocated the inclusion of only one result from each study because the assumption of independence (Vanderwerf, 1992). However, the omission of multiple results in each study would cause the loss of information, which may be a more serious problem than violation the assumption of independence (Gurevitch & Hedges, 1993). Thus, we included more than one results from a single study in this meta-analysis, whose reliability (or feasibility) had been tested by previous meta-analyses (Maestre et al., 2005). Actually, we also compared the results with using all data and with using one data from each study, and found these patterns were unchanged.

3. Results

3.1. Changes of N forms in the atmosphere and soil

According to the literature survey, although the global estimate varies largely among models, the atmospheric N deposition has substantially increased since the 1850s (Table 1). The total N deposition has increased by a factor of about 3 at present (135±5 Tg N yr^{-1}) since 1850 (51±1.8 Tg N yr^{-1}). This accelerated N deposition is mainly owing to the large increase of reduced (NH_x) and oxidised (NO_y) N (Table 1, modelled by TM4-ECPL). The global total N deposition is not expected to change much in the nearly future (2050, 138±6 Tg N yr^{-1}). However, the ratio of reduced to oxidised N (i.e., NH_x/NO_y) modelled by TM4-ECPL would be increased up to 2.0 at 2050, which is almost doubled from 2005. The fast increase in global NH_x/NO_y in the future was consistent with that derived from the MsTMIP environmental data. However, the trend of NH_x/NO_y was decreasing in some regions, such as the southeastern China and western Europe (Fig. 2).

In the manipulative experiments, the ratio of NH_4^+-N/ NO_3^--N in the soil was differently influenced by N deposition (Fig. 3). Manipulative N addition significantly decreased the ratio of NH_4^+/NO_3^- by stimulating NO_3^--N (+356.0%) more than NH_4^+-N (+60.0%; Fig. 3a).

3.2. Responses of plant growth to additive N forms vary with plant functional types

Across all the plant species, plant growth was more increased by the addition of NH_4^+-N (6.3% g^{-1} N) than NO_3^--N (1.0% g^{-1} N) (Q_b =36.8, P < 0.001; Fig. 4a, Table 2). This higher positive effect of NH_4^+-N than NO_3^--N was found in all plant functional groups except for grasses, which oppositely respond less to NH_4^+-N (6.9% g^{-1} N) than NO_3^--N (11.1% g^{-1} N) addition (Q_b =3.5,

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P =0.06; Table 2). The positive effects of NH_4^+-N addition were comparable between woody (5.6% g^{-1} N) and herbaceous (6.9% g^{-1} N) species (Q_b =1.7, *P* >0.1; Table 3), but a smaller response of herbaceous (1.6% g^{-1} N) and woody species (0.6% g^{-1} N) (Q_b =0.6, *P* >0.1; Table 3) was detected to NO_3^--N addition. The positive effects of NH_4^+-N addition among different functional types had no significant difference, with the rank of grasses (6.9% g^{-1} N), forbs (6.9% g^{-1} N), trees (6.5% g^{-1} N) and shrubs (3.2% g^{-1} N) (Q_b =8.2, *P* =0. 08; Table 3). However, grasses (11.1% g^{-1} N) respond more than shrubs (1.9% g^{-1} N), trees (1.6% g^{-1} N) and forbs (-0.8% g^{-1} N) to NO_3^--N addition (Q_b =26.0, *P* <0. 001; Table 3).

Across all species, the response of plant growth to NH_4NO_3 addition (5.5% g^{-1} N) was comparable to NH_4^+-N addition (Fig. 4b). No significant difference was detected between NH_4NO_3 and NH_4^+-N effect for each plant functional type, except trees (*P* <0.05). Urea addition showed greater positive effects across all species (11.0% g^{-1} N) than both NH_4^+-N and NO_3^--N addition. Its positive impacts were the largest in all plant functional types (e.g., 9.0% g^{-1} N and 15.4% g^{-1} N in woody and herbaceous species, respectively) (Table 2).

3.3. Responses of plant growth to NH_4^+- and NO_3^--N addition vary with plant tissues

NO_3^--N addition significantly stimulated the growth of above-ground (8.4% g^{-1} N), whereas NH_4^+-N addition showed greater effect on the below-ground growth (5.9% g^{-1} N; Fig. 5a, Table 2). NH_4^+-N addition significantly increased below-ground (7.3% g^{-1} N and 9.4% g^{-1} N) but not above-ground growth of both woody and herbaceous species. In contrast, NO_3^--N addition significantly increased the above-ground (4.0% g^{-1} N and 12.1% g^{-1} N) but not below-ground growth of both woody and herbaceous species (Fig. 5b, c).

4. Discussions

4.1. Future trends of global atmospheric N deposition and soil N availability

Once the anthropogenic reactive N compounds are emitted into the atmosphere, they are deposited to the biosphere quickly (Galloway et al., 2008). The major compounds of reactive N are NH_x and NO_y. The survey of modeling projections in this study shows an increasing NH_x/NO_y ratio globally in the coming decades (Table 1). This trend suggests that the impacts of future N deposition on terrestrial plant growth could be more dominated by NH_4^+-N than NO_3^--N. However, it should be noted that the projections in Table 1 are highly uncertain,

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because model simulations in different projects are usually driven by different purposes and assumptions. Also, because both NH_x and NO_y are short-lived gases, and thus a global average cannot reflect the large spatial variation of the trends in the NH_4^+/NO_3^- ratio (Fig. 2). For example, the wet N deposition across the US has been dominated by NO_3^--N in the 1980s but has recently been shifted to be dominated by NH_4^+-N (Li et al., 2016). In China, however, both the ratios of NH_x/NO_y and NH_4^+/NO_3^- have been significantly reduced since 1980s (Liu et al., 2013, 2016).

Because NH_4^+-N could be transformed into NO_3^--N via nitrification, so it remains unclear that how the change of NH_4^+-N/NO_3^--N in the atmospheric N deposition will affect the soil N availability to plants. It has been demonstrated that the increasing atmospheric N deposition not only stimulates the availability of mineral N, but also leads to a shifted NH_4^+-N/NO_3^--N ratio in the soil (De Graaf et al., 1998; Seinfeld & Pandis, 2016). For example, a synchronous change of NH_4^+-N/NO_3^--N ratio and pH in throughfall and soil has been observed in a 22-year measurement in Netherland (Boxman et al., 2008). These results suggest that terrestrial plants will experience a different soil NH_4^+-N/NO_3^--N ratio because of the changing N deposition pattern (Seinfeld and Pandis, 2016). As shown in this study, the soil NH_4^+-N/NO_3^--N ratio tends to reduce under experimental N addition. Thus, the different responses of plant growth to NH_4^+-N and NO_3^--N addition is critical for future projection of terrestrial vegetation growth and ecosystem productivity.

4.2. Different responses of plant growth to NH_4^+- and NO_3^--N addition among plant functional types

There has been a long history of research investigating the physiological and ecological consequences of plant species' preference for acquiring NH_4^+-N or NO_3^--N (Lee & Stewart, 1978; Raven et al., 1992; Kronzucker et al., 1997; Sheppard et al., 2014; Tho et al., 2017). In this study, the sensitivity of plant growth to NH_4^+-N addition is about 6-fold higher than that to NO_3^--N addition (Fig. 4). The larger effect of NH_4^+-N than NO_3^--N addition on plant growth is reasonable, because ammonium is more energetically favorable and less energetically costly than nitrate, which has to be reduced to ammonium before assimilation (Guo et al., 2007). For example, ammonium was reported to be superior to nitrate for growth of rice (Qian et al., 2004) or *Cyperus laevigatus* (Piwpuan et al., 2013) and other macrophytes (Konnerup & Brix, 2010; Tho et al., 2017). Thus, the NH_4^+-N uptake by plants often exceeds that of NO_3^--N when N is the limiting nutrient (ven den Berg et al., 2005; De Schrijver et al., 2008). A higher uptake rate of NH_4^+-N means a higher tissue N concentration and then a higher content of proteins in the tissue, which can be reflected in the photosynthetic capacity of the plants (Brück & Guo, 2006; Konnerup & Brix, 2010). Larger stimulation of CO_2 assimilation rate by NH_4^+-N than that of NO_3^--N addition has been found in many studies (Claussen & Lenz, 1999; Terce-Laforgue et al., 2004). Given to the increasing trend of ammonium-dominance in N deposition in most regions (Table 1 and Fig. 2), terrestrial plant growth is likely to be more enhanced by future N enrichment due to its higher sensitivity to NH_4^+-N than NO_3^--N addition.

Although plants acquire NH_4^+-N more efficiently than NO_3^--N in terms of energy cost, the presence of free NH_4^+ in plant cells is very toxic (Britto et al., 2001). For example, ammonium supplied as the sole nitrogen source usually inhibits plant growth compared to nitrate or a mixture of nitrate and ammonium (Gerendás et al., 1997; Guo et al., 2002). A numerous of pot control experiments and field isotope labelling studies have also pointed out that higher NH_4^+ uptake rates may accomplish by potential risk of cell acidification, deficiencies of metal ions, and inhibition on root growth (Li et al., 2014; Sarasketa et al., 2016). Also, NH_4^+-N addition could result in reduction in leaf expansion rate and root water uptake capacity, which may lead to a low carbon accumulation and an inhibition on growth (Guo et al., 2002; Brück & Guo, 2006). Furthermore, NH_4^+-N addition show a higher stimulation on plant growth but a lower variability among plant functional types than NO_3^--N addition (Fig. 4). It suggests that future N deposition with more NH_4^+-N input will more evenly enhance the growth of different plant functional types. It is interesting that NO_3^--N addition only enhances growth of grasses but not of other plant functional types (Fig. 4), suggesting the nitrate-dominated N deposition could affect grassland more than other types of ecosystem. It should be also noted that the ratio of NH_x/NO_y in N deposition shows contrasting trends between the grasslands in the North America and Europe (Fig. 2). These results call for more research efforts on the different impacts of NH_4^+-N and NO_3^--N addition on community composition in the future.

4.3. Different responses of plant growth to NH_4^+- and NO_3^--N addition among plant tissues

It has been known that ammonium has to be assimilated immediately at the root, while nitrates could be stored in the vacuoles before the assimilation (Marchner, 2012). Due to this immediate assimilation, ammonium uptake demands a higher quantity of carbon skeletons. As a consequence, a higher fraction of root carbon would be consumed by plants for acquiring NH_4^+-N than NO_3^--N (Cramer & Lewis, 1993; Gerendás et al., 1997). This could lead to root growth suppression under NH_4^+-N supply (Bloom et al., 1992; Walch-Liu et al., 2000; Esteban et al.,

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2016). However, inconsistent with previous studies, our meta-analysis results showed that below-ground growth is stimulated more by NH_4^+-N addition (5.9% g^{-1} N), whereas above-ground growth is more sensitive to NO_3^--N addition (8.4% g^{-1} N) across all growth forms.

This unexpected response suggests more resources allocation to root under the increased NH_4^+-N supply. More carbon supply to root could reduce some risks, such as the NH_4^+ toxicity (Roosta & Schjoerring, 2008; Vega-Mas et al., 2015), to plant growth. In addition, reduction of leaf growth, thus, may represent a protective mechanism in order to keep a balance between lowered root water-uptake capacity and the high carbon demand for control of net NH_4^+ uptake by roots. Furthermore, plants grown with NH_4^+-N environment are more sensitive to light stress than that grown under NO_3^--N supply (Zhu et al., 2000; Setién et al., 2013). It suggests that plants under NH_4^+-N supply would invest more resources into below-ground for acquiring NH_4^+ and water. These results indicate that plants have evolved different specialized strategies to adapt to the changing N environments.

4.4. Implications and limitations

The findings in this study have some important implications for the widespread N-addition manipulative experiments. First, NH_4NO_3 and urea are widely used as the major fertilizers in most field ecological experiments. We found the NH_4NO_3 addition shows a similar effect as the NH_4^+-N addition, but detected a greater stimulation of urea addition on plant growth (Fig. 4b). This suggests the N effect on plant growth in the manipulative experiments could be larger than that in the natural ecosystems, especially in those regions with increasing deposition of NO_3^--N. Second, the ratio of NH_4^+-N/NO_3^--N in the soil is increased under the addition of NH_4NO_3 or urea (Fig. 3d). However, the increasing ratio of NH_4^+-N/NO_3^--N in wet N deposition usually eventually enhances the NH_4^+-N/NO_3^--N ratio in the soil (Boxman et al., 2008; Seinfeld & Pandis, 2016). Due to the contrasting impacts of NH_4^+-N and NO_3^--N addition on biomass allocation, attentions should be paid to the scaling of experimental results up to the real ecosystems. Third, plant growth is more sensitive to NH_4^+-N than NO_3^--N addition in most plant functional types except for grasses. It suggests that the composition of N deposition could be important in affecting the community structure in grassland ecosystems.

It should be noted that there are some limitations in this study. First, the meta-analysis combines results from previous studies to calculate a weighted average of the measure, or identify patterns based on results from different studies. However, the weighted effect could be

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affected by factors such as the criteria for searching studies and the uneven sample size among individual studies. To further test the robustness of the key findings in this study, we studied the experiments which simultaneously addition of NH_4^+- and NO_3^--N (totally 42 paired data and 12 species from 7 independent studies, Supporting information Table S2). The results (Supporting information Fig. S1) were consistent with the general patterns that found in Fig. 4. Second, this study tried to discuss the plant preference for N forms under the background of the compositional shift in atmospheric N deposition. However, the increasing NH_x/NO_y ratio in the atmospheric N deposition does not necessary lead to enhanced NH_4^+-N/NO_3^--N in the soil, because NH_4^+ could be quickly transformed to NO_3^- by nitrification. Although there is evidence of associated changes between ratios of atmospheric NH_x/NO_y and soil NH_4^+-N/NO_3^--N at the site level (e.g., Boxman et al., 2008), further research is still needed. Third, some of the discussion is based on the global trends of atmospheric NH_x/NO_y, but the projection itself has great uncertainty.

5. Summary

In general, this study found a higher response of plant growth to ammonium than nitrate addition in terrestrial ecosystem. The higher impact of ammonium than nitrate was detected in all plant functional types except for grasses. The ammonium addition mainly stimulated below-ground growth but nitrate addition only significantly enhanced above-ground growth. These findings indicate that the different preference for N forms in terrestrial plants complicates the predictions of future changes ecosystem structure and functions under the N enrichment. There is a large spatial variation of the trend of NH_x/NO_y at the globe, so plants that grow in the real ecosystems could respond to future N enrichment differently from that have been reported by the manipulative field experiments. Thus, we recommend future manipulative experiments with N addition to consider the compositional features of local N deposition and the different preferences of plant species for acquiring ammonium and nitrate.

Author contribution

L. Y. and J. X. designed the study. X. X. collected and analyzed the data. L. Y. wrote the manuscript with contributions from all co-authors.

Competing interests

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The authors declare that they have no conflict of interest.

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564 **Table 1** Annual atmospheric deposition of total nitrogen flux (TN, Tg N yr^{-1}) and its
565 components (reduced N: NH_x, oxidized N: NO_y, and organic N: ON) from 1850 to 2050,
566 estimated by different models based on different anthropogenic emission inventories.

567

	NH_x	NO_y	ON	TN	Models (emission inventories)	References
1850	18	6	32	56	TM4-ECPL (ACCMIP)	Kanakidou *et al.* (2014)
	17	12	19	48	TM4-ECPL (ACCMIP)	Kanakidou *et al.* (2016)
	17	12	20	49	TM4-ECPL (ACCMIP)	Kanakidou *et al.* (2016)
	17	6	28	51	TM4-ECPL (ACCMIP)	Kanakidou *et al.* (2016)
1990	41	39.9	/	81	MOGUNTIA	Holland *et al.* (1997)
2000	64.4	52	/	116.4	ACCENT IPCC-AR4 (CTM 2000)	Dentener *et al.* (2006)
	58	53	/	111	GISS-E2-R (ACCMIP)	Lamarque *et al.* (2013)
	60	49	/	109	NCAR-CAM3.5 (ACCMIP)	Lamarque *et al.* (2013)
	61	52	/	113	STOC-HadAM3 (ACCMIP)	Lamarque *et al.* (2013)
	60	50	/	110	Multi-model means (ACCMIP)	Lamarque *et al.* (2013)
	64	51	/	115	PhotoComp (ACCMIP)	Lamarque *et al.* (2013)
2005	65	51	38	154.0	TM4-ECPL (RCP6)	Kanakidou *et al.* (2014)
	53	38	41	132.0	TM4-ECPL (GAINS)	Kanakidou *et al.* (2014)
	53	46	27	126	TM4-ECPL (RCP6)	Kanakidou *et al.* (2016)
	53	46	33	132	TM4-ECPL (RCP6)	Kanakidou *et al.* (2016)
	53	40	36	129	TM4-ECPL (RCP6)	Kanakidou *et al.* (2016)
2030	80	38	/	118	ACCENT IPCC-AR4 (IIASA CLE)	Dentener *et al.* (2006)
	84.7	79.2	/	164	ACCENT IPCC-AR4 (SRES-A2)	Dentener *et al.* (2006)
2050	84	53	37	173.0	TM4-ECPL (GAINS)	Kanakidou *et al.* (2014)
	63	29	42	134.0	TM4-ECPL (RCP6)	Kanakidou *et al.* (2014)
	64	26	38	128.0	TM4-ECPL (RCP8.5)	Kanakidou *et al.* (2014)
	68	37	27	132	TM4-ECPL (RCP6)	Kanakidou *et al.* (2016)
	68	37	33	138	TM4-ECPL (RCP6)	Kanakidou *et al.* (2016)
	64	30	36	129	TM4-ECPL (RCP6)	Kanakidou *et al.* (2016)
	70	39	27	135	TM4-ECPL (RCP8.5)	Kanakidou *et al.* (2016)

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Table 2 Between-group heterogeneity (Q_b) and probability (P) of nitrogen effect on plant growth across various nitrogen form (NO_3^--N and NH_4^+-N; NH_4NO_3 and urea), NO_3^-- or NH_4^+-N effect on above- and below-ground growth within each plant functional type.

	NO_3^--N vs NH_4^+-N		NH_4NO_3 vs Urea		AGB vs BGB under NO_3^--N		AGB vs BGB under NH_4^+-N	
	Q_b	P	Q_b	P	Q_b	P	Q_b	P
Seed plant	36.8	<0.001	33.0	<0.001	44.7	<0.001	12.2	<0.001
Woody	7.1	<0.01	49.0	<0.001	0.2	0.64	4.5	<0.05
Herb	35.4	<0.001	12.4	<0.001	51.3	<0.001	0.3	0.60
Trees	7.2	<0.01	34.2	<0.001	0.6	0.43	1.6	0.20
Shrubs	0.1	0.72	1.5	0.22			35.7	<0.001
Grasses	3.5	0.06	11.3	<0.001	0.1	0.74	0.0	1.00
Forbs	29.2	<0.001	0.1	0.70	26.3	<0.001	0.3	0.60

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Table 3 Between-group heterogeneity (Q_b) and probability (P) of nitrogen effect on plant growth across different functional types (woody and herb, among trees, shrubs, grasses and forbs) with each nitrogen form.

	Woody vs Herb		Plant functional types	
	Q_b	P	Q_b	P
NO_3^--N	0.6	0.44	26	<0.001
NH_4^+-N	1.7	0.19	6.6	0.08
NH_4NO_3	41.2	<0.001	45.1	<0.001
Urea	9.3	<0.01	15.1	<0.01

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Figure legend

Figure 1 The global distribution of study sites in this meta-analysis.

Figure 2 The trend of global gridded (0.5°×0.5°) NH_x/NO_y over 2010-2100 based on the environmental driver data sets for the Multi-scale Synthesis and Terrestrial Model Intercomparison Project (MsTMIP) project.

Figure 3 The frequency distribution of the natural logrithm of the response ratio ($\log_e$RR, a) and the percentage changes (means ± 95% CI, b) for the concentration of ammonium (NH_4^+-N), nitrate (NO_3^--N) and the ratio of ammonium to nitrate (NH_4^+/NO_3^-) in the soil under N addition.

Figure 4 The percentage changes in plant growth under NH_4^+-N and NO_3^--N fertilization (a) and under NH_4NO_3 and Urea (b) for different growth forms and plant functional types. Values are means ± 95% CI.

Figure 5 Comparison the effects of NH_4^+-N and NO_3^--N on plant above-ground (ΔAGB) and below-ground growth (ΔBGB) across all the plant species (a) and within growth forms (b-c). Open shapes for NH_4^+-N effects and closed ones for NO_3^--N effects. Values are means ± 95% CI.

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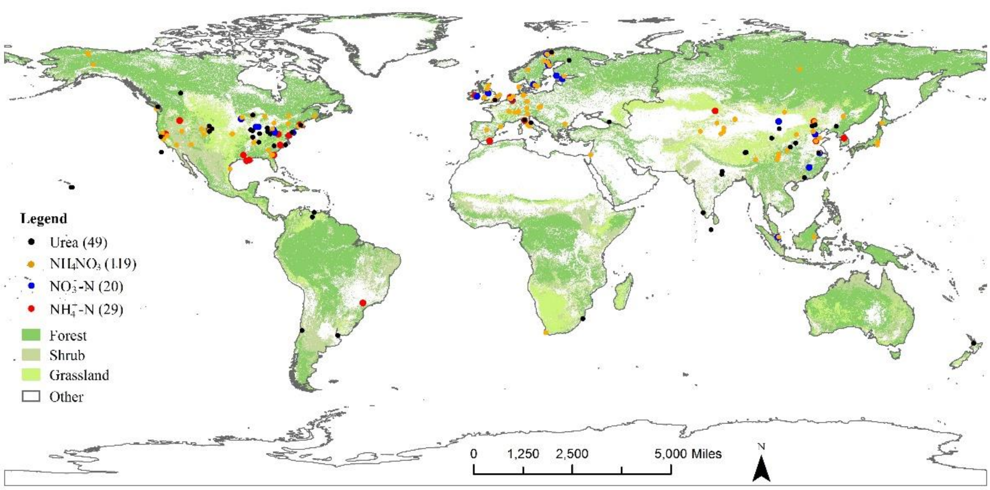
Figure 1 The global distribution of 217 study sites in this meta-analysis.



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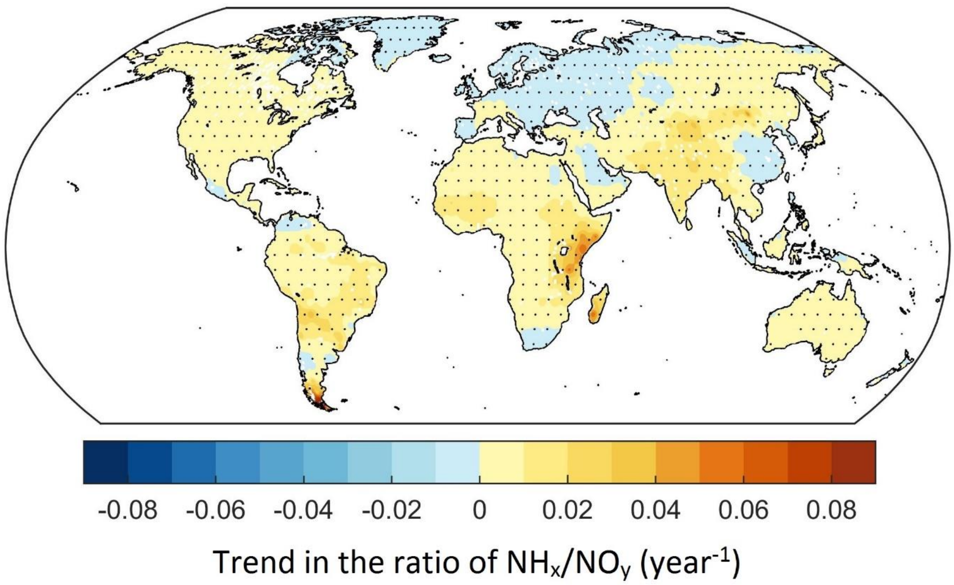

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599 **Figure 3** The frequency distribution of the natural logarithm of the response ratio (log_eRR, a) and the percentage changes (means ± 95% CI, b) for the concentration of ammonium (NH_4^+-N), nitrate (NO_3^--N) and the ratio of ammonium to nitrate (NH_4^+/NO_3^-) in the soil under N addition, warming, and CO_2 enrichment treatment, respectively.

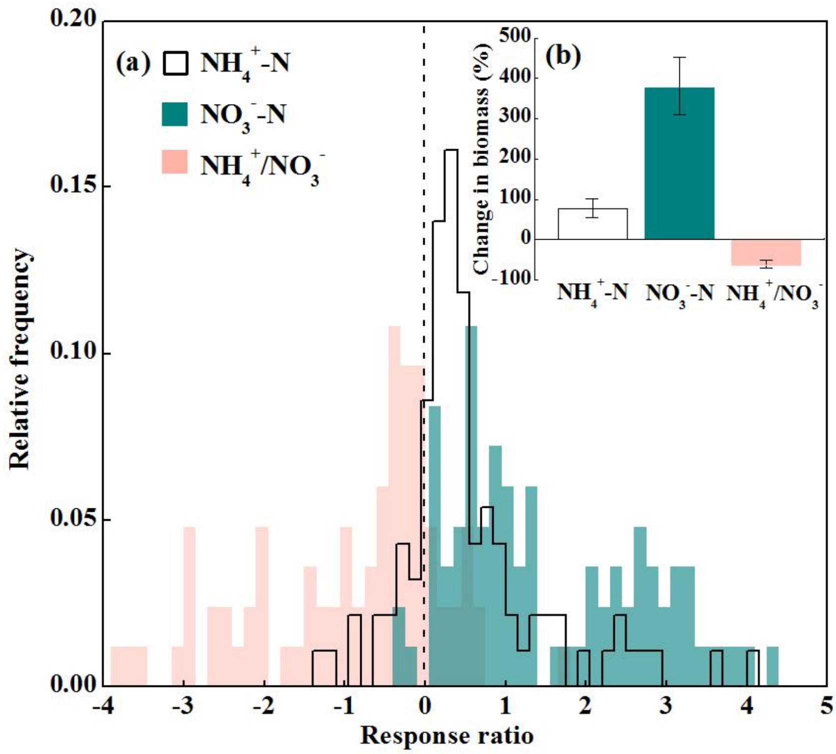

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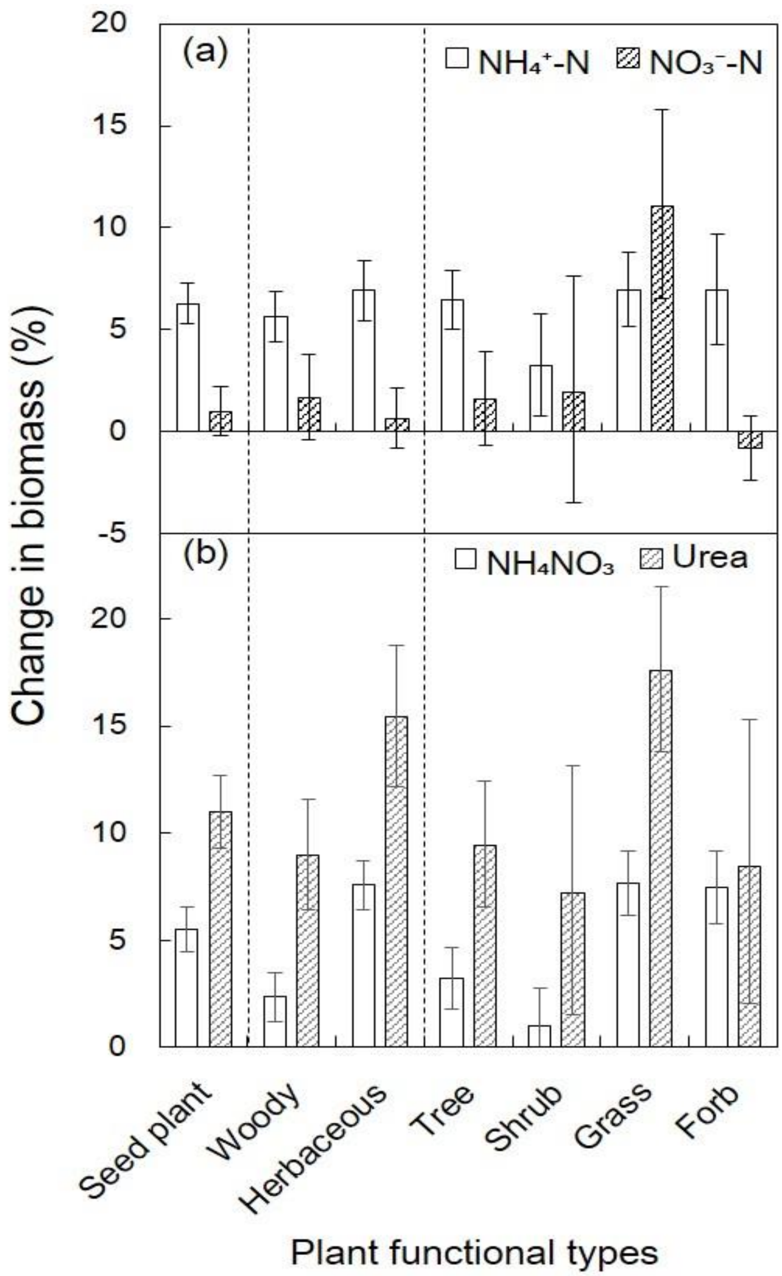
Figure 4 The percentage changes in plant growth under NH_4^+-N and NO_3^--N fertilization (a) and under NH_4NO_3 and Urea (b) for different growth forms and plant functional types. Values are means ± 95% CI.



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608 **Figure 5** Comparison the effects of NH_4^+-N and NO_3^--N on plant above-ground (ΔAGB) and below-ground growth (ΔBGB) across all the plant species (a) and within growth forms (b-c). Open shapes for NH_4^+-N effects and closed ones for NO_3^--N effects. Values are means ± 95% CI.