

Effect of nitrogen addition on the variations in the natural abundance of nitrogen isotopes of plant and soil components

Jun Liu · Chao Wang · Bo Peng · Zongwei Xia ·
Ping Jiang · Edith Bai 

Received: 17 February 2016 / Accepted: 6 October 2016 / Published online: 17 October 2016
© Springer International Publishing Switzerland 2016

Abstract

Background Anthropogenic nitrogen (N) addition has dramatically increased and significantly affected global nitrogen cycling. The natural abundance of stable N isotope ratios ($\delta^{15}\text{N}$) has been used as an indicator of the N status of an ecosystem. However, how plant and soil $\delta^{15}\text{N}$ signatures would respond to N addition is still unclear.

Methods and aims Herein, we synthesized the data of 951 observations from 48 individual studies associated with responses of plant and soil $\delta^{15}\text{N}$ values to N addition and conducted a meta-analysis to explore a general pattern of N addition effects on $\delta^{15}\text{N}$ values of plant and soil.

Results Our results showed that $\delta^{15}\text{N}$ values of plant, soil total N, and soil NO_3^- were significantly increased by N addition, while $\delta^{15}\text{N}$ value of soil N_2O was significantly decreased and $\delta^{15}\text{N}$ value of soil NH_4^+ was not significantly changed. The $\delta^{15}\text{N}$ value of soil total N of different ecosystems showed similar responses to N addition, whereas $\delta^{15}\text{N}$ values of different plant types showed

different responses. Increasing treatment duration significantly increased the effects of inorganic N addition on $\delta^{15}\text{N}$ values of shrubs and soil NH_4^+ but did not affect the responses of $\delta^{15}\text{N}$ values of soil total N and NO_3^- . With increasing inorganic N addition rate, only $\delta^{15}\text{N}$ value of plant was significantly increased, but no significant relationship was found between the effect of N addition on other components and N addition rate because of the input of isotopically depleted sources.

Conclusions Our study revealed a comprehensive picture of the effects of N addition on $\delta^{15}\text{N}$ signatures in terrestrial ecosystems and could help us understand how plant and soil $\delta^{15}\text{N}$ signatures change with N addition and how these signatures can be used as an indicator of ecosystem N status under increasing N deposition or fertilization.

Keywords ^{15}N · Signature · Nitrogen deposition · Nitrogen fertilizer · Nitrogen stable isotope · Meta-analysis

Responsible Editor: Liz Shaw.

Electronic supplementary material The online version of this article (doi:10.1007/s11104-016-3081-0) contains supplementary material, which is available to authorized users.

J. Liu · C. Wang · B. Peng · Z. Xia · P. Jiang · E. Bai (✉)
CAS Key Laboratory of Forest Ecology and Management,
Institute of Applied Ecology, No. 72 Wenhua Road, Shenyang,
Liaoning 110016, China
e-mail: baie@iae.ac.cn

J. Liu
University of Chinese Academy of Sciences, Beijing 100049,
China

Abbreviations

N	Nitrogen
N_2O	Nitrous oxide
NH_4NO_3	Ammonium nitrate
NH_4Cl	Ammonium chloride
NH_4SO_4	Ammonium sulfate
KNO_3	Potassium nitrate
NaNO_3	Sodium nitrate
SE	Standard error
SD	Standard deviation
CI	Confidence intervals

Introduction

Owing to human activities, global nitrogen (N) deposition has dramatically increased and is expected to reach 200 Tg N yr⁻¹ by 2050 (Galloway et al. 2008). N fertilization in agricultural ecosystems has steadily increased as well and is expected to reach 103 Tg N yr⁻¹ by 2050 (Bouwman et al. 2013). Excess N inputs could relieve N limitation in some ecosystems, but may lead to an imbalance of the N cycle, causing many negative effects. N cycling is complex, with multiple transformation processes, feedbacks, and interactions with other biogeochemical cycles. The natural abundance of stable N isotope ratios (¹⁵N:¹⁴N expressed as δ¹⁵N) has been used as an indicator of the N status of an ecosystem or an N transformation process (Bai and Houlton 2009; Craine et al. 2009; Kalcsits et al. 2014) and could be a convenient method to study how increasing reactive N would affect N cycling.

The variation of δ¹⁵N value among different components in terrestrial ecosystems is mainly caused by isotope fractionation and isotope mixing. For a reaction or a biogeochemical process, light isotope ¹⁴N is usually more abundant in the product, and heavy isotope ¹⁵N is usually enriched in the substrate (Evans 2001). For an ecosystem N pool, its δ¹⁵N value is determined by both input processes and output processes (Brenner 2001; Amundson 2003). Atmospheric N deposition and biological N fixation are generally isotopically depleted sources compared to δ¹⁵N values of soils and plants (Amundson 2003). Therefore, if N output rates remain constant, the increase of N input rates would decrease δ¹⁵N values of soils and plants. However, the N output processes such as denitrification and leaching generally have strong isotope fractionation against ¹⁵N (Houlton et al. 2006; Bai and Houlton 2009; Brookshire et al. 2012). If the rates of these output processes change under increasing N deposition, the responses of plant and soil δ¹⁵N values would be more complex (Högberg et al. 2014; Kalcsits et al. 2014; Kriszan et al. 2009). For instance, long-term N fertilization was found to enhance N losses through gaseous pathway and to significantly increase soil δ¹⁵N value (Kriszan et al. 2009). Therefore, soil and plant δ¹⁵N signatures could provide information on how ecosystem output processes respond to increasing N inputs. In addition, foliar δ¹⁵N value has been widely recognized as a useful integrator to characterize the N status of an ecosystem and higher foliar δ¹⁵N value generally indicates higher N availability (Evans 2001; Robinson 2001; Craine

et al. 2009; Craine et al. 2015). Thus, examination of ecosystem δ¹⁵N change under N addition treatment could provide valuable information on how N fluxes, assimilation, and allocation would respond to increasing N inputs into terrestrial ecosystems.

Over the past three decades, a series of individual relevant studies on the effects of N addition on the natural abundance of δ¹⁵N value have been conducted. However, inconsistencies among results have hampered our ability to draw general conclusions. Two primary reasons are probably responsible for this problem: (1) these studies were conducted in various ecosystems (e.g., forest, shrubland, grassland, and agriculture) and different types of plants were tested (e.g., tree, shrub, grass, crop, moss, and lichen) and (2) the experimental setup differed among different studies. For example, several kinds of fertilizers, different rates of N addition, and varying length of treatment duration were implemented in these studies. To improve the utilization of δ¹⁵N signature as an indicator or a predictor of N cycling change under increasing N deposition or fertilization, here we synthesized the results across these individual studies and conducted a meta-analysis following previous suggestions (Hedges et al. 1999).

By compiling data from 48 individual studies, we conducted a meta-analysis to identify the general patterns of the responses of plant and soil δ¹⁵N signatures to simulated N deposition and to investigate the differences among different experimental scenarios (e.g., ecosystem/plant types, plant functional types, treatment duration, N addition rates, and N fertilizer types), which could help us better predict how ecosystem N status would respond to increasing N deposition or fertilization.

Materials and methods

Data compilation

In total, 48 papers with 951 observations (an observation is composed of both control and N addition treatment data for one examined variable) were selected from the Web of Science resource (Supporting Information, Reference S1 and Table S1). The search terms “N isotope” and “nitrogen addition,” “N-15” and “nitrogen input,” “nitrogen natural abundance” and “deposition,” “nitrogen addition” and “isotope signature,” and “nitrogen loss” and “¹⁵N” were used. Any selected articles should meet the following criteria: at least one of our selected variables was reported;

the means and sample sizes for both control and N addition treatments had to be provided; and for multifactorial studies, only control and N addition treatment data were selected and the interacting effects were excluded.

For each study, we noted the ecosystem type (or plant type), N addition rate, fertilizer type, treatment duration, the response variables, and other background information (e.g., soil type and pH, mean annual precipitation, and ambient N deposition). Furthermore, specific name and plant functional types (e.g., broadleaf/needle, evergreen/deciduous, perennial/annual, C₃/C₄, N₂-fixing/non-N₂-fixing) were noted for the sampled plants. Meanwhile, the change of corresponding N content was also extracted if reported. We used Getdata software to extract the data which were expressed in figures.

In order to better understand the responses of $\delta^{15}\text{N}$ signature to experimental treatments, we grouped our data according to ecosystem/plant type (forest, shrubland, grassland, cropland/trees, shrubs, herbs, crops, moss and lichen), N addition rate (<100, 100–200, and ≥ 200 kg N ha⁻¹ yr⁻¹), fertilizer type (urea, NH₄NO₃, NH₄Cl/(NH₄)₂SO₄, KNO₃/NaNO₃, and manure), and treatment duration (<3, 3–10, and >10 years). We also grouped plants into different organs (leaf, wood, grain, and root) and functional types.

Statistical analyses

Due to the negative values of $\delta^{15}\text{N}$, the commonly used “effect size”—the natural log of the response ratio (Hedges et al. 1999), cannot be applied. We used the Hedges’ d as the effect size instead (Hedges and Olkin 1985) to quantify the response of $\delta^{15}\text{N}$ signatures to N addition. We assumed that other factors were constant and only N input rates were different between the treatments (N addition and control). The d value was calculated as Eq. 1.

$$d = \frac{(X_e - X_c)}{S} J \quad (1)$$

$$S = \sqrt{\frac{(N_e - 1)(S_e)^2 + (N_c - 1)(S_c)^2}{N_c + N_e - 2}} \quad (2)$$

$$J = 1 - \frac{3}{4(N_c + N_e - 2) - 1} \quad (3)$$

where X_e and X_c are the mean values of $\delta^{15}\text{N}$ (‰) under N addition treatment and control, respectively; S is the

pooled standard deviation (Eq. 2); and J is used to correct the bias when sample sizes are small (Eq. 3). N_e and N_c are the sample sizes for the N addition treatment and control groups, respectively. S_e and S_c are the standard deviations (SDs) for the N addition treatment and control groups, respectively. If only standard error (SE) was reported, we calculated SD by multiplying SE by the square roots of sample size N ($\text{SD} = \text{SE} \sqrt{N}$). If neither SD nor SE was reported, the arithmetic mean SD calculated from each data set was used (Wiebe et al. 2006).

The variance of d (v) was approximated using the following equation:

$$v = \frac{N_c + N_e}{N_c N_e} + \frac{d^2}{2(N_c + N_e)} \quad (4)$$

A non-parametric weighting function was used to weight individual studies (Hedges et al. 1999). We calculated the weighting factor w of each observation by the inverse of the pooled variance ($1/v$). Because most studies contained two or more observations for one variable, we adjusted the weight by the total number of observations per study in order to reduce the weight from the same study (Bai et al. 2013) and used the final weight (w') to calculate the mean effect sizes (\bar{d}') for all observations (Eqs. 5–7).

$$w' = w/n \quad (5)$$

$$d' = w' \times d \quad (6)$$

$$\bar{d}' = \frac{\sum_i d'_i}{\sum_i w'_i} \quad (7)$$

where n is the total number of observations in the same study, d' is the weighted effect size, and d'_i and w'_i are the d' and w' of the i th observation, respectively.

In order to determine whether N addition had a significant effect on a variable, we used a fixed-effect model completed by Metawin 2.1 software (Rosenberg et al. 2000). Confidence intervals (CIs) of the weighted effect size were generated using bootstrapping with 60,000 iterations. If the 95 % CI values did not overlap with 0, we deemed that N addition had a significant effect on a variable.

Otherwise, the effect was not considered to be significant.

The difference of plant and soil $\delta^{15}\text{N}$ values between N addition treatment and control under different fertilize types (Δ_{t-c} , ‰) was calculated according to the following equation:

$$\Delta_{t-c} = X_e - X_c \quad (8)$$

At last, a continuous randomized-effect model was used to find out if there was any correlation between the effect size of N addition on $\delta^{15}\text{N}$ signature and the duration and addition rate of N addition treatment. The total heterogeneity of d' among studies (Q_T) was partitioned into the difference among group cumulative effect sizes (Q_M) and the residual error (Q_E) (Rosenberg et al. 2000).

Results

Effects of N addition on plant $\delta^{15}\text{N}$ signatures

Plant $\delta^{15}\text{N}$ value was significantly increased by N addition, and the mean effect size and actual ^{15}N change were 1.1093 (95 % CI 0.0527~1.6321) and 1.25‰, respectively (Fig. 1a and Table 1). When different plant types were considered, $\delta^{15}\text{N}$ values of trees, shrubs, herbs, and moss/lichen were still higher under N addition treatment, and the mean effect sizes were 0.4372 (95 % CI 0.0356~0.871), 0.7003 (95 % CI 0.1055~1.3464), 0.7922 (95 % CI 0.4648~1.1603), and 1.0913 (95 % CI 0.5072~1.7823), respectively (Fig. 1b). However, $\delta^{15}\text{N}$ value of crops showed a non-significant response to N addition (Fig. 1b).

Different experimental methods showed different effects on plant $\delta^{15}\text{N}$ signature (Fig. 1c, d, e). Plant $\delta^{15}\text{N}$ value was most increased when the ammonia salt (NH_4Cl or $(\text{NH}_4)_2\text{SO}_4$) was applied, and the mean effect size was 1.9843 (95 % CI 0.1108~2.0403) (Fig. 1d). NH_4NO_3 , nitrate salt (KNO_3 or NaNO_3), and manure addition treatments all had a positive effect on plant $\delta^{15}\text{N}$ value. Whereas, urea showed a negative effect on plant $\delta^{15}\text{N}$ value (mean effect size = -0.4812, 95 % CI -0.8889~0.0107) (Fig. 1d). The continuous randomized-effect model showed that inorganic N addition rate had a significantly positive correlation with the effect size of N addition on all plant $\delta^{15}\text{N}$ values

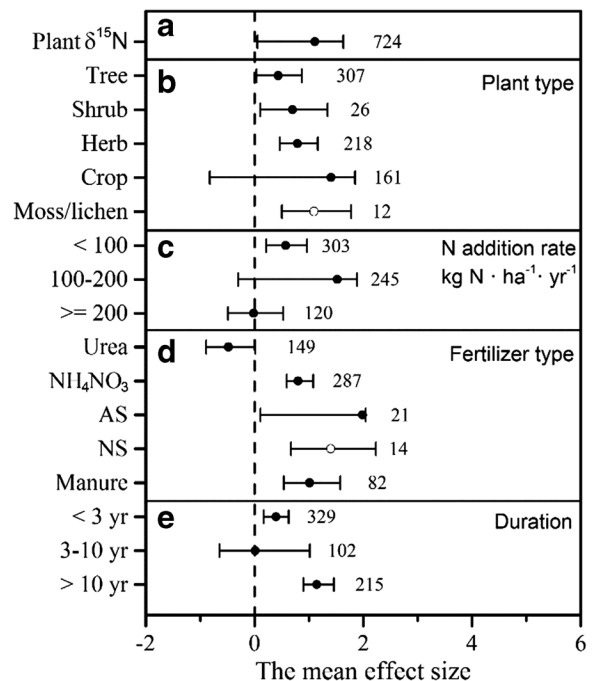


Fig. 1 The mean effect size of nitrogen addition on the natural abundance of plant $\delta^{15}\text{N}$ and the difference among different plant types (b), nitrogen addition rates ($\text{kg N ha}^{-1} \text{ yr}^{-1}$) (c), fertilizer types (d), and duration of treatment (year) (e). The error bars represent the 95 % confidence intervals (CIs). The solid points are the variables with >20 observations, and the hollow points are the variables with <20 observations. The dashed line was drawn at mean effect size = 0. The effect of nitrogen addition was considered to be significant if the 95 % CI of the effect size did not cover zero. The sample size for each variable was shown next to the point. AS ammonium salt ($(\text{NH}_4)_2\text{SO}_4/\text{NH}_4\text{Cl}$), NS nitrate salt ($\text{KNO}_3/\text{NaNO}_3$)

(Table 2). However, when the rates of N addition were divided into three levels (<100, 100–200, and $\geq 200 \text{ kg N ha}^{-1} \text{ yr}^{-1}$), we found that plant $\delta^{15}\text{N}$ value was significantly increased under the lowest level of N addition (<100 $\text{kg N ha}^{-1} \text{ yr}^{-1}$), and non-significant change was found under the other two levels (Fig. 1c). On the other hand, the continuous randomized-effect model showed that the duration of inorganic N addition treatment had a significantly positive correlation with the effect size of N addition on $\delta^{15}\text{N}$ value of shrubs (Table 2). We further partitioned duration into short (<3 years), medium (3–10 years), and long terms (>10 years) and found that the increase of plant $\delta^{15}\text{N}$ value was highest when long-term duration (>10 years) of N addition was applied (mean effect size = 1.1452, 95 % CI 0.8996~1.4620). No significant correlation was found between organic N addition rate and plant $\delta^{15}\text{N}$ value (Table 2).

Table 1 The mean plant and soil $\delta^{15}\text{N}$ values under N addition treatment with different fertilizer types and control and their differences ($\delta^{15}\text{N}_{\text{treatment}} - \delta^{15}\text{N}_{\text{control}}$, Δ_{t-c}) and $\delta^{15}\text{N}$ values of the source fertilizer ($\delta^{15}\text{N}_{\text{fertilizers}}$ based on values in Table S2)

Component	Group	$\delta^{15}\text{N}_{\text{fertilizer}}$ (‰)	$\delta^{15}\text{N}_{\text{treatment}}$ (‰)	$\delta^{15}\text{N}_{\text{control}}$ (‰)	Δ_{t-c} (‰)	Number of observations
Plant	All plant		1.51 (3.46)	0.26 (3.47)	1.25	724
Fertilize type	Urea	-1.50 (2.90)	1.40 (2.77)	0.42 (3.55)	0.98	149
	NH_4NO_3	0.76 (2.86)	0.20 (1.95)	-1.26 (2.50)	1.46	287
	$(\text{NH}_4)_2\text{SO}_4/\text{NH}_4\text{Cl}$	-0.40/1.16 (-/1.79)	3.61 (2.44)	3.38 (2.98)	0.23	21
	$\text{KNO}_3/\text{NaNO}_3$	5.87 (3.32)	4.56 (3.17)	3.23 (2.61)	1.33	14
	Manure	10.19 (7.90)	5.55 (5.45)	2.84 (2.79)	2.71	82
Soil total N	All Soil total N		4.23 (4.37)	4.04 (4.55)	0.19	228
Fertilize type	Urea	-1.50 (2.90)	3.75 (2.40)	3.02 (3.16)	0.73	39
	NH_4NO_3	0.76 (2.86)	2.84 (2.07)	2.19 (2.31)	0.65	59
	$(\text{NH}_4)_2\text{SO}_4$	-0.40 (-)	5.39 (1.05)	5.67 (0.98)	-0.28	8
	Manure	10.19 (7.90)	6.42 (1.56)	4.41 (2.18)	2.01	44

The numbers in the parentheses are the standard deviations

Different plant organs showed different responses to N addition (Fig. 2a). N addition had a significantly positive effect on $\delta^{15}\text{N}$ values of leaf, wood, and root and a significantly negative effect on $\delta^{15}\text{N}$ value of grain (mean effect size = -1.0029, 95 % CI -1.4772~-0.0296). Similarly, different plant functional types also showed different responses to N addition (Fig. 2b, c, d). Trees and shrubs were classified into broadleaf and needle or evergreen and deciduous functional types (Fig. 2b). Plant $\delta^{15}\text{N}$ value in the needle group was significantly increased by N addition with a mean effect size of 1.1243 (95 % CI 0.8821~1.7542), but no significant change was found for the broadleaf group. Both the evergreen and the deciduous groups showed positive change of plant $\delta^{15}\text{N}$ value under N addition, and the mean effect sizes were 0.5468 (95 % CI 0.0920~1.0525) and 0.2502 (95 % CI -0.0304~0.5429), respectively. Grasses and crops were classified into perennial and annual functional groups, and we found that $\delta^{15}\text{N}$ value of perennial plants was significantly increased by N addition (mean effect size = 0.7483, 95 % CI 0.4038~1.3110), and $\delta^{15}\text{N}$ value of annual plants had no significant change (Fig. 2c). When plants were divided into C_3 and C_4 groups according to their photosynthesis pathways, the effect size of N addition on $\delta^{15}\text{N}$ value of C_3 plants was obviously higher than that of C_4 plants, although neither was significant (Fig. 2d). When plants were divided into non- N_2 -fixing and N_2 -fixing functional groups, the change of $\delta^{15}\text{N}$ value by N addition was higher for non- N_2 -fixing plants than for N_2 -fixing plants (Fig. 2d).

Effects of N addition on soil $\delta^{15}\text{N}$ signatures

The $\delta^{15}\text{N}$ values of soil total N and soil NO_3^- were significantly increased by N addition with mean effect sizes of 0.6685 (95 % CI 0.3894~0.9626) and 0.6841 (95 % CI 0.1742~1.2628), respectively. On the contrary, there was a negative effect of N addition on $\delta^{15}\text{N}$ values of soil NH_4^+ (mean effect size = -0.2007, 95 % CI -1.2467~0.6482) and soil N_2O (mean effect size = -1.5639, 95 % CI -2.3180~-0.9896) (Fig. 3a). There were only two observations for $\delta^{15}\text{N}$ value of soil leachate, which were not enough for evaluation (Fig. 3a).

All of the four ecosystem types (forest, shrubland, grassland, and cropland) showed a positive effect of N addition on $\delta^{15}\text{N}$ value of soil total N (Fig. 3b). The $\delta^{15}\text{N}$ value of soil total N was significantly increased by urea, NH_4NO_3 , and manure fertilization but was significantly decreased by $(\text{NH}_4)_2\text{SO}_4$ addition (Fig. 3d). The mean effect size and actual ^{15}N discrimination were 1.7046 and 2.01‰ when manure was applied, reaching the highest level (Fig. 3d and Table 1). The continuous randomized-effect model and classified N addition rate analysis both showed that the effect of N addition on $\delta^{15}\text{N}$ value of soil total N was independent of N addition rate (Table 2 and Fig. 3c). Although the continuous randomized-effect model showed that inorganic and organic N addition duration had little correlation with the effect size of N addition on $\delta^{15}\text{N}$ value of soil total N (Table 2), long-term duration (>10 years) did have a significantly more positive effect on $\delta^{15}\text{N}$ value of soil

Table 2 Relationships between the effect size of nitrogen addition on plant (or soil) $\delta^{15}\text{N}$ and N addition rate (or treatment duration)

	Q_T	Q_M	Q_E	Slope	<i>P</i> value
Inorganic N addition rate					
Plant	223.399	29.662	193.738	0.0096	0.00000
Tree	32.606	0.145	32.461	-0.0018	0.70336
Shrub	8.404	0.003	8.401	0.0037	0.52644
Herb	36.717	0.031	36.686	-0.0008	0.86021
Crop	33.707	1.677	32.030	0.0060	0.19532
Soil total N	36.323	0.966	35.358	-0.0041	0.32577
Soil NO_3^-	4.833	0.038	4.795	0.0022	0.84610
Soil NH_4^+	9.684	3.740	5.944	-0.0225	0.05312
Inorganic N treatment duration					
Plant	94.299	2.984	91.314	0.0001	0.08407
Tree	32.606	1.011	31.595	0.0001	0.31467
Shrub	12.329	3.882	8.447	0.0057	0.04880
Herb	30.812	0.684	30.127	0.0002	0.40814
Crop	4.501	0.121	4.380	-0.0001	0.72764
Soil total N	29.269	1.430	27.838	0.0001	0.23171
Soil NO_3^-	8.239	0.659	7.581	0.0002	0.41700
Soil NH_4^+	10.687	6.756	3.931	0.0006	0.00934
Organic N addition rate					
Plant	21.265	0.245	21.020	-0.0009	0.62040
Herb	10.914	0.037	10.876	-0.0005	0.84649
Crop	9.687	2.669	7.017	0.0204	0.10231
Soil total N	10.610	1.845	8.764	0.0050	0.17435
Organic N treatment duration					
Plant	35.123	1.343	33.780	0.0002	0.24651
Herb	10.914	2.952	7.961	0.0005	0.08576
Crop	21.916	0.048	21.867	-0.0001	0.82592
Soil total N	6.586	1.449	5.137	0.0003	0.22862

Statistical results were reported as total heterogeneity in effect sizes among studies (Q_T), the difference among group cumulative effect sizes (Q_M), and the residual error (Q_E) from continuous randomized-effect model meta-analyses. The relationship is significant when $P < 0.05$

total N with a mean effect size of 0.9524 (95 % CI 0.5861~1.4946) compared to the two treatments with short duration (Fig. 3e).

Discussion

Effects of N addition on soil $\delta^{15}\text{N}$ signatures

In this meta-analysis, $\delta^{15}\text{N}$ value of soil total N was significantly increased by N addition (Fig. 3a). The $\delta^{15}\text{N}$ value of soil total N is regulated by both N input and N output fluxes. The $\delta^{15}\text{N}$ value of inorganic N

fertilizer generally is close to zero (Table S2), which is lower than soil $\delta^{15}\text{N}$ value in this meta-analysis (Table 1). N addition would have caused a decrease of soil $\delta^{15}\text{N}$ value if output fluxes did not change; so the increasing trend of soil $\delta^{15}\text{N}$ value under N addition we observed must be caused by output fluxes. Our speculation was based on the mass balance model by Brenner et al. (2001), which showed that at steady state, soil $\delta^{15}\text{N}$ is determined by the weighted mean isotopic ratio of inputs divided by the proportion of ^{15}N to ^{14}N leaving the soil (Eq. A11 in Brenner et al. 2001). Because N addition generally decreases the weighted mean isotopic ratio of inputs (Bateman and Kelly 2007), higher soil

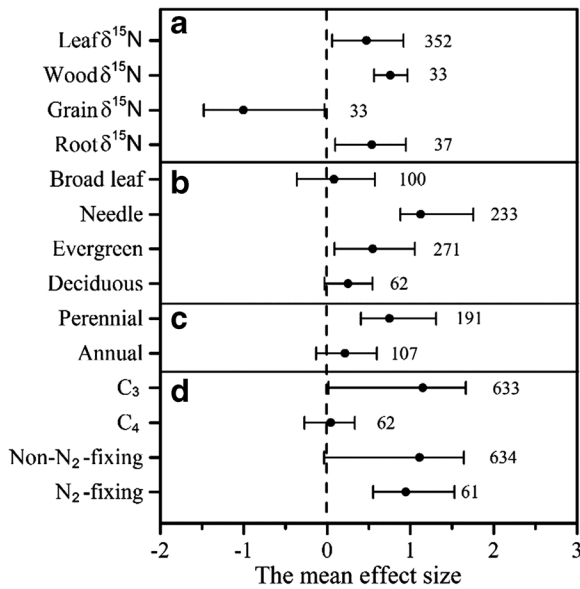


Fig. 2 The mean effect size of nitrogen addition on plant $\delta^{15}\text{N}$ natural abundance of different plant organs (a) and functional types (b–d). The error bars represent the 95 % confidence intervals (CIs). The effect of nitrogen addition was considered to be significant if the 95 % CI of the effect size did not cover zero. The sample size for each variable was shown next to the point

$\delta^{15}\text{N}$ value under N addition must be caused by lower proportion of ^{15}N to ^{14}N leaving the soil. Therefore, we speculate that the losses of ^{15}N -depleted pools through leaching and gaseous emissions increased following N addition. However, studies on $\delta^{15}\text{N}$ values of gaseous losses and soil leachate are extremely rare and difficult and we could not find enough observation data for this meta-analysis. A previous study suggested that the $\delta^{15}\text{N}$ value of volatilized ammonia ranged from -28 to -0.3‰ , which was more depleted than $\delta^{15}\text{N}$ value of the originally added urea-N (1.2‰) (Frank et al. 2004). Koba et al. (2012) found that both NH_4^+ in stream water and soil-emitted N_2O were extremely ^{15}N depleted (the $\delta^{15}\text{N}$ values were -9.2 and -14.3‰ , respectively) compared to soil $\delta^{15}\text{N}$ value ($+3.9\text{‰}$) in an N-saturated subtropical forest. These N output fluxes usually lead to an isotope fractionation against ^{15}N , leaving heavier ^{15}N in soil (Robinson 2001). In addition, plant N uptake also could cause isotope discrimination, enriching soil $\delta^{15}\text{N}$ value in a short term (Evans 2001). Therefore, this result suggested that soil $\delta^{15}\text{N}$ value was more affected by N loss fluxes and plant N uptake than by N inputs.

The $\delta^{15}\text{N}$ value of soil NO_3^- was significantly increased by N addition, while $\delta^{15}\text{N}$ value of soil NH_4^+ was not significantly changed (showing a decreasing

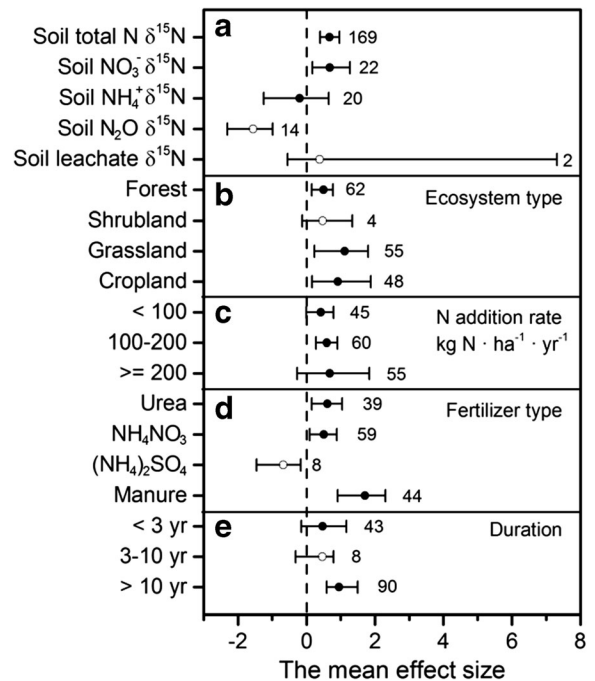
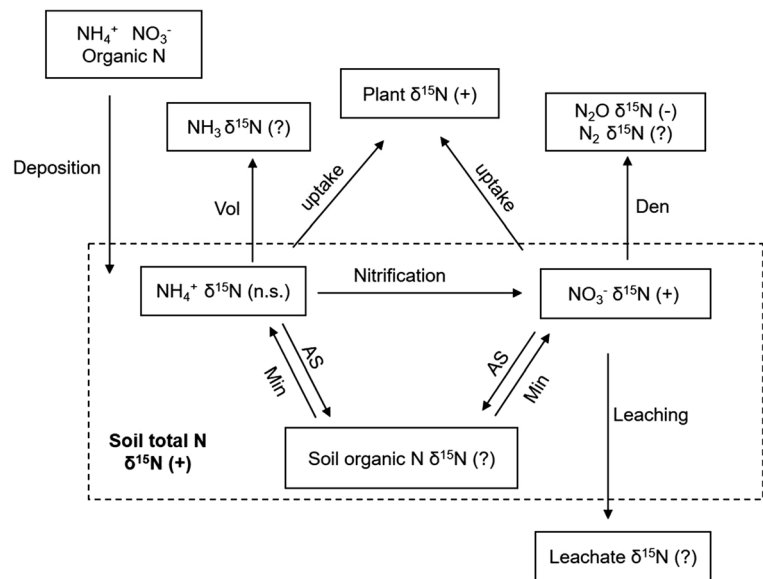


Fig. 3 The mean effect size of nitrogen addition on soil $\delta^{15}\text{N}$ natural abundance and the difference among different ecosystems (b), nitrogen addition rates ($\text{kg N} \cdot \text{ha}^{-1} \cdot \text{yr}^{-1}$) (c), fertilizer types (d), and duration of treatment (year) (e). The error bars represent the 95 % confidence intervals (CIs). The solid points are the variables with >20 observations, and the hollow points are the variables with <20 observations. The dashed line was drawn at mean effect size = 0. The effect of nitrogen addition was considered to be significant if the 95 % CI of the effect size did not cover zero. The sample size for each variable was shown next to the point

trend) by N addition (Fig. 3a). When the integrated $\delta^{15}\text{N}$ value of input fluxes is higher than the integrated $\delta^{15}\text{N}$ value of output fluxes for an N pool, its $\delta^{15}\text{N}$ value is increased and vice versa. The input fluxes (nitrification and direct N addition (including N deposition)) and the output fluxes (immobilization, denitrification, leaching, and plant uptake) of soil NO_3^- together determine the size of this pool. The integrated $\delta^{15}\text{N}$ value of nitrification and N addition derived NO_3^- should generally have been reduced by N addition (Robinson 2001), so the increase of $\delta^{15}\text{N}$ value of soil NO_3^- by N addition was most likely caused by the output fluxes. This result indicated that N addition decreased the integrated $\delta^{15}\text{N}$ value of output fluxes for NO_3^- , which was most likely caused by the increase of ^{15}N -depleted outputs such as denitrification and leaching (Robinson 2001). Although N addition increased $\delta^{15}\text{N}$ value of soil NO_3^- (3.412‰), it was still lower than soil $\delta^{15}\text{N}$ value (4.082‰). Therefore, the increased losses of this pool

Fig. 4 Conceptual model of $\delta^{15}\text{N}$ response to nitrogen addition based on our meta-analysis. *Min* mineralization, *AS* assimilation, *Vol* ammonia volatilization, *Den* denitrification. The *plus sign* means a significant positive effect, the *minus sign* means a significant negative effect, the *question mark* means not clear at present, and *n.s.* means not significant



would still cause the enrichment of soil $\delta^{15}\text{N}$ value under N addition. In addition, the observation number is too low for studies on N addition effects on $\delta^{15}\text{N}$ value of soil NO_3^- and more studies are needed to evaluate its responses. In our meta-analysis, the two observations of N addition on soil leachate were too few, which were not enough to evaluate (Fig. 3a). We suggest that more studies should be carried out on this topic. On the other hand, the decrease of $\delta^{15}\text{N}$ value of N_2O by N addition in the meta-analysis could further support the result that the increased $\delta^{15}\text{N}$ value of soil NO_3^- was mainly caused by denitrification (Fig. 3a). N losses via NO_x and N_2 also contribute to the enrichment of soil $\delta^{15}\text{N}$ value, whose effects could be much bigger than the effects of N_2O emission (Robinson 2001). However, due to the low observation numbers on NO_x and N_2 losses under N addition, we did not include these fluxes in our study.

For soil NH_4^+ , the input fluxes are mineralization and direct N addition (including N deposition), while the output fluxes are immobilization, nitrification, ammonia volatilization, and plant uptake. As discussed above, the increase of output fluxes would have enriched $\delta^{15}\text{N}$ value of soil NH_4^+ , so the unchanged soil NH_4^+ under N addition was most likely the balanced result between input and output fluxes. We speculate that the increase of N mineralization under N addition (Lu et al. 2011) produced more ^{15}N -depleted NH_4^+ , reducing $\delta^{15}\text{N}$ value of soil NH_4^+ . Therefore, our results suggest that N addition has accelerated many N-cycling processes such

as denitrification and mineralization, which are reflected by the change of natural abundance of nitrogen isotopes.

The $\delta^{15}\text{N}$ value of soil total N was significantly increased by N addition in forest, grassland, and cropland but was not affected by N addition in shrubland (Fig. 3b). There were only four observations in shrubland, which may be the potential reason of the discrepancy. More studies should be carried out in shrubland to better understand the responses of nitrogen cycling to N addition in this ecosystem type.

Our results indicated that $\delta^{15}\text{N}$ value of soil total N was increased most when manure was applied among all types of fertilizer (Fig. 3d). Manure is more enriched in ^{15}N than inorganic fertilizer in this meta-analysis (Table S2), which is due to the isotope fractionation during digestion processes and ammonium volatilization (Kerley and Jarvis 1996; Choi et al. 2002). Urea and NH_4NO_3 application also increased $\delta^{15}\text{N}$ value of soil total N, but application of $(\text{NH}_4)_2\text{SO}_4$ showed a different effect (Fig. 3d). Previous studies indicated that the growth of ammonia-oxidizing bacteria was restrained, and thus, nitrification was inhibited when $(\text{NH}_4)_2\text{SO}_4$ was applied (Tong and Xu 2012). This inhibition of microbial activities by $(\text{NH}_4)_2\text{SO}_4$ might be the potential reason of the decrease of soil $\delta^{15}\text{N}$ value under $(\text{NH}_4)_2\text{SO}_4$ treatment.

The continuous randomized-effect model showed no correlation between the effect size of N addition on soil $\delta^{15}\text{N}$ value and N addition rate or treatment duration (Table 1). However, when treatment duration was

classified into three categories, $\delta^{15}\text{N}$ value was significantly higher under longer-term (>10 years) treatment compared to the other two shorter terms. These results indicated that increasing N addition rate had minor effects on soil $\delta^{15}\text{N}$ value, but with increasing treatment duration, soil $\delta^{15}\text{N}$ value may be more affected by N addition.

It should be noted that other factors, such as water availability, nutrient status, microbial community composition, and soil physical properties, can also affect plant and soil ^{15}N signatures (Högberg 1997; Robinson 2001; Pardo and Nadelhoffer 2010). While N addition treatment changed N input rates, those above-mentioned factors may also be affected, changing the effect size of N addition treatment. Therefore, it is important to examine if other factors also changed when N addition treatment was applied and caution should be taken when explaining the responses of plant and soil $\delta^{15}\text{N}$ to N inputs due to the complexity of natural ^{15}N abundance.

Effects of N addition on plant $\delta^{15}\text{N}$ signatures

N addition significantly increased plant $\delta^{15}\text{N}$ value (Fig. 1). The source of plant N of non- N_2 -fixing plants is soil N; therefore, the increase of plant $\delta^{15}\text{N}$ value was directly linked to the rise of soil $\delta^{15}\text{N}$ value (Fig. 3). In addition, the isotope fractionation during plant N uptake (Evans 2001) might have been decreased by N addition, enriching plant $\delta^{15}\text{N}$ value. When soil N availability was lower, plants might depend more on mycorrhizal fungi, which usually transfer ^{15}N -depleted N to plants (Hobbie and Colpaert 2003). Therefore, N addition increased N availability, weakening the role of mycorrhizal fungi, which could increase plant $\delta^{15}\text{N}$ value. Different plant types did not show different response, except for the big variation of the effect size of N addition on crop $\delta^{15}\text{N}$ value (Fig. 1). The effect size of inorganic N addition on $\delta^{15}\text{N}$ value of shrubs showed a positive correlation with treatment duration, and the effect size of inorganic N addition on $\delta^{15}\text{N}$ value of all types of plants showed a positive correlation with N addition rate (Table 1), which suggested that cumulative enrichment of plant $\delta^{15}\text{N}$ value might happen with increasing inorganic N addition duration and rate. However, no significant correlation was found between organic N addition rate (or treatment duration) and plant $\delta^{15}\text{N}$ value. One reason was because studies on organic N addition were mostly done in croplands with high N addition rates.

Therefore, the lack of data under low N addition levels and long-term treatments caused the insignificant relationship between organic N addition rate (or treatment duration) and plant (or soil) $\delta^{15}\text{N}$ values. In addition, the mean $\delta^{15}\text{N}$ value of manure was 10.19‰ with high-standard variation (Table S2), which made the variations of plant or soil $\delta^{15}\text{N}$ values more complex and uncertain.

It is worth noting that under urea addition, plant $\delta^{15}\text{N}$ value was decreased (Fig. 1), although soil $\delta^{15}\text{N}$ value was significantly increased (Fig. 3). We speculate that the following three potential reasons were responsible for this phenomenon. First, substantial ^{15}N -depleted NH_3 would be volatilized when urea was applied (Elliot and Fox 2014), and studies suggested that plant shoot and leaves can directly absorb NH_3 from the air when its partial pressure exceeded a certain threshold (Farquhar et al 1980; Ping et al. 2000; Frank et al. 2004; Boaretto et al. 2013). Second, during the hydrolysis of urea, some ^{15}N -depleted NH_4^+ was quickly used by plant roots (Bateman and Kelly 2007), while the rest NH_4^+ underwent N transformation processes which enriched soil N pools. Lastly, many plants can take up urea intactly (Witte 2011), making urea different from other N forms as N sources.

The $\delta^{15}\text{N}$ value of different plant organs showed various responses to N addition (Fig. 2a). Both leaf and root $\delta^{15}\text{N}$ values were significantly increased by N addition with similar mean effect sizes. Therefore, although there could be intra-plant isotope discrimination (Evans 2001), the effect of N addition on this discrimination was minimal based on our meta-analysis. The effect size of N addition on wood $\delta^{15}\text{N}$ value was also positive (Fig. 2a), although wood $\delta^{15}\text{N}$ value reflects cumulative N with plant growth (Hietz et al. 2010). Interestingly, we observed that $\delta^{15}\text{N}$ value of crop grains was significantly reduced by N addition. Grain $\delta^{15}\text{N}$ value is generally more negative than shoot and root $\delta^{15}\text{N}$ values (Yun et al. 2011). Therefore, the reduction of grain $\delta^{15}\text{N}$ value by N addition hinted increased isotopic discrimination during N transport to grain. The ^{15}N fractionation processes such as assimilation, reallocation, and transformation to grain may be affected by N addition (Serret et al. 2008; Zhou et al. 2013).

For trees and shrubs, $\delta^{15}\text{N}$ value of needle plants was increased most by N addition, while $\delta^{15}\text{N}$ value of broadleaf plants was unresponsive to N addition (Fig. 2b). In general, broadleaf plants are more ^{15}N enriched than needle plants (Martinelli et al. 1999;

Fang et al. 2011). So, our results indicated that needle $\delta^{15}\text{N}$ value may be more sensitive to N addition. A previous study also demonstrated that leaf $\delta^{15}\text{N}$ values showed site-specific and species-specific responses to N addition (Silva et al. 2015). Of course, other factors, such as N preference, may also contribute to this phenomenon (Evans 2001).

For herbs and crops, we classified them into perennial and annual function groups. We found that $\delta^{15}\text{N}$ value of perennial plants was significantly increased while $\delta^{15}\text{N}$ value of annual plants was unchanged by N addition (Fig. 2c). This was probably due to their different N utilization strategies. A meta-analysis pointed out that the increase of N in annual plants was higher than that in perennial plants under N addition, probably because annual plants had advantages of absorbing added N (Xia and Wan 2008). Because added N was generally more ^{15}N -depleted N compared to soil N, N addition effects on $\delta^{15}\text{N}$ value of annual plants were thus weakened.

The effect size of N addition on $\delta^{15}\text{N}$ value of C_3 plants was bigger than that on $\delta^{15}\text{N}$ value of C_4 plants (Fig. 2d). The underlying mechanism is still unclear. Similar to non- N_2 -fixing plants, $\delta^{15}\text{N}$ value of N_2 -fixing plants was significantly increased by N addition (Fig. 2d). Plant $\delta^{15}\text{N}$ values of N_2 -fixing plants are generally close to 0‰, because of their N source from atmospheric N_2 . However, N_2 fixation is a process with high energy consumption (Gutschick 1981; Fisher et al. 2010), and with N addition, N_2 -fixing plants would shift a proportion of their N sources from atmospheric N_2 to soil N to avoid high energetic cost. Previous studies have found that nodule formation and nitrigenase activity were inhibited by N addition, and the function of biological N_2 fixation could be reduced (Streeter and Wong 1988; Vessey and Waterer 1992). Therefore, $\delta^{15}\text{N}$ value of N_2 -fixing plants was also significantly changed by N addition.

Conclusion

Based on our meta-analysis of 48 studies, the conceptual model of plant and soil $\delta^{15}\text{N}$ signatures to N addition is presented in Fig. 4. This is only a conceptual model, which reflects general patterns but may not fit all cases equally well. In summary, N addition generally increased both soil and plant $\delta^{15}\text{N}$ values, which suggested that N addition would increase N losses via gases

or leaching pathways. The $\delta^{15}\text{N}$ value of soil NO_3^- was significantly increased by N addition, while $\delta^{15}\text{N}$ value of soil NH_4^+ showed a decreasing trend under N addition, although it was not significant. The $\delta^{15}\text{N}$ value of needle plants was increased the most among all types of trees and shrubs. The $\delta^{15}\text{N}$ value of perennial grasses showed higher responses to N addition than that of annual grasses. The significantly positive relationships between inorganic N rate and the effect size of inorganic N addition on $\delta^{15}\text{N}$ value of all types of plants indicated that a cumulative enrichment of plant $\delta^{15}\text{N}$ value might happen with increasing N addition duration. Our study revealed a comprehensive picture of the effects of N addition on $\delta^{15}\text{N}$ signatures in terrestrial ecosystems, which could help us to understand how plant and soil $\delta^{15}\text{N}$ values respond to N addition and to better use $\delta^{15}\text{N}$ signature as an indicator to predict how ecosystem N status would respond to increasing N deposition or fertilization. Ecosystem $\delta^{15}\text{N}$ signatures may provide information on thresholds of atmospheric N levels and should be more explored in global climate change studies.

Acknowledgments This work was supported by the National Basic Research Program of China (973 Program; 2014CB954400), the National Natural Science Foundation of China (31522010), and Key Research Program of Frontier Sciences, CAS (QYZDB-SSW-DQC006).

References

- Amundson R, Austin AT, Schuur EAG, Yoo K, Matzek V, Kendall C, Uebersax A, Brenner D, Baisden WT (2003) Global patterns of the isotopic composition of soil and plant nitrogen. *Glob Biogeochem Cycles* 17:1031
- Bai E, Houlton BZ (2009) Coupled isotopic and process-based modeling of gaseous nitrogen losses from tropical rain forests. *Glob Biogeochem Cycles* 23:GB2011
- Bai E, Li S, Xu W, Li W, Dai W, Jiang P (2013) A meta-analysis of experimental warming effects on terrestrial nitrogen pools and dynamics. *New Phytol* 199:441–451
- Bateman AS, Kelly SD (2007) Fertilizer nitrogen isotope signatures. *Isot Environ Healt Stud* 43:237–247
- Boaretto RM, Mattos D, Quaggio JA, Cantarella H, Trivelin PCO (2013) Absorption of $^{15}\text{NH}_3$ volatilized from urea by *Citrus* trees. *Plant Soil* 365:283–290
- Bouwman L, Goldewijk KK, Van Der Hoek KW, Beusen AHW, Van Vuuren DP, Willems J, Rufino MC, Stehfest E (2013) Exploring global changes in nitrogen and phosphorus cycles in agriculture induced by livestock production over the 1900–2050 period. *Proc Natl Acad Sci U S A* 110:20882–20887

- Brenner DL, Amundson R, Baisden WT, Kendall C, Harden J (2001) Soil N and ^{15}N variation with time in a California annual grassland ecosystem. *Geochim Cosmochim Acta* 65:4171–4186
- Brookshire ENJ, Hedin LO, Newbold JD, Sigman DM, Jackson JK (2012) Sustained losses of bioavailable nitrogen from montane tropical forests. *Nat Geosci* 5:123–126
- Choi W-J, Lee S-M, Ro H-M, Kim K-C, Yoo S-H (2002) Natural ^{15}N abundances of maize and soil amended with urea and composted pig manure. *Plant Soil* 245:223–232
- Craine JM, Brookshire ENJ, Cramer MD, Hasselquist NJ, Koba K, Marin-Spiotta E, Wang L (2015) Ecological interpretations of nitrogen isotope ratios of terrestrial plants and soils. *Plant Soil*: 1–26
- Craine JM, Elmore AJ, Aidar MPM, Bustamante M, Dawson TE, Hobbie EA, Kahmen A, Mack MC, McLaughlan KK, Michelsen A, Nardoto GB, Pardo LH, Peñuelas J, Reich PB, Schuur EAG, Stock WD, Templer PH, Virginia RA, Welker JM, Wright IJ (2009) Global patterns of foliar nitrogen isotopes and their relationships with climate, mycorrhizal fungi, foliar nutrient concentrations, and nitrogen availability. *New Phytol* 183:980–992
- Elliot JR, Fox TR (2014) Ammonia volatilization following fertilization with urea or ureaform in a thinned loblolly pine plantation. *Soil Sci Soc Am J* 78:1469–1473
- Evans RD (2001) Physiological mechanisms influencing plant nitrogen isotope composition. *Trends Plant Sci* 6:121–126
- Fang H, Yu G, Cheng S, Zhu T, Zheng J, Mo J, Yan J, Luo Y (2011) Nitrogen-15 signals of leaf-litter-soil continuum as a possible indicator of ecosystem nitrogen saturation by forest succession and N loads. *Biogeochemistry* 102:251–263
- Farquhar GD, Firth PM, Wetselaar R, Weir B (1980) On the gaseous exchange of ammonia between leaves and the environment: determination of the ammonia compensation point. *Plant Physiol* 66:710–714
- Fisher JB, Sitch S, Malhi Y, Fisher RA, Huntingford C, Tan SY (2010) Carbon cost of plant nitrogen acquisition: a mechanistic, globally applicable model of plant nitrogen uptake, retranslocation, and fixation. *Glob Biogeochem Cycles* 24:GB1014
- Frank DA, Evans RD, Tracy BF (2004) The role of ammonia volatilization in controlling the natural ^{15}N abundance of a grazed grassland. *Biogeochemistry* 68:169–178
- Galloway JN, Townsend AR, Erismann JW, Bekunda M, Cai Z, Freney JR, Martinelli LA, Seitzinger SP, Sutton MA (2008) Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. *Science* 320:889–892
- Gutschick VP (1981) Evolved strategies in nitrogen acquisition by plants. *Am Nat* 607:637
- Hedges LV, Gurevitch J, Curtis PS (1999) The meta-analysis of response ratios in experimental ecology. *Ecology* 80:1150–1156
- Hedges LV, Olkin I (1985) *Statistical method for meta-analysis*. Academic press, New York
- Hietz P, Dünisch O, Wanek W (2010) Long-term trends in nitrogen isotope composition and nitrogen concentration in Brazilian rainforest trees suggest changes in nitrogen cycle. *Environ Sci Technol* 44:1191–1196
- Hobbie EA, Colpaert JV (2003) Nitrogen availability and colonization by mycorrhizal fungi correlate with nitrogen isotope patterns in plants. *New Phytol* 157:115–126
- Högberg P (1997) Tansley review no. 95 ^{15}N natural abundance in soil-plant systems. *New Phytol* 137:179–203
- Högberg P, Johannisson C, Högberg MN (2014) Is the high ^{15}N natural abundance of trees in N-loaded forests caused by an internal ecosystem N isotope redistribution or a change in the ecosystem N isotope mass balance? *Biogeochemistry* 117:351–358
- Houlton BZ, Sigman DM, Hedin LO (2006) Isotopic evidence for large gaseous nitrogen losses from tropical rainforests. *Proc Natl Acad Sci U S A* 103:8745–8750
- Kalcsits LA, Buschhaus HA, Guy RD (2014) Nitrogen isotope discrimination as an integrated measure of nitrogen fluxes, assimilation and allocation in plants. *Physiol Plantarum* 151:293–304
- Kerley SJ, Jarvis SC (1996) Preliminary studies of the impact of excreted N on cycling and uptake of N in pasture systems using natural abundance stable isotopic discrimination. *Plant Soil* 178:287–294
- Koba K, Fang Y, Mo J, Zhang W, Lu X, Liu L, Zhang T, Takebayashi Y, Toyoda S, Yoshida N, Suzuki K, Yoh M, Senoo K (2012) The ^{15}N natural abundance of the N lost from an N-saturated subtropical forest in southern China. *J Geophys Res* 117:1–13
- Kriszan M, Amelung W, Schellberg J, Gebbing T, Kühbauch W (2009) Long-term changes of the $\delta^{15}\text{N}$ natural abundance of plants and soil in a temperate grassland. *Plant Soil* 325:157–169
- Lu M, Yang Y, Luo Y, Fang C, Zhou X, Chen JK, Yang X, Li B (2011) Responses of ecosystem nitrogen cycle to nitrogen addition: a meta-analysis. *New Phytol* 189:1040–1050
- Martinelli LA, Piccolo MC, Townsend AR, Vitousek PM, Cuevas E, McDowell W, Robertson GP, Santos OC, Treseder K (1999) Nitrogen stable isotopic composition of leaves and soil: tropical versus temperate forests. *Biogeochemistry* 46:45–65
- Ping J, Bremer E, Janzen HH (2000) Foliar uptake of volatilized ammonia from surface-applied urea by spring wheat. *Commun Soil Sci Plant* 31:165–172
- Pardo LH, Nadelhoffer KJP (2010) Using nitrogen isotope ratios to assess terrestrial ecosystems at regional and global scales. In: West JB, Bowen GJ, Dawson TE, Tu KP (eds) *Isoscapes: understanding movement, pattern, and process on Earth through isotope mapping*. Springer-Verlag, New York
- Robinson D (2001) $\delta^{15}\text{N}$ as an integrator of the nitrogen cycle. *Trends Ecol Evol* 16:153–162
- Rosenberg MS, Adams DC, Gurevitch J (2000) *MetaWin: statistical software for meta-analysis*. Sinauer Associates, Sunderland
- Serret MD, Ortiz-Monasterio I, Pardo A, Araus JL (2008) The effects of urea fertilisation and genotype on yield, nitrogen use efficiency, $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ in wheat. *Ann Appl Biol* 153: 243–257
- Silva LCR, Gómez-Guerrero A, Doane TA, Horwath WR (2015) Isotopic and nutritional evidence for species- and site-specific responses to N deposition and elevated CO_2 in temperate forests. *J Geophys Res: Biogeosci* 120:1110–1123
- Streeter J, Wong PP (1988) Inhibition of legume nodule formation and N_2 fixation by nitrate. *Crit Rev Plant Sci* 7:1–23
- Tong D, Xu R (2012) Effects of urea and $(\text{NH}_4)_2\text{SO}_4$ on nitrification and acidification of Ultisols from southern China. *J Environ Sci* 24:682–689
- Vessey JK, Waterer J (1992) In search of the mechanism of nitrate inhibition of nitrogenase activity in legume nodules: recent developments. *Physiol Plant* 84:171–176
- Wiebe N, Vandermeer B, Platt RW, Klassen TP, Moher D, Barrowman NJ (2006) A systematic review identifies a lack

- of standardization in methods for handling missing variance data. *J Clin Epidemiol* 59:342–353
- Witte CP (2011) Urea metabolism in plants. *Plant Sci* 180:431–438
- Xia JY, Wan SQ (2008) Global response patterns of terrestrial plant species to nitrogen addition. *New Phytol* 179:428–439
- Yun S-I, Lim S-S, Lee G-S, Lee S-M, Kim H-Y, Ro H-M, Choi W-J (2011) Natural ^{15}N abundance of paddy rice (*Oryza sativa* L.) grown with synthetic fertilizer, livestock manure compost, and hairy vetch. *Biol Fertil Soils* 47:607–617
- Zhou W, Hu CS, Li J, Christie P, He XH, Ju XT (2013) Natural ^{15}N abundance in winter wheat amended with urea and compost: a long-term experiment. *Pedosphere* 23:835–843