Responses of ecosystem nitrogen cycle to nitrogen addition: a meta-analysis

Meng Lu[1,2,3]*, Yuanhe Yang[2]*, Yiqi Luo[1,2], Changming Fang[1], Xuhui Zhou[2], Jiakuan Chen[1], Xin Yang[3] and Bo Li[1]

[1]Coastal Ecosystems Research Station of the Yangtze River Estuary, Ministry of Education Key Laboratory for Biodiversity Science and Ecological Engineering, The Institute of Biodiversity Science, Fudan University, 220 Handan Road, Shanghai 200433, China; [2]Department of Botany and Microbiology, University of Oklahoma, Norman, OK 73019, USA; [3]Department of Environmental Science and Engineering, Fudan University, 220 Handan Road, Shanghai 200433, China

Authors for correspondence:
Yiqi Luo
Tel: +1 405 325 8578
Email: yluo@ou.edu

Bo Li
Tel: +86 21 65642178
Email: bool@fudan.edu.cn

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Summary

- Anthropogenic nitrogen (N) addition may substantially alter the terrestrial N cycle. However, a comprehensive understanding of how the ecosystem N cycle responds to external N input remains elusive.
- Here, we evaluated the central tendencies of the responses of 15 variables associated with the ecosystem N cycle to N addition, using data extracted from 206 peer-reviewed papers.
- Our results showed that the largest changes in the ecosystem N cycle caused by N addition were increases in soil inorganic N leaching (461%), soil NO_3^- concentration (429%), nitrification (154%), nitrous oxide emission (134%), and denitrification (84%). N addition also substantially increased soil NH_4^+ concentration (47%), and the N content in belowground (53%) and aboveground (44%) plant pools, leaves (24%), litter (24%) and dissolved organic N (21%). Total N content in the organic horizon (6.1%) and mineral soil (6.2%) slightly increased in response to N addition. However, N addition induced a decrease in microbial biomass N by 5.8%.
- The increases in N effluxes caused by N addition were much greater than those in plant and soil pools except soil NO_3^-, suggesting a leaky terrestrial N system.

Introduction

Humans have approximately doubled the input of reactive nitrogen (N) to the Earth's land surface (Galloway *et al.*, 2008; Gruber & Galloway, 2008; Schlesinger, 2009). The increase of anthropogenic reactive N emissions via agricultural fertilization and combustion of fossil fuel has induced significant atmospheric N deposition, with an average rate of 105 Tg N yr^{-1} (Vitousek *et al.*, 1997; Galloway *et al.*, 2008). The enhanced N input may exert strong effects on both the structure (Clark & Tilman, 2008; Bobbink *et al.*, 2010) and the functioning (Reay *et al.*, 2008; Janssens *et al.*, 2010) of terrestrial ecosystems. As the fundamental components of ecosystem functioning, terrestrial carbon (C) and N cycles may be sensitive to enhanced N deposition (Gruber & Galloway, 2008). It is well known that plant growth is usually constrained by soil N availability in most terrestrial ecosystems (Vitousek & Howarth, 1991; LeBauer & Treseder, 2008). However, it is still uncertain whether this N-induced stimulation of plant growth results in ecosystem C and N accumulation (Neff *et al.*, 2002; Reay *et al.*, 2008). Therefore, improved understanding of the responses of ecosystem C and N cycles to N addition is much needed to enable prediction of the effects of N fertilization and deposition on terrestrial ecosystems.

A number of meta-analyses have examined the effects of additional N input on both C pools (e.g. Treseder, 2008; Xia & Wan, 2008; Liu & Greaver, 2010) and fluxes (e.g. Knorr *et al.*, 2005; LeBauer & Treseder, 2008; Liu & Greaver, 2009; Janssens *et al.*, 2010) in terrestrial ecosystems. However, little is known about how the ecosystem N pools and fluxes respond to atmospheric N deposition. The lack of a comprehensive understanding of the effects of N addition on the ecosystem N cycle greatly limits our ability to explore the responses of the ecosystem C cycle to N fertilization and deposition, as C and N cycles are coupled in

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*These authors contributed equally to this work.

terrestrial ecosystems (Gruber & Galloway, 2008). Therefore, to gain insights into the responses of ecosystem C and N cycles to additional N input, it is imperative to examine how the ecosystem N cycle responds to N fertilization and deposition.

Numerous individual studies have been conducted to examine how the ecosystem N cycle responds to N fertilization or deposition. Previous studies have demonstrated that ecosystem N pools (Mack *et al.*, 2004), microbial biomass N and enzyme activities (Ajwa *et al.*, 1999), and nitric oxide (NO) and nitrous oxide (N_2O) emissions (Butterbach-Bahl *et al.*, 1997) can all be significantly influenced by the external N input. However, experimental results from various individual studies are highly variable, particularly for the soil N pool. For instance, the total N pool in mineral soil has been reported to exhibit an increase (Fisk & Schmidt, 1996), a decrease (Mack *et al.*, 2004) or an insignificant change (Johnson *et al.*, 2000) in response to external N input. Similarly, the responses of microbial biomass N and associated fluxes (i.e. N mineralization, nitrification, and denitrification) to N addition are also highly variable. For example, net N mineralization may increase (Brenner *et al.*, 2005), decrease (Kowaljow & Mazzarino, 2007) or show minor changes (Riley, 1998) in response to N addition. Thus, a general pattern of the responses of N pools and fluxes to N fertilization and deposition is still unavailable.

The highly diverse results from individual experiments are unlikely to reveal a general pattern that can be applied to various ecosystems. However, the results across individual studies can be synthesized to reveal a central tendency of changes in ecosystem N cycle induced by the additional N input (Hedges & Olkin, 1985). By compiling data from 206 individual studies, we conducted a meta-analysis to identify the central tendency of the effects of N addition on ecosystem N cycle. More specifically, this study aimed to investigate the responses of N pool sizes (including plant, litter and microbial biomass, organic horizon and mineral soil pools) to N addition; to explore the responses of N fluxes (i.e. net N mineralization, nitrification, denitrification and leaching) to the external N input; and to examine whether ecosystem types and other factors affect the responses of the ecosystem N cycle to N addition.

Materials and Methods

Data compilation

We selected 206 papers from 2000 peer-reviewed publications that reported N dynamics in response to N fertilization (Supporting Information Notes S1). The compiled database included the responses to N addition of 15 variables related to N pools, fluxes, and other associated parameters (Table S1). The following five criteria were applied to select appropriate studies. (1) N fertilizers were directly added to terrestrial ecosystems and at least one of our selected variables was measured. (2) The treatment and control plots were started with the same plant species and soil types. For crop rotation experiments, the selected data were obtained using the same tillage management system, crop species and rotation sequences. (3) The N application rate, experimental duration and soil depth were clearly recorded and the measurements of treatment and control groups were conducted at the same temporal and spatial scales. (4) To investigate the long-term effect of N addition on the soil N pool, experiments shorter than 1 yr were excluded to avoid short-term noise. (5) The means, standard deviations or standard errors and sample sizes of the chosen variables were directly reported or could be indirectly calculated from the chosen papers. It should be noted that measurements for different N application rates were considered as independent observations if more than one level of N addition was applied in the same experiment (Curtis & Wang, 1998; Liu & Greaver, 2009). The latest sampling was used if more than one measurement at different temporal scales was available for the same experiment (Treseder, 2008; Liu & Greaver, 2009).

The aboveground plant N pool was obtained from direct measurements of aboveground plant N content or indirectly calculated from aboveground plant biomass and N concentration. The belowground plant N pool was quantified using the reported root N content. The litter N pool was determined from the litter N content or the N content of returned residues in agricultural ecosystems, and direct measurements of litter N stock in nonagricultural ecosystems. The soil N pool was calculated for the organic horizon and mineral soil, respectively. The soil N concentration was also used to represent the soil N pool size because of the insignificant effects of N addition on soil bulk density (Fig. S2). To reveal the effects of N addition on ecosystem N fluxes, we extracted data from the studies that directly reported the average or cumulative net N mineralization, N immobilization, nitrification, denitrification, inorganic N and/or NO_3^- leaching, and N_2O emission in response to external N input. In addition, data on mean annual temperature (MAT) and mean annual precipitation (MAP) at each study site were either extracted from the published papers or, if they were not reported in the paper, from the global database at http://www.worldclim.org/ using latitude and longitude coordinates.

Considering that agricultural and nonagricultural ecosystems may respond differently to N addition, we examined the effects of N addition on the ecosystem N cycle for these two ecosystems separately. Given that only a few studies of N-fixing plants were found for the N addition experiment in nonagricultural ecosystems, plant species were grouped into N-fixing and non-N-fixing plants within agricultural ecosystems to examine their responses to N addition. We also grouped our data according to N application rate (0–5, 5–10 and > 10 g N m^{-2} yr^{-1}) and experimental duration (0–5, 5–10 and > 10 yr) to explore their effects on the responses of the ecosystem N cycle to N addition. In agricultural ecosystems, fertilization and control groups received the same irrigation treatment. In nonagricultural ecosystems, experiments involving irrigation treatments were excluded as only experiments comparing fertilization treatments (with or without N fertilization) were selected. Thus, irrigation treatments in agricultural ecosystems should not greatly affect the general patterns observed in this meta-analysis.

Statistical analyses

The response ratio (RR) was used to reflect the effects of N addition on terrestrial ecosystem N pools and fluxes (Hedges *et al.*, 1999). The RR, the ratio of the mean value of the chosen variable in the N addition group ($\overline{Xt}$) to that in the control group ($\overline{Xc}$), is an index of the effect of N addition on the corresponding variable (Eqn 1). More specifically, the mean, standard deviation (*S*) or standard error, and sample size for each treatment were extracted to calculate the logarithm of RR, the variance (v), the weighting factor (w_{ij}), the weighted response ratio (RR_{++}) and the 95% confidence interval (CI) of RR_{++} for the purpose of statistical tests (Eqns 2–6) (Curtis & Wang, 1998; Gurevitch & Hedges, 1999; Luo *et al.*, 2006). The frequency distribution of $\log_e$RR was assumed to follow normal distribution and fitted by a Gaussian function (Eqn 7, Luo *et al.*, 2006). If the 95% CI values of RR_{++} for a variable did not cover zero, the effects of N addition on the variable were considered to differ significantly between two treatments. Otherwise, they were not considered to differ significantly. We also used a *t*-test to examine whether the RR_{++} of a variable differed significantly between agricultural and nonagricultural ecosystems, between N-fixing and non-N-fixing plants, among different N application rates, and among various experimental durations. The per cent change in a variable was estimated by$(e^{RR_{++}} - 1) \times 100\%$. In addition, the relationships between $\log_e$RR and environmental and/or forcing factors were examined using a single-factor regression approach.

$$\log_e \mathrm{RR} = \log_e(\overline{Xt}/\overline{Xc}) = \log_e(\overline{Xt}) - \log_e(\overline{Xc}), \qquad \text{Eqn 1}$$

$$v = \frac{S_t^2}{n_t \overline{X}_t^2} + \frac{S_c^2}{n_c \overline{X}_c^2}, \qquad \text{Eqn 2}$$

$$w_{ij} = \frac{1}{v}, \qquad \text{Eqn 3}$$

$$\mathrm{RR}_{++} = \frac{\sum_{i=1}^{m} \sum_{j=1}^{ki} w_{ij} \mathrm{RR}_{ij}}{\sum_{i=1}^{m} \sum_{j=1}^{ki} w_{ij}}, \qquad \text{Eqn 4}$$

$$S(\mathrm{RR}_{++}) = \sqrt{\frac{1}{\sum_{i=1}^{m} \sum_{j=1}^{ki} w_{ij}}}, \qquad \text{Eqn 5}$$

$$95\%\ \mathrm{CI} = \mathrm{RR}_{++} \pm 1.96 S(\mathrm{RR}_{++}), \qquad \text{Eqn 6}$$

$$y = a \exp\left[-\frac{(x-\mu)^2}{2\sigma^2}\right], \qquad \text{Eqn 7}$$

(x, the mean of $\log_e$RR in an individual interval; y, the frequency in an interval; a, the expected number of $\log_e$RR values at $x = \mu$; μ and σ^2, the mean and variance of the normal distribution of $\log_e$RR, respectively.)

Results

Responses of N pools and fluxes to N addition

N-induced changes in N pools exhibited great variability across the studies, ranging from a minimum $\log_e$RR of −0.084 to a maximum of 1.31 in the leaf, from −0.083 to 1.69 in the aboveground plant, from −0.40 to 1.73 in the belowground plant, and from −0.16 to 1.25 in litter (Fig. 1). On average, the overall effects of N addition on plant N pools were positive, with an increase of 23.9% in leaf N pool ($P < 0.05$; Fig. 1a), 44.2% in the aboveground plant N pool ($P < 0.05$; Fig. 1b), 53.2% in the belowground plant N pool ($P < 0.05$; Fig. 1c), and 24.2% in the litter N pool ($P < 0.05$; Fig. 1d).

N addition significantly decreased microbial biomass N by 5.8% ($P < 0.05$; Fig. 2a), while total N pools in both the organic horizon and mineral soil increased by 6.1 and 6.2% under N enrichment, respectively ($P < 0.05$; Fig. 2b,c). Also, averaged dissolved organic N (DON) increased by 21.1% in the N addition group in comparison with the control group ($P < 0.05$; Fig. 2d). In addition, N addition significantly increased soil inorganic N (SIN) by 114.8% ($P < 0.05$; Fig. 2e), with a 47.2% rise in the NH_4^+ pool and a 428.6% rise in the NO_3^- pool (Fig. S2). Among all N pool variables, the N-induced increases in organic horizon and mineral soil N were among the smallest (Fig. 2f).

Both N influx and efflux were stimulated under N addition (Fig. 3). Compared with those in control groups, soil net N mineralization, nitrification, denitrification and inorganic N leaching increased in the N fertilization groups by 24.9, 153.9, 84.3 and 460.9%, respectively ($P < 0.05$; Fig. 3). Moreover, N_2O emissions increased by 133.6% in response to N addition ($P < 0.05$; Fig. 3c). However, N immobilization showed only a minor change with external N input (Fig. 3d).

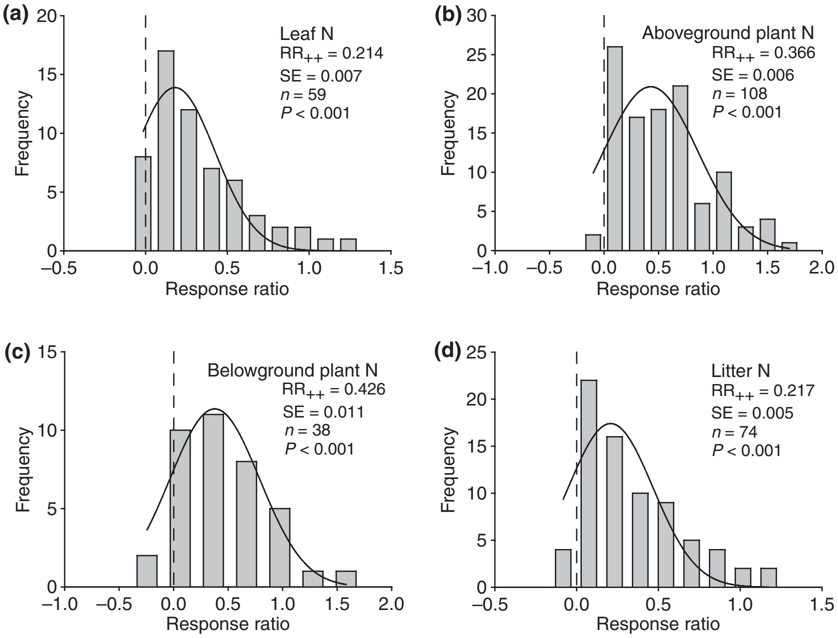

Fig. 1 The frequency distributions of the natural logarithm of the response ratio ($\log_e$RR) for leaf (a), aboveground plant (b), belowground plant (c), and litter (d) nitrogen (N) pool responses to N addition. The solid curve is a Gaussian distribution fitted to the frequency data. The *x*-axis is $\log_e$RR and the *y*-axis is frequency. The vertical dashed line is at $\log_e$RR = 0.

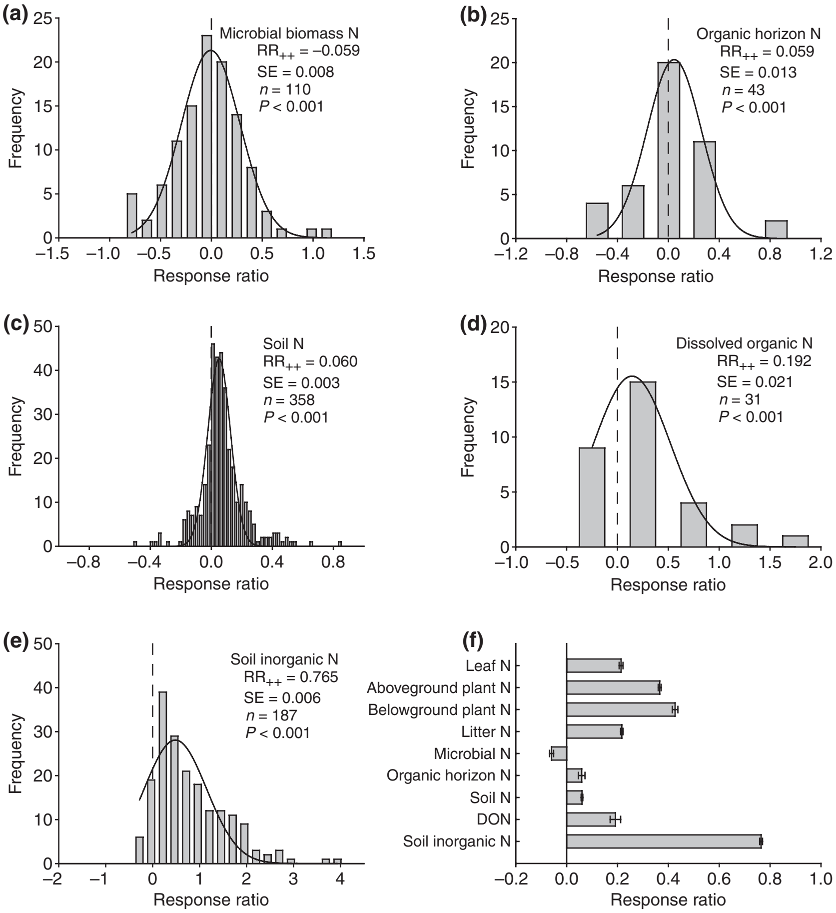

Fig. 2 The frequency distributions of the natural logarithm of the response ratio ($\log_e$RR) for microbial biomass nitrogen (N) (a), organic horizon N pool (b), soil N pool (c), dissolved organic N (d) and soil inorganic N (e) responses to N addition, and the weighted response ratio (RR_{++}) for the responses to N addition of nine variables related to the ecosystem N pool (f). The solid curve is a Gaussian distribution fitted to the frequency data. The *x*-axis is $\log_e$RR and the *y*-axis is frequency. The vertical dashed line is at $\log_e$RR = 0.

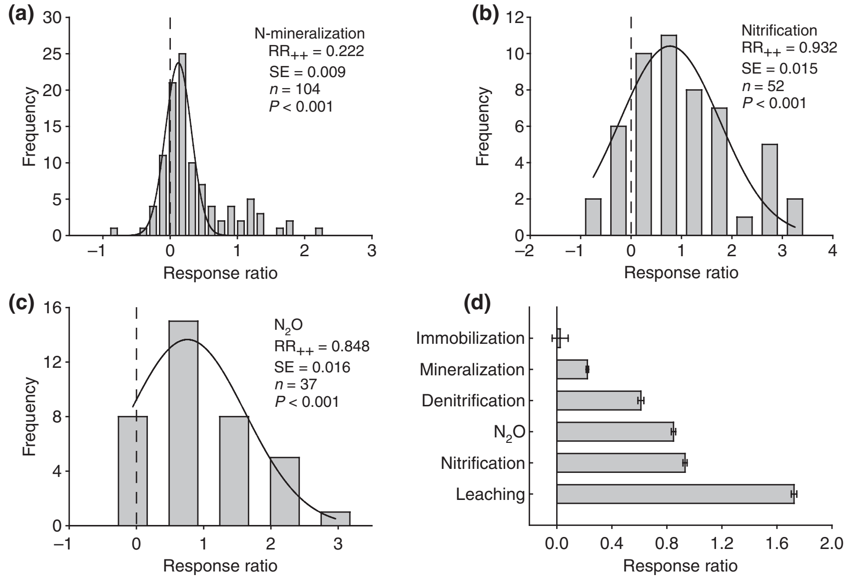

Fig. 3 The frequency distributions of the natural logarithm of the response ratio ($\log_e RR$) for net nitrogen (N) mineralization (a), nitrification (b) and N_2O emission (c) responses to N addition, and the weighted response ratio (RR_{++}) for N immobilization, mineralization, denitrification, N_2O flux, nitrification and soil inorganic leaching (d) responses to N addition. The solid curve is a Gaussian distribution fitted to the frequency data. The *x*-axis is $\log_e RR$ and the *y*-axis is frequency. The vertical dashed line is at $\log_e RR = 0$.

Differential responses in agricultural and nonagricultural ecosystems

N-induced changes in N pools and fluxes in agricultural ecosystems were different from those in nonagricultural ecosystems (Fig. 4). The increments of leaf, aboveground plant and litter N pools in agricultural ecosystems were larger than those in nonagricultural ecosystems ($P < 0.05$), while the increment of SIN in agricultural ecosystems was significantly smaller than that in nonagricultural ecosystems ($P < 0.05$). Moreover, the increments of net N mineralization, nitrification, and inorganic N leaching in nonagricultural ecosystems were greater than those in agricultural ecosystems ($P < 0.05$). However, the increases in belowground plant N, soil N pool and DON showed no significant differences between agricultural and nonagricultural ecosystems ($P > 0.1$). In addition, N addition decreased microbial biomass N in both agricultural and nonagricultural ecosystems ($P < 0.05$), and the changes in microbial biomass N did not reveal significant differences between agricultural and nonagricultural ecosystems ($P = 0.32$).

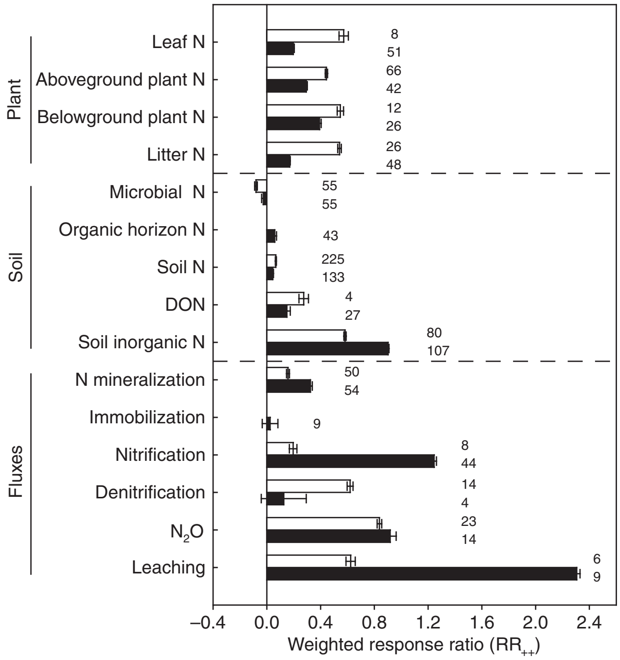

Fig. 4 The weighted response ratio (RR_{++}) for the responses to nitrogen (N) addition of 15 variables related to the ecosystem N cycle in agricultural (open bars) and nonagricultural (closed bars) ecosystems. Bars represent RR_{++} ± SE. The vertical line is drawn at $\log_e RR = 0$. The sample size for each variable is shown next to the bar. DON, dissolved organic N.

Factors affecting the responses of N pools and fluxes to N addition

Both aboveground plant and litter N pools in non-N-fixing crops exhibited larger responses than those in N-fixing crops under N addition ($P < 0.05$; Fig. 5). Moreover, an increase in the N application rate from 0–5 to 5–10 g N m^{-2} yr^{-1} led to greater increases in leaf, aboveground plant and litter N pools ($P < 0.05$; Fig. 6). In addition, litter N accumulation tended to increase, while changes in microbial biomass N tended to decrease with experimental duration ($P < 0.05$; Fig. 7, Tables S2, S3).

N-induced changes in the aboveground plant N pool slightly increased with latitude ($r^2 = 0.07$, $P < 0.05$), but were negatively correlated with MAT ($r^2 = 0.21$, $P < 0.001$) and MAP ($r^2 = 0.14$, $P < 0.05$) (Fig. 8). The changes in the aboveground plant N pool were positively related to N application rate ($r^2 = 0.07$, $P < 0.05$).

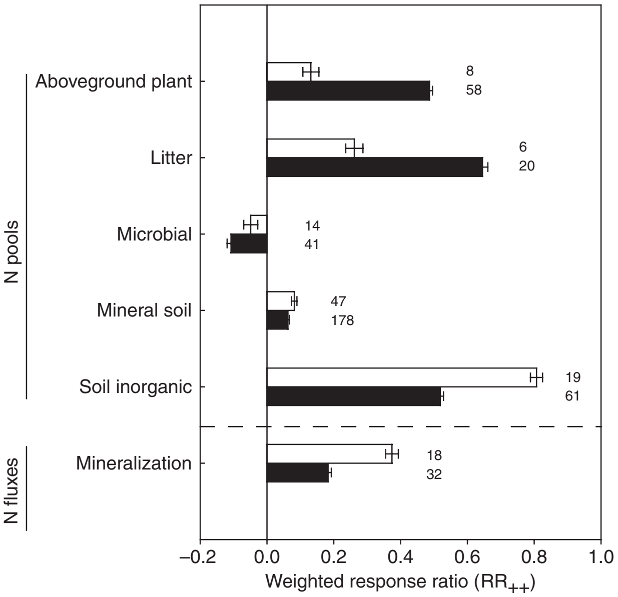

Fig. 5 The weighted response ratio (RR_{++}) for the responses to nitrogen (N) addition of six variables related to the ecosystem N cycle, with two functional groups of N fixation (open bars, N-fixing; closed bars, non-N-fixing). Bars represent RR_{++} ± SE. The vertical line is drawn at $\log_e RR = 0$. The sample size for each variable is shown next to the bar.

However, N-induced changes in the aboveground plant N pool did not show any significant correlations with experimental duration or the cumulative amount of N. Moreover, the relationships between the $\log_e$RR of the aboveground plant N pool and environmental factors did not differ significantly between agricultural and nonagricultural systems, except that the changes in the aboveground plant N pool were not significantly correlated with N application rate in nonagricultural ecosystems ($P = 0.13$; Fig. 8d). Both environmental and forcing factors also regulated the responses of the litter N pool and soil N pool to N addition. Specifically, the $\log_e$RR of the litter N pool significantly increased with the cumulative amount of N and experimental duration, but significantly decreased with MAT ($P < 0.05$) (Table S2). The $\log_e$RR of the organic horizon N pool significantly increased with the $\log_e$RR of the litter N pool ($r^2 = 0.46$, $P < 0.05$) (Table S3). However, the $\log_e$RR of the soil N pool only increased with the $\log_e$RR of the belowground plant N pool ($r^2 = 0.36$, $P < 0.05$) (Fig. S1), and slightly decreased with soil depth ($r^2 = 0.02$, $P < 0.05$) (Table S3).

Discussion

Response of ecosystem N pools to N addition

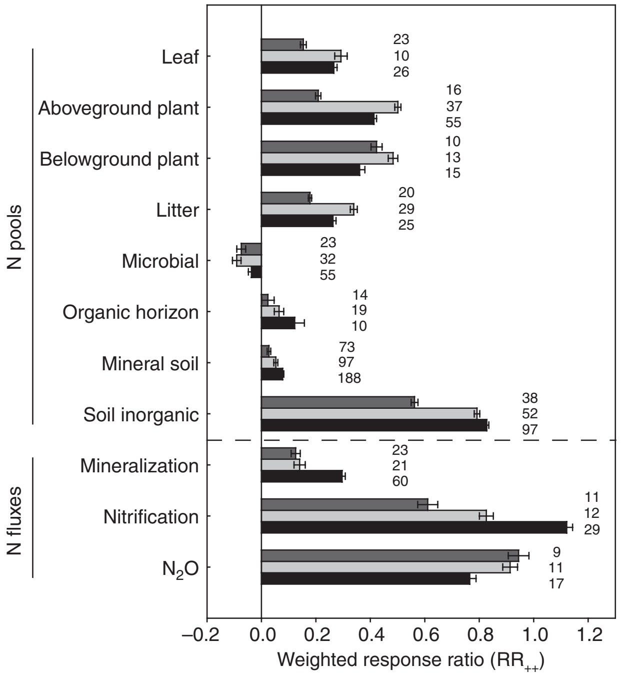

Fig. 6 The weighted response ratio (RR_{++}) for the responses to nitrogen (N) addition of 11 variables related to N pools and fluxes, with three N application rates (0–5 N m^{-2} yr^{-1}, dark grey bars; 5–10 N m^{-2} yr^{-1}, light grey bars; > 10 g N m^{-2} yr^{-1}, black bars). Bars represent RR_{++} ± SE. The vertical line is drawn at $\log_e RR = 0$. The sample size for each variable is shown next to the bar.

N addition stimulated N sequestration in both plant and litter pools. The net N accumulation in plants under N addition could be attributed to the increased plant biomass and plant N concentration. As a growth-limiting factor, the external input of N usually leads to increases in both plant N concentration (Xia & Wan, 2008) and net primary production (LeBauer & Treseder, 2008) in terrestrial ecosystems, and thus results in increased plant N accumulation. The increased litter N pool is logically consistent with N accumulation in both above- and belowground plant pools. As the major input to litter, the N-induced increase in plant N content could ultimately lead to net N accumulation in litter (Vanotti *et al.*, 1995; Mack *et al.*, 2004). Our results also showed that the changes in litter N pool were positively correlated with those in the aboveground plant N pool ($r^2 = 0.54$, $P < 0.01$) (Fig. S1), indicating that the accumulation of the litter N pool could be driven by the increases in the aboveground plant N pool under N addition.

By contrast, N addition decreased microbial biomass N. In ecosystems there may be a number of processes that lead to a decline in the microbial N pool. First, the increased amount of soil inorganic N caused by N addition can react with soil organic matter and result in the accumulation of recalcitrant compounds (Soderstrom *et al.*, 1983; Fog, 1988), which may be unavailable for microbial growth in the N addition scenario (Treseder, 2008; Janssens *et al.*, 2010). Secondly, N addition significantly decreased soil pH by 3.5% across various ecosystems (Fig. S2). N-induced soil acidification may result in calcium and magnesium leaching

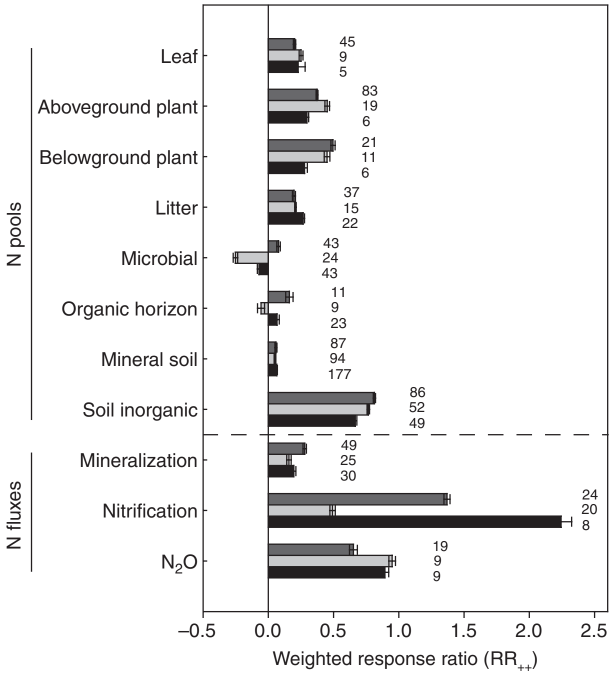

Fig. 7 The weighted response ratio (RR_{++}) for the responses to nitrogen (N) addition of 11 variables related to nitrogen (N) pools and fluxes, with three experimental durations (0–5 yr, dark grey bars; 5–10 yr, light grey bars; > 10 yr, black bars). Bars represent RR_{++} ± SE. The vertical line is drawn at $\log_e RR = 0$. The sample size for each variable is shown next to the bar.

and other corresponding changes in soil physical–chemical properties, which may limit microbial biomass growth (Vitousek *et al.*, 1997; Treseder, 2008). In addition, it has also been reported that the potential N saturation in the N addition scenario may constrain the activities of β-glucosidase in mineral soil, causing a decline in microbial C acquisition and a decrease in microbial biomass (DeForest *et al.*, 2004).

N addition led to N sequestration in both the organic horizon and mineral soil, but to a much lower degree than in plant and litter pools. The smaller response of the soil N pool to experimental N addition compared with the plant N pool may reflect the difference in the sizes of these N pools (Batjes, 1996). It usually takes longer to increase N content in a large pool than in a small one. The smaller response of the soil N pool may also be partly attributable to minor increases in soil organic mass (Liu & Greaver, 2010) and low stoichiometrical flexibility in the narrow-range C : N ratio in soil organic matter. Nevertheless, our analysis showed that the changes in the soil N pool were significantly correlated with those in the belowground plant N pool ($r^2 = 0.36$, $P < 0.05$) (Fig. S1), indicating that belowground plant N dynamics may contribute to changes in the mineral soil N pool. However, the N added to the soil could be lost via stimulated N fluxes, such as nitrification, denitrification, N_2O emission and inorganic N leaching. Thus, N addition resulted in low increases in total N pools in both the organic horizon and mineral soil.

Enhanced N fluxes in response to N addition

N addition stimulated net N mineralization in terrestrial ecosystems. An increase in N mineralization may be induced by increases in DON and the soil N pool (Chapin *et al.*, 2002; Booth *et al.*, 2005), and decreases in the C: N ratio in mineral soil (Barrios *et al.*, 1996). We found that DON and the total N pool in mineral soil increased by 21.1 and 6.2%, respectively (Fig. 2), while the C : N ratio in mineral soil significantly decreased by 1.9% in response to N addition. As a consequence, increased substrate quantity and quality under N enrichment may exert positive effects on N mineralization.

External N input increased nitrification in terrestrial ecosystems. An increase in nitrification may be driven by changes in soil NH_4^+ concentration and soil C : N ratio under N enrichment. Previous studies have suggested that soil NH_4^+ availability is one of the most important factors that determine the nitrification rate (Robertson, 1989). It has also been reported that net nitrification in mineral soil is negatively correlated with the C : N ratio in the soil (Barrios *et al.*, 1996). Thus, the N-induced increase in NH_4^+ availability and decrease in C : N ratio in mineral soil (Fig. S2) may stimulate nitrification in terrestrial ecosystems under N enrichment.

Denitrification was also accelerated in terrestrial ecosystems under N enrichment. It is well known that oxygen concentration, NO_3^- availability, and organic C supply are the three main factors that control the denitrification rate (Del Grosso *et al.*, 2000). Thus, the enhanced litter input (Fig. 1d) and soil NO_3^- concentration (Fig. S2) under N enrichment could result in the acceleration of the denitrification process. In addition, soil N_2O emission and soil inorganic leaching significantly increased in response to N addition. The increases in N_2O flux might have resulted from the accelerated nitrification and denitrification processes, while the increase in N leaching may have been largely a result of the increased soil NO_3^- availability. Overall, the increase in substrate availability (especially soil NH_4^+ and NO_3^- concentrations) is the key factor that drives N losses under N enrichment.

Different responses of N pools in agricultural versus nonagricultural ecosystems

The increments of leaf, aboveground plant and litter N pools in agricultural ecosystems were larger than those in nonagricultural ecosystems (Fig. 4). There may be at least three explanations for these differences. First, the higher increases in the N pool observed in agricultural ecosystems may be a result of the higher N application rate in these systems

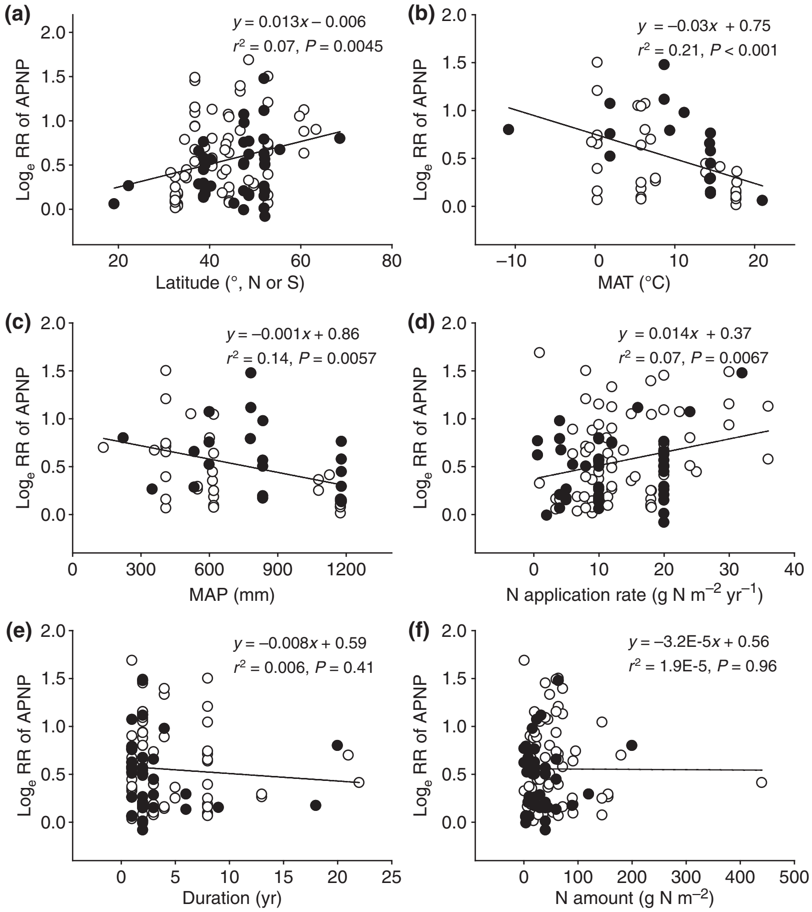

Fig. 8 Relationships between the natural logarithm of the response ratio ($\log_e$RR) for the response to nitrogen (N) addition of the aboveground plant nitrogen (N) pool (APNP) and latitude (a), mean annual temperature (MAT) (b), mean annual precipitation (MAP) (c), N application rate (d), experimental duration (e), and cumulative N amount (f). Open circles, agriculture; closed circles, nonagriculture.

compared with natural ecosystems. In this meta-analysis, the averaged N application rate in agricultural ecosystems was greater than that in nonagricultural ecosystems (14.9 versus 11.7 g N m^{-2} yr^{-1}). Our meta-analysis also indicated that the response ratio of the aboveground plant N pool increased with increasing N application rate (Fig. 8d). Thus, the higher N application rate in agricultural ecosystems may lead to larger responses of N pools to N addition. Secondly, crops grown in agricultural fields have usually been selected to use N more luxuriously and be more responsive to a high N application rate, and to have high photosynthetic rates (Chapin *et al.*, 2002). Specifically, long-term cropped cultivars have been bred to have higher NH_4^+ and NO_3^- uptake rates, better utilization of a high inorganic N concentration in soil, and higher potential growth rates than most wild plants (Engels & Marschner, 1995). Thus, the increase in N pools in crop species may be greater than that in wild species. Thirdly, the use of N-fixing crops such as soybean (*Glycine max*) could make a significant contribution to the increase in soil N availability (Engels & Marschner, 1995), and the subsequent transfer of external fixed N to non-N-fixing crops may benefit crop growth (Ledgard & Giller, 1995), and thus could lead to a higher growth rate than that of most wild plants. In addition, crop management, tillage and irrigation treatments may favour N accumulation in both plant and litter pools in agricultural ecosystems.

Effects of other factors on the responses of the ecosystem N cycle to N addition

Both aboveground plant and litter N pools in non-N-fixing crops exhibited larger responses to N addition than those in N-fixing crops (Fig. 5), indicating that non-N-fixing crops may be more limited by N and/or have higher potential growth rates than N-fixing crops. There could be at least three reasons for these differences. First, it is well established that a portion of the net photosynthetic production of N-fixing plants is consumed by the nodulation/N-fixation process (Imsande, 1988). Thus, compared with non-N-fixing crops, N-fixing crops could be expected to have lower increases in the aboveground plant N pool under N enrichment because of the extra energy cost. Secondly, high soil N availability, especially soil NO_3^- availability, may restrain root-hair infection processes in N-fixing crops so that nodule development and growth associated with N fixation processes may be inhibited under N enrichment (Streeter, 1988; Carroll & Mathews, 1990). As a consequence, excessive N input may inhibit N-fixing plant growth by restraining plant N uptake processes, and lead to decreases in biomass, plant N concentration, photosynthetic rate, and seed production (Imsande, 1989). Thirdly, soil acidification can exert a larger negative effect on the persistence and production of N-fixing crops via aluminium and/or manganese toxicity than on the persistence and production of non-N-fixing crops, which directly take up inorganic N from the soil (Ledgard & Giller, 1995). Thus, N-induced soil acidification (Fig. S2) may inhibit plant growth of N-fixing crops more than that of non-N-fixing crops.

An increase in the N application rate from 0–5 to 5–10 g N m^{-2} yr^{-1} led to increases in leaf, aboveground plant and litter N pools (Fig. 6), largely driven by positive effects on leaf N concentration and photosynthetic rate (Field & Mooney, 1986; Reich *et al.*, 1997). However, no significant difference was observed between medium and high N application rates (i.e. from 5–10 to > 10 g N m^{-2} yr^{-1}) (Fig. 6). Such a response is consistent with the relationship between plant production and N input rate reported by Kelly & Levin (1986). The results obtained in this study, together with the earlier analysis by Kelly & Levin (1986), suggest that a high external N input rate may exceed plant growth demand, which may cause ecosystem N saturation and inhibit further increases in plant growth (Aber *et al.*, 1998). In addition, the N application rate had minor effects on belowground plant, microbial and soil N pools. The lack of effect on the belowground plant N pool might explain the nonsignificant differences in the total N pool in mineral soil among the treatments of different N input rates.

Experimental duration had a positive effect on litter N accumulation, but had a negative effect on the changes in microbial biomass N (Fig. 7). Our results were consistent with previous observations by Treseder (2008), who demonstrated that microbial biomass decreased with experimental duration. The negative relationship between microbial biomass and experimental duration may be attributed to a progressive inhibition of microbial growth by additional N input (Waldrop & Zak, 2006). Over the long term, the increased amount of soil inorganic N caused by N addition could react with soil organic matter and lead to the accumulation of recalcitrant compounds (Soderstrom *et al.*, 1983; Fog, 1988), which may inhibit microbial growth and thus restrain C efflux processes (Treseder, 2008; Janssens *et al.*, 2010). This suggests that, as a result of a decline in microbial biomass, more N was accumulated in litter as experimental duration increased.

The N-induced changes in the aboveground plant N pool were negatively correlated with both MAT and MAP (Fig. 8), possibly because of the stronger N limitation of plant growth in high-latitude regions than in low-latitude regions (Walker & Syers, 1976). The negative correlations between $\log_e$RR of the aboveground plant N pool and climatic factors could also be ascribed to the enhanced N losses driven by increases in temperature and precipitation. An increase in temperature usually stimulates microbial activities associated with N loss processes, such as nitrification and denitrification (Engels & Marschner, 1995; Chapin *et al.*, 2002). Previous studies indicated that both NO and N_2O production increased as temperature increased between 4 and 32°C, and the observed optimum temperature for N_2O production was *c.* 28°C (Kesik *et al.*, 2006). A similar optimum temperature was observed for nitrification and denitrification (25–30°C; Saad & Conrad, 1993). Thus, higher MAT may accelerate N loss processes and mitigate N effects on plant growth. Also, increased MAP usually accelerates soil NO_3^- leaching and results in N losses from terrestrial ecosystems (Austin & Vitousek, 1998). As a result, a decrease in soil N availability induced by increased rainfall may also have a negative effect on N accumulation in plants.

In summary, our meta-analysis revealed that both N pools and fluxes were substantially altered under N enrichment. Four subsystems, that is, the aboveground plant subsystem, the litter/organic horizon subsystem, the belowground plant/soil subsystem and the substrate subsystem, were examined to investigate how N addition regulates key processes of the N cycle in terrestrial ecosystems (Fig. 9). N addition substantially increased inorganic N in mineral soil. The increase in soil N availability stimulated plant growth and resulted in N accumulation in plant, litter and soil, indicating additional N sequestration in terrestrial ecosystems under N fertilization and deposition. However, the concurrent increase in N_2O emission and SIN leaching under N fertilization tended to export the inorganic N out of

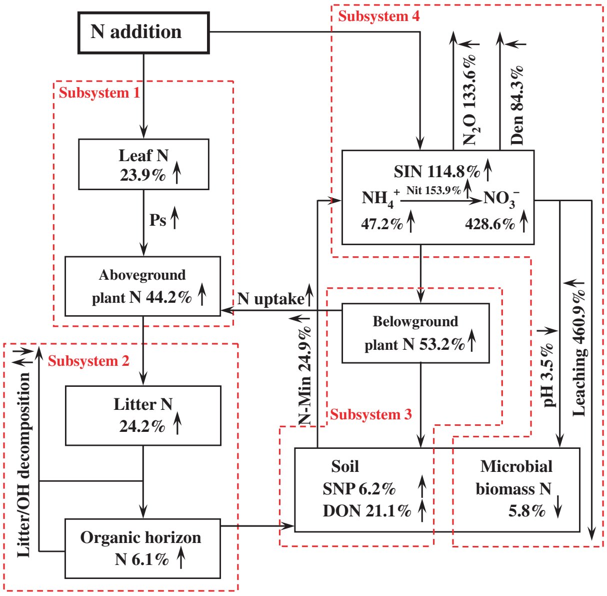

Fig. 9 A conceptual framework for the responses of ecosystem nitrogen (N) cycle to N addition. ↑, increase in response to N addition; ↓, decrease in response to N addition; Ps, photosynthesis; SIN, soil inorganic N; N-min, net N mineralization; Nit, nitrification; Den, denitrification; SNP, soil N pool; DON, dissolved organic N.

the systems rather than benefit plant uptake over the long term, suggesting a leaky terrestrial N system. This leaky N system may have a series of environmental consequences. The increase in N_2O, which is one of the most important greenhouse gases, may accelerate global warming. In addition, the enhanced NO_3^- leaching will cause soil acidification, eutrophication and other serious ecological problems.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Relationships of the $\log_e$ response ratio ($\log_e$RR) of the leaf nitrogen (N) pool (leaf NP) with the N application rate (a), the $\log_e$RR of the aboveground plant N pool (APNP) with the $\log_e$RR of the belowground plant N pool (BPNP) (b), the $\log_e$RR of the litter N pool with the $\log_e$RR of the APNP (c), and the $\log_e$RR of the soil N pool with the $\log_e$RR of the BPNP (d).

Fig. S2 The frequency distributions of the $\log_e$ response ratio ($\log_e$RR) of soil NH_4^+ concentration (a), NO_3^- concentration (b), the soil carbon (C): nitrogen (N) ratio (c), the soil pH value (d), and the soil bulk density (e) in response to N addition.

Notes S1 A list of 206 papers from which data were extracted for this meta-analysis.

Table S1 The weighted response ratio (RR_{++}) and number of data sets (in parentheses) of 15 variables extracted from each of the papers

Table S2 Regression analysis of the $\log_e$ response ratio ($\log_e$RR) of the leaf nitrogen (N) pool (leaf NP), the belowground plant N pool (BPNP), and the litter N pool (LNP) against latitude, mean annual temperature (MAT), mean annual precipitation (MAP), N application rate (N rate), experimental duration (duration), and cumulative N fertilization amount (N amount)

Table S3 Regression analysis of the $\log_e$ response ratio ($\log_e$RR) of microbial biomass N (MBN), organic horizon N pool (OHNP), and soil N pool (SNP) against latitude, mean annual temperature (MAT), mean annual precipitation (MAP), N application rate (N rate), experimental duration (duration), cumulative N fertilization amount (N amount), soil depth, and the $\log_e$RR of the litter N pool (LNP)