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# Nitrogen addition affects chemical compositions of plant tissues, litter and soil organic matter

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**Abstract.** Increasing nitrogen (N) deposition or fertilization has been found to significantly affect carbon (C) cycling. However, a comprehensive understanding of how different C chemical components of plant, litter, and soil would respond to external N addition is still lacking. We compiled data of 1,160 observations from 52 individual studies and conducted a meta-analysis of N addition effects on 18 variables related to C chemical compositions in terrestrial ecosystems. Results showed that plant lignin (+7.13%), plant protein (+25.94%), and soil lignin (+7.30%) were significantly increased by N addition, and plant hemicellulose (−4.39%) was significantly decreased, whereas plant fiber, plant cellulose, plant non-structural carbohydrate (NSC), litter lignin, and litter cellulose were not significantly changed. The effects of N addition on C chemical composition varied among different ecosystems/plant types and different forms of N addition. Increasing treatment duration did not significantly change the effects of N addition on the chemical composition of plant, litter, and soil C. With increasing N addition rate, the effect of N addition on plant lignin, plant fiber, plant cellulose, and plant protein increased, while the effect of N addition on plant hemicellulose, plant NSC, and litter cellulose became more negative. Our meta-analysis provided a systematic evaluation of the responses of different C chemical components to N addition in the plant–litter–soil continuum. Results suggest that the change of plant and soil C chemical composition under N addition may be beneficial for ecosystem C sequestration and could affect ecosystem structure and function in the future.

**Key words:** carbon cycle; cellulose; lignin; meta-analysis; nitrogen deposition; non-structural carbohydrate.

## INTRODUCTION

With the increase of fertilizer application and fossil fuel combustion, more reactive nitrogen (N) gets into terrestrial ecosystems via atmospheric N deposition, and global mean N deposition rate is expected to reach 200 Tg N/yr by 2050, which is twice the current level (Galloway et al. 2008). Previous studies suggested that more N inputs into terrestrial ecosystems could effectively stimulate plant growth, promoting carbon (C) storage in terrestrial ecosystems (Coyle and Coleman 2005). In addition to the quantity of C, increasing N deposition could also change the quality of C in terrestrial ecosystems by altering the chemical components of plants or the decomposition process of plant C. For instance, increasing N deposition has been found to affect plant lignin content and lignin decomposition in soil (Knorr et al. 2005, Dias et al. 2013). These changes of C chemical composition could have important impacts on plant community, soil fauna and the whole C cycling process (Piccolo 1996, Magill and Aber 1998). Therefore,

it is essential to comprehensively understand how N deposition could influence C quality of both the input (plant C) and the output (C decomposition) processes.

The chemical composition of plant C not only is involved in plant morphological construction, such as cellulose, lignin, etc. (Campbell and Sederoff 1996, Somerville 2006), but also is essential to plant growth and metabolism, such as protein and non-structural carbohydrate (NSC, comprised of starch and sugars; Dietze et al. 2014). When plant C gets into soil via litter and roots, it is used by soil microbes and soil fauna such as earthworms. The chemical composition of soil C could affect soil C storage, soil fertility, and the soil microbial and faunal community (Piccolo 1996, Prescott et al. 2000).

To date, several meta-analyses or review papers have examined the effects of N addition on C pools and fluxes in terrestrial ecosystems (Knorr et al. 2005, Hyvönen et al. 2008, Lu et al. 2011b). However, there is still no meta-analysis on how the ecosystem C chemical composition responds to increasing atmospheric N deposition, although numerous individual studies have investigated the effects of simulated nitrogen deposition on the C chemical composition of plant, litter, and soil organic matter (Dias et al. 2013, Farrer et al. 2013, Tu et al. 2015, Wu et al. 2015). These studies were conducted in various ecosystems and

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whether the results were different among different ecosystems is unclear. In addition, several kinds of N fertilizers (e.g.,  $\text{NH}_4\text{NO}_3$ , urea,  $\text{NaNO}_3$ , and  $(\text{NH}_4)_2\text{SO}_4$ ) were applied and they may have different effects. The duration of N addition treatment also varied from several months to a dozen years. We can employ a comprehensive analysis of all these studies by a meta-analysis to explore if there is a general pattern of N addition effects on ecosystem C chemical composition (Hedges et al. 1999).

We compiled data of 1,160 observations from 52 individual studies. The objectives were (1) to identify the general direction and magnitude of N addition effects on the C chemical composition of plant, litter, and soil, and (2) to investigate the differences among different settings of simulated N deposition experiments (e.g., ecosystem types, plant types, treatment duration, N-addition rates, and N fertilizer types).

## MATERIALS AND METHODS

### Data compilation

Journal articles published before July 2015 were searched using the Web of Science resource. The search terms were “carbon” and “nitrogen addition” or “lignin” and “nitrogen” or “cellulose” and “nitrogen.” A total of 1,160 observations taken from 52 papers (Appendix S1) were selected and the compiled database included 18 variables related to the chemical composition of C (Data S1: Table S1). The following four criteria were applied to select proper studies: (1) only field N addition studies were selected and laboratory incubation studies were not included; (2) at least one of our selected variables was measured; (3) for multifactorial studies, only control and N addition treatment data were used and the interacting effects were excluded; and (4) the means and sample sizes of the chosen variables had to be reported.

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, ambient N deposition, and so on) for each study. Fresh litter and decomposed litter were marked separately; and decomposed litter means the litter had undergone decomposition process. Data were extracted using Getdata software (<http://www.getdata-graph-digitizer.com/index.php>) if the figure was used in the original publication. The standard deviation (SD) was either reported or calculated from the standard error (SE) and sample size ( $\text{SD} = \text{SE} \sqrt{n}$ ).

In order to better identify the responses of variables to experimental treatments, each study was grouped into one of these four ecosystems (or plant types) (forest [trees], shrubland [shrubs], grassland [herbs], and cropland [crops]), one of three levels of N addition rates ( $<100 \text{ kg N} \cdot \text{ha}^{-1} \cdot \text{yr}^{-1}$ ,  $100\text{--}200 \text{ kg N} \cdot \text{ha}^{-1} \cdot \text{yr}^{-1}$ , and  $\geq 200 \text{ kg N} \cdot \text{ha}^{-1} \cdot \text{yr}^{-1}$ ), one of three fertilizer types (ammonium nitrate ( $\text{NH}_4\text{NO}_3$ ), urea, and others), and one of three treatment durations ( $<3 \text{ yr}$ ,  $3\text{--}10 \text{ yr}$ , and

$>10 \text{ yr}$ ). In addition, the crop species included  $\text{C}_3$  plants (*Cynara cardunculus* L., *Brassica napus* L., *Megathyrus maximus*, *Mombaca grass*, *Gossypium hirsutum* L.) and  $\text{C}_4$  plants (*Miscanthus x giganteus* and *Zea mays*).

### Statistical analyses

The natural log of the response ratio ( $R$ ), defined as the effect size (Hedges et al. 1999), was used to measure the response of different chemical components of C to N addition.  $R$  was calculated as the ratio of its value in the N addition group ( $\bar{X}_t$ ) to that in the control group ( $\bar{X}_c$ ) (Eq. 1), and both sides of Eq. 1 were ln-transformed in order to meet analytical criteria:

$$\ln R = \ln(\bar{X}_t / \bar{X}_c) = \ln(\bar{X}_t) - \ln(\bar{X}_c) \quad (1)$$

The variance of  $\ln R$  ( $v$ ) was approximated using the following formula:

$$v = \frac{S_t^2}{n_t \bar{X}_t^2} + \frac{S_c^2}{n_c \bar{X}_c^2} \quad (2)$$

where  $S_t$  and  $S_c$  are the standard deviations (SD) for the N addition treatment and control groups, respectively;  $n_t$  and  $n_c$  are the sample sizes for the N addition treatment and control groups, respectively. If neither SD nor SE was reported, we estimated the missing SD by multiplying the reported mean by the average coefficient of variation (CV) calculated from each data set.

We used a nonparametric weighting function to weight individual studies (Hedges et al. 1999). The weighting factor  $w$  of each observation was calculated as the inverse of the pooled variance ( $1/v$ ). If a study contained two or more observations for one variable, we adjusted the weight by the total number of observations per site to reduce the weight from the same site; and the final weight ( $w'$ ) was calculated according to Eq. 3 (Bai et al. 2013). Finally, The mean effect size ( $\ln R'$ ) of all observations was estimated according to Eqs. 4 and 5

$$w' = w/n \quad (3)$$

$$\ln R' = W' \times \ln R \quad (4)$$

$$\overline{\ln R'} = \frac{\sum_i \ln R'_i}{\sum_i w'_i} \quad (5)$$

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

A fixed-effect model was used in our meta-analysis to determine whether N addition had a significant effect on a variable, using Metawin 2.1 software (Rosenberg et al. 2000). Confidence intervals (CIs) of the weighted effect size were generated using bootstrapping with 9999 iterations.

N addition was considered to have a significant effect on a variable if the 95% CI values did not overlap with 0. Otherwise, the effect was not considered to be

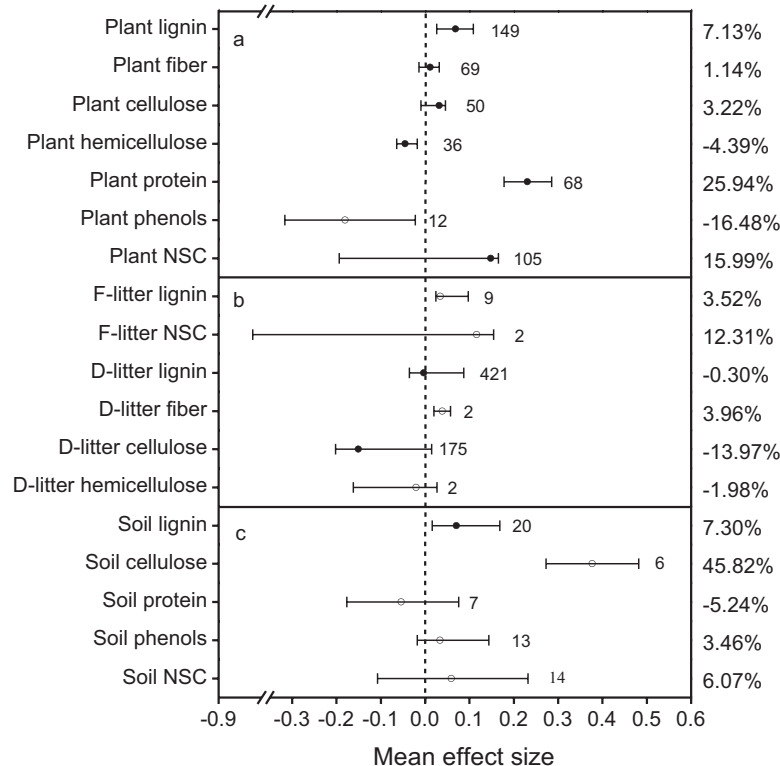


FIG. 1. The mean effect size of nitrogen addition on the chemical composition of carbon in (a) plant, (b) litter, and (c) soil. Error bars represent 95% confidence intervals (CIs). Solid points represent variables with >20 observations and open points represent variables with <20 observations. The dashed 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 is shown next to the point. The data on right-hand y-axis represent the mean percentage change for each variable (%). NSC, non-structural carbohydrate; F-litter, fresh litter; D-litter, decomposed litter.

significant. In order to express the changes of C chemical composition more clearly and simply, we transformed the mean effect size back to the percentage of change

$$(e^{\overline{\ln R'}} - 1) \times 100\%. \quad (6)$$

For better understanding the relationships between the effect size of N addition on C chemical composition and the duration and addition rate of N treatment, a continuous randomized-effect model was used on the variables with >20 observations. The total heterogeneity of  $\ln R'$  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

Among the variables with more than 20 observations (lignin, fiber, cellulose, hemicellulose, protein, phenols, NSC), plant protein was most significantly changed by N addition, and the mean effect size was 0.2306 (95% CI: 0.1778–0.2852). The mean effect size of N addition on plant lignin was 0.0689 (95% CI: 0.0263 to 0.1085), and the 95% CI did not cover zero (Fig. 1a), showing a significantly positive effect of N addition on these variables (Fig. 1a). There

was a negative effect of N addition on plant hemicelluloses with a mean effect size of  $-0.0449$  (95% CI:  $-0.0642$  to  $-0.0178$ ). The effect of N addition on plant fiber, plant cellulose, and plant NSC was not significant, and the mean effect size was 0.0113 (95% CI:  $-0.0136$  to 0.0316), 0.0317 (95% CI:  $-0.0093$  to 0.0459), and 0.1483 (95% CI:  $-0.1938$  to 0.1653), respectively. The effect of N addition on decomposed litter lignin and cellulose was not significant and the mean effect size was  $-0.003$  (95% CI:  $-0.0356$  to 0.0872), and  $-0.1505$  (95% CI:  $-0.2022$  to 0.0147), respectively (Fig. 1b). The mean effect size of N addition on soil lignin was 0.0705 (95% CI: 0.0162 to 0.1682; Fig. 1c).

The number of observations for plant phenols, fresh litter lignin, fresh litter NSC, decomposed litter fiber, decomposed litter hemicellulose, soil cellulose, soil protein, soil phenols, and soil NSC was <20 (Fig. 1). Based on the limited number of observations, we found a positive effect of N addition on fresh litter lignin, decomposed litter fiber, and soil cellulose, and a negative effect of N addition on plant phenols (mean effect size =  $-0.1801$ ; 95% CI:  $-0.3162$  to  $-0.0219$ ), and non-significant effects on the other five variables.

Different ecosystem types showed different responses to N addition (Figs. 2 and 3). For plant lignin, fiber, and

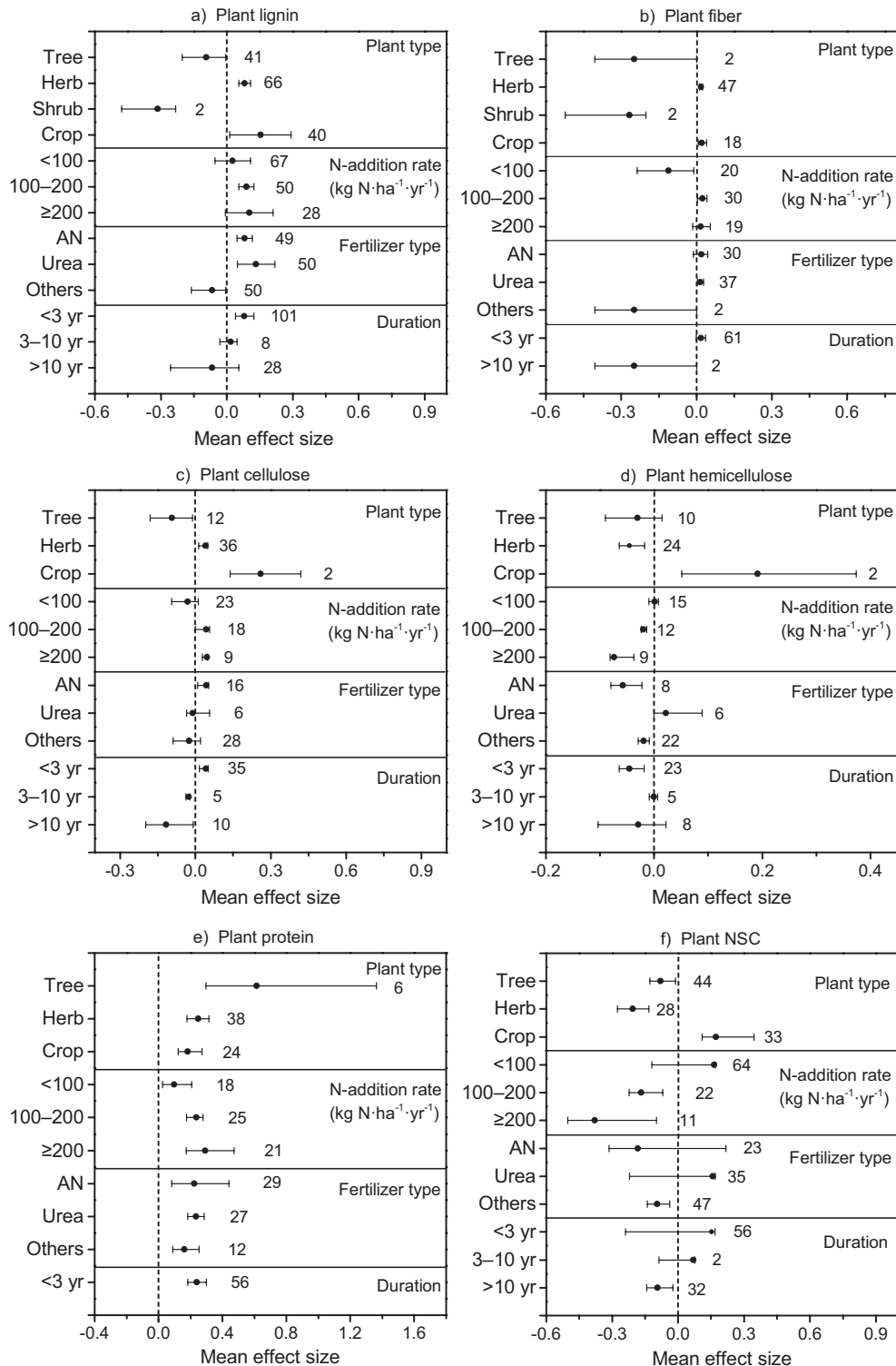


FIG. 2. The effect of nitrogen addition on plant (a) lignin, (b) fiber, (c) cellulose, (d) hemicellulose, (e) protein, and (f) NSC and the difference among different plant types, nitrogen addition rates (kg·ha<sup>-1</sup>·yr<sup>-1</sup>), fertilizer types, and duration of treatment (yr). Error bars represent 95% confidence intervals (CIs). 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 is shown next to the point. NSC, non-structural carbohydrate; AN, ammonium nitrate.

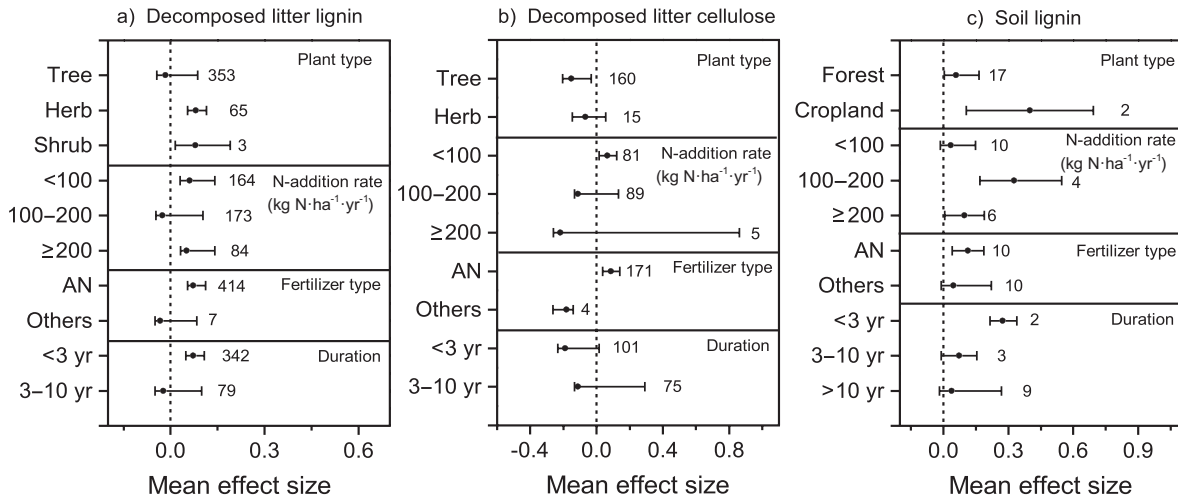


FIG. 3. The mean effect size of nitrogen addition on decomposed litter lignin (a), decomposed litter cellulose (b), and soil lignin (c), and the difference among different ecosystem types, nitrogen addition rates (kg·ha<sup>-1</sup>·yr<sup>-1</sup>), fertilizer types, and duration of treatment (yr). Error bars represent 95% confidence intervals (CIs). 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 is shown next to the point. AN, ammonium nitrate.

cellulose, N addition significantly decreased their content in trees and shrubs, but increased their content in herbs and crops (Fig. 2a–c). For plant protein, the effect of N addition was consistent among all three plant types (trees, herbs, and crops; Fig. 2e). Plant NSC significantly decreased in trees and herbs under N addition, but

significantly increased in crops (Fig. 2f). The effect of N addition on plant hemicellulose was similar to the effect on plant NSC, except for the nonsignificant effects on plant hemicellulose in trees (Fig. 2d). For the response of decomposed litter lignin to N addition, both herbs (mean effect size = 0.0808; 95% CI: 0.0542 to 0.1145) and

TABLE 1. Relationships between the effect size of nitrogen addition on carbon chemical composition and the duration and rate of N addition treatment

| Component           | $Q_T$   | $Q_M$  | $Q_E$   | Slope   | $P$     |
|---------------------|---------|--------|---------|---------|---------|
| Treatment duration  |         |        |         |         |         |
| Plant lignin        | 112.277 | 2.335  | 109.942 | <0.0001 | 0.12647 |
| Plant fiber         | 98.210  | 34.613 | 63.596  | <0.0001 | <0.0001 |
| Plant cellulose     | 55.428  | 9.374  | 46.054  | <0.0001 | 0.00220 |
| Plant hemicellulose | 17.539  | 0.030  | 17.508  | <0.0001 | 0.86219 |
| Plant protein       | 88.842  | 0.479  | 88.363  | –0.0001 | 0.48876 |
| Plant NSC           | 46.483  | 1.505  | 44.978  | <0.0001 | 0.21997 |
| D-Litter lignin     | 56.060  | 15.209 | 40.851  | <0.0001 | 0.00010 |
| D-Litter cellulose  | 84.318  | 3.534  | 80.784  | <0.0001 | 0.06012 |
| Soil lignin         | 11.055  | 0.007  | 11.048  | <0.0001 | 0.93515 |
| N-addition rate     |         |        |         |         |         |
| Plant lignin        | 121.253 | 6.481  | 114.772 | 0.0005  | 0.00900 |
| Plant fiber         | 63.288  | 7.338  | 55.950  | 0.0003  | 0.00675 |
| Plant cellulose     | 40.864  | 9.505  | 31.359  | 0.0003  | 0.00205 |
| Plant hemicellulose | 82.372  | 61.176 | 21.195  | –0.0002 | <0.0001 |
| Plant protein       | 80.905  | 21.267 | 59.639  | 0.0011  | <0.0001 |
| Plant NSC           | 114.484 | 8.465  | 106.019 | –0.0008 | 0.00362 |
| D-Litter lignin     | 56.060  | 0.097  | 55.962  | 0.0001  | 0.75486 |
| D-Litter cellulose  | 84.318  | 13.442 | 70.876  | –0.0007 | 0.00025 |
| Soil lignin         | 16.632  | 0.125  | 16.507  | –0.0001 | 0.72371 |

Notes: 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-effects model meta-analyses. The relationship is significant when  $P < 0.05$ .

shrubs (mean effect size = 0.0797; 95% CI: 0.0154 to 0.1907) had a positive response (Fig. 3a), and trees (mean effect size = -0.0160; 95% CI: -0.0438 to 0.0866) had a nonsignificant response. The effect of N addition on decomposed litter cellulose has been studied in trees and herbs, and a negative response was found in trees (mean effect size = -0.1530; 95% CI: -0.2053 to -0.0324; Fig. 3b). The effect of N addition on soil lignin has been mainly studied in forest ecosystems (mean effect size = 0.0596; 95% CI: 0.0041 to 0.1654), and there were only two observations in cropland (Fig. 3c).

Our results indicated that N addition rates had a significantly positive correlation with the effect size of N addition on plant lignin, plant fiber, plant cellulose, plant protein, and a significantly negative correlation with the effect size of N addition on plant hemicellulose, plant NSC, and decomposed litter cellulose (Table 1). In order to see if there was a nonlinear effect of N addition rate on these variables, we also divided N addition rate into three groups (<100 kg N·ha<sup>-1</sup>·yr<sup>-1</sup>, 100–200 kg N·ha<sup>-1</sup>·yr<sup>-1</sup>, and ≥200 kg N·ha<sup>-1</sup>·yr<sup>-1</sup>). At an N addition rate of 100–200 kg N·ha<sup>-1</sup>·yr<sup>-1</sup>, plant lignin showed a significant positive response (mean effect size = 0.0889; 95% CI: 0.0541–0.1230), whereas, it showed a nonsignificant response at N addition rates of <100 or ≥200 kg N·ha<sup>-1</sup>·yr<sup>-1</sup> (Fig. 2a). At an N addition rate of <100 kg N·ha<sup>-1</sup>·yr<sup>-1</sup>, plant fiber was significantly decreased by N addition (mean effect size = -0.1121; 95% CI: -0.2367 to -0.0110); but it was significantly increased at an N addition rate of 100–200 kg N·ha<sup>-1</sup>·yr<sup>-1</sup>, and was unchanged at N addition rates of ≥200 kg N·ha<sup>-1</sup>·yr<sup>-1</sup> (Fig. 2b). Only at higher rates of N addition (100–200 and ≥200 kg N·ha<sup>-1</sup>·yr<sup>-1</sup>) did plant cellulose show a significantly positive response to N addition (Fig. 2c). All three N addition rates had a significantly positive effect on plant protein (Fig. 2e). With increasing level of N addition rate, plant hemicellulose and NSC were gradually decreased by N addition (Fig. 2d,f). Lignin in decomposed litter increased at N addition rates of <100 and ≥200 kg N·ha<sup>-1</sup>·yr<sup>-1</sup>, and was unchanged at 100–200 kg N·ha<sup>-1</sup>·yr<sup>-1</sup> (Fig. 3a). Cellulose in decomposed litter showed a significantly positive response only at the N addition rate of <100 kg N·ha<sup>-1</sup>·yr<sup>-1</sup>

(Fig. 3b). Soil lignin was not changed at the rate of <100 kg N·ha<sup>-1</sup>·yr<sup>-1</sup>, whereas it increased significantly at higher N addition rates (Fig. 3c).

Different types of fertilizer showed different effects on the examined variables (Figs. 2 and 3). Under NH<sub>4</sub>NO<sub>3</sub> treatment, plant lignin (mean effect size = 0.0814; 95% CI: 0.0458–0.1157), cellulose (mean effect size = 0.0438; 95% CI: 0.0084–0.0525), protein (mean effect size = 0.2245; 95% CI: 0.0816–0.4402) significantly increased, and plant hemicellulose (mean effect size = -0.0579; 95% CI: -0.0801 to -0.0223) significantly decreased. Meanwhile, plant fiber and NSC did not significantly change. The effect of urea was significant on plant lignin (mean effect size = 0.1332; 95% CI: 0.0485–0.2184), fiber (mean effect size = 0.0152; 95% CI: 0.0027–0.0283), and protein (mean effect size = 0.2349; 95% CI: 0.1823–0.2849), but was not significant on other plant variables. The majority of studies on decomposed litter lignin, decomposed litter cellulose and soil lignin were treated by NH<sub>4</sub>NO<sub>3</sub>, and we found that all the three variables significantly increased under NH<sub>4</sub>NO<sub>3</sub> treatment (Fig. 3).

The continuous randomized-effect model showed that the duration of N addition treatment had only weak correlation with the effect size of N addition for all examined variables (Table 1). We further partitioned duration into short (<3 yr), medium (3–10 yr), and long terms (>10 yr) and found duration had a consistent effect on plant lignin, plant fiber, plant cellulose, plant NSC, and decomposed litter lignin. The effects of N addition on these variables all showed a decreasing trend with increasing duration of the N addition treatment (Figs. 2a,b,f and 3a). On the contrary, the effect of N addition on decomposed litter cellulose increased with increasing duration (Fig. 3a,b). Short-term (<3 yr) N addition significantly increased plant lignin, plant cellulose, plant protein, decomposed litter lignin, and soil lignin, but significantly decreased plant hemicellulose. At medium-term N addition (3–10 yr), many significant effects disappeared, and only plant cellulose showed a significant decrease as a response to N addition (Figs. 2 and 3). Finally, at the long-term duration (>10 yr), all of the plant variables except for protein showed a reduction as a response to

TABLE 2. Comparison of the plant chemical compounds between N<sub>2</sub>-fixing alfalfa and non-N<sub>2</sub>-fixing crops/grasses under control and N addition treatment.

| Chemical compound       | N <sub>2</sub> -fixing plants, alfalfa | Percentage of dry mass                                 |                                                                                   |
|-------------------------|----------------------------------------|--------------------------------------------------------|-----------------------------------------------------------------------------------|
|                         |                                        | Non- N <sub>2</sub> -fixing crops and grasses, control | Non- N <sub>2</sub> -fixing crops and grasses, N <sub>2</sub> -addition treatment |
| Crude protein           | 18.87 ± 3.78                           | 13.97 ± 5.24*                                          | 17.15 ± 5.60 n.s.                                                                 |
| Neutral detergent fiber | 44.00 ± 8.15                           | 43.88 ± 17.79 n.s.                                     | 43.89 ± 17.32 n.s.                                                                |
| Acid detergent lignin†  | 6.79 ± 1.60                            | 3.86 ± 4.21**                                          | 3.24 ± 1.41**                                                                     |
| Cellulose               | 20.08 ± 5.09                           | 33.34 ± 6.93**                                         | 34.18 ± 6.73**                                                                    |

Notes: Data used for calculation appears in Data S1; Table S2. Values are mean ± SD.

\*  $P < 0.05$ ; \*\*  $P < 0.01$  for significant differences between N<sub>2</sub>-fixing plants (alfalfa) and non- N<sub>2</sub>-fixing crops and grasses (control or N addition treatment). n.s., not significant.

† Some sources did not state what method was used to measure lignin content.

N addition (Fig. 2), whereas soil lignin showed an increasing trend under N addition.

One-way ANOVA analysis found no significant difference ( $P > 0.05$ ) between  $N_2$ -fixing plants (alfalfa) and non- $N_2$ -fixing crops and grasses under N addition for crude protein. However, crude protein in  $N_2$ -fixing plants (alfalfa) was significantly higher than that in non- $N_2$ -fixing crops and grasses (under control;  $P < 0.05$ , Table 2). Neutral detergent fiber content was not different among  $N_2$ -fixing (alfalfa), non- $N_2$ -fixing plants under control, and non- $N_2$ -fixing plants under N addition treatment ( $P > 0.05$ ). Acid detergent lignin content in  $N_2$ -fixing plants (alfalfa) was much higher than that in non- $N_2$ -fixing plants under both control and N addition treatment ( $P < 0.01$ ). On the contrary,  $N_2$ -fixing plants (alfalfa) had lower cellulose compared to non- $N_2$ -fixing plants (under both control and N addition treatment) ( $P < 0.01$ ).

## DISCUSSION

### *N addition effect on plant protein and NSC*

The clearest trend we found was the response of plant protein to N addition (+25.94%). The increase of plant protein by N addition was independent of ecosystem type, fertilizer type, N addition rate, or treatment duration (Fig. 2e). It is well known that N is an important element for protein synthesis. Under N addition treatment, enhanced plant uptake and utilization of N is expected (Lu et al. 2011a), resulting in the observed increase of plant protein content. The positive correlation between N addition rate and plant protein content (Table 1) indicated that plants may assimilate excess N and reserve it as protein for future requirements (Millard 1988, Warren et al. 2003). The rise of plant protein by N addition may be a double-edged sword. On one hand, it can effectively promote plant growth (Proe and Millard 1994), and the redundant N can be stored as protein to reduce its physiological toxicity and for future needs (Millard 1988). On the other hand, it can improve plant digestibility, which could increase the risk of diseases and pests, and the consumption by herbivores (Schoeneweiss 1975, Roelofs et al. 1985, Erelli et al. 1998, Strengbom et al. 2002).

Effect of N addition on plant NSC had the biggest variation (Fig. 2f) although the mean effect size on plant NSC was positive (Fig. 1). NSC is produced by photosynthesis (Dietze et al. 2014) and its accumulation or consumption depends on the C source-sink balance expressed as photosynthesis vs. growth and respiration (Chapin et al. 1990, Hoch et al. 2003). N addition could improve plant photosynthetic rate (Brown et al. 1996a, Schaberg et al. 1997, Nakaji et al. 2001), which could increase plant NSC. On the other hand, N addition may also damage the photosynthetic system (Goyal and Huffaker 1984), or increase energy and C skeletons consumption due to N assimilation (Invers et al. 2004), or stimulate plant growth, which all could decrease plant NSC. Therefore, we observed highly different responses in different studies.

However, we found that generally plant NSC in trees and herbs was decreased by N addition, while plant NSC in crops was increased instead (Fig. 2f). We speculate that this was because crops are more adapted to high N input. Because NSC is of vital importance for plant resistance to cold and drought conditions (Ericsson et al. 1996, Woodruff and Meinzer 2011, Rosas et al. 2013), increasing N deposition may have adverse effect on trees and herbs under extreme conditions.

A previous study suggested that there was no significant difference of N concentration and biomass between  $C_3$  and  $C_4$  plants under N addition (Xia and Wan 2008). But there were only three studies which focused on the effects of N addition on the C chemical composition of  $C_4$  crops, which were not enough to evaluate different responses between  $C_3$  and  $C_4$  plants.

### *N addition effect on plant structural carbohydrate*

We found N addition increased plant cellulose although the effect was not significant (Fig. 1). However, the effect of N addition on cellulose in herbs and crops was significantly positive. The increase of plant NSC and protein under N addition may contribute to this phenomenon. The biosynthesis of cellulose is an enzymatic reaction which links thousands of glucose molecules into giant chains (Brown et al. 1996b). Therefore, the increase of substrate (NSC derivative) and enzyme (protein) could promote the production of cellulose.

Cellulose is the most abundant organic matter in plants, which roughly accounts for one-third of the total biomass of many plants (Brown et al. 1996b, Somerville 2006). It is the main material of digestion for ruminant herbivores. Therefore the increase of cellulose induced by N addition may affect the development of prataculture (Sanz et al. 2011). The use of cellulose as a renewable resource to produce clean energy (Brown et al. 1996b, Kumar and Murthy 2013) may also benefit from the increase of plant cellulose content under N addition. It should be noted that with increasing duration of the N addition treatment, there seems to be a trend of decrease of plant cellulose (Fig. 2c) although linear correlation analysis did not show a significant effect (Table 1). More long-term studies should be carried out to better understand if this is the case.

Lignin is an aromatic heteropolymer and the second most abundant plant biopolymer after cellulose (Zhao and Dixon 2011), and is important for mechanical support, water transport, and defense in vascular plants (Campbell and Sederoff 1996). Our meta-analysis showed that N addition significantly increased plant lignin by 7.13% (Fig. 1). Similar to cellulose synthesis, lignin synthesis is also an energetically costly process (Amthor 2003, Rogers et al. 2005), which could benefit from the increase of carbohydrate under N addition. We found lignin of woody species (trees and shrubs) significantly decreased under N addition, whereas lignin of herbs and crops significantly increased (Fig. 2a). Although we are unclear of the underlying reasons, we speculate that the following three points

may contribute to this phenomenon. First, the structure and the biosynthetic pathway of lignin are quite different between woody plants and herbaceous plants (Zhao and Dixon 2011). Second, the degree of lignification of woody plants is much higher compared to that of herbaceous plants (Amthor 2003). Third, lignin in woody plants has much greater longevity than lignin in herbaceous plants because many grasses are annual or biennial and most perennial grasses lose their aboveground biomass annually. In addition, the observations may not be enough to reveal an inherent rule in our meta analysis. Nevertheless, we should be alert that the increase of lignin in herbs by N addition may be harmful to forage or biofuel production (Hamelinck et al. 2005, Gallagher et al. 2011).

We found that plant hemicellulose significantly decreased by 4.39% under N addition (Fig. 1a), and the effect was significantly negatively correlated with N addition rate (Table 1). Plant hemicelluloses are polysaccharides and can strengthen the cell walls by interaction with cellulose and, in some walls, with lignin (Scheller and Ulvskov 2010). The decrease of hemicellulose under N addition corresponded to the increase of lignin. A previous study suggested that the relationship between hemicellulose and lignin was that “as one falls, another rises accordingly, and vice versa”, which was because the loss of hemicellulose in cell matrix can be replaced by lignin (Allison et al. 2012). Under N addition, the reduction of plant hemicellulose may be harmful to the mechanical support system.

Generally, we found the effect of N addition on plant fiber was minimal and nonsignificant (Figs. 1 and 2b). Plant fiber is essentially a biocomposite in which rigid cellulose microfibrils are embedded in a matrix mainly composed of lignin and hemicelluloses (Jawaid and Khalil 2011, Philip et al. 2013). Hence, the result might be a consequence of enhanced cellulose and lignin and reduced hemicellulose under N addition treatment (Fig. 1).

It is worth mentioning that all of the four plant structural carbohydrates and NSC in trees were decreased by N addition, whereas all of the six studied C chemical compositions in crops were significantly increased (Fig. 2). In this meta-analysis, the average N addition rate was higher for crops than for trees (Data S1: Table S1). Crops are adapted to high N inputs while trees are mostly adapted to low ambient N deposition; so externally added N input could be detrimental to tree growth, reducing their structural carbohydrates. Therefore, it is possible that N treatment settings (i.e., N addition rate or N fertilizer type) were different among different ecosystems, especially between crops and other ecosystems, which may have contributed to the different effects we found among different ecosystems.

$\text{NH}_4\text{NO}_3$  and urea were the most frequently used fertilizer types and their effects were analyzed separately in this meta-analysis. Our results indicated that they had different effects on most plant variables (Fig. 2). This was probably because nitrate in  $\text{NH}_4\text{NO}_3$  could cause many biochemical reactions, altering plant N uptake kinetics (Piwpuan et al. 2013). Previous studies found nitrate changed the

carbohydrate metabolic process and C partitioning in plants (Marques et al. 1983, Cramer and Lewis 1993) and promoted plant cation absorption, but inhibited plant phosphorus absorption (Ruan et al. 2000). Generally, plants consume more energy to take up nitrate than ammonium (Wang and Macko 2011). These could all contribute to the different effect between  $\text{NH}_4\text{NO}_3$  and urea.

#### *N addition effect on litter and soil C chemical composition*

We found that N addition significantly increased fresh litter lignin content by 3.52% (Fig. 1b). Fresh litter was directly from plant and has not been exposed to decomposers yet. Therefore, fresh litter should have similar response to N addition with plants. Decomposed litter is affected by the decomposition process and therefore may respond differently from fresh litter to N addition. For example, lignin in decomposed litter was unchanged although lignin in fresh litter was increased by N addition (Fig. 1b). Litter lignin is a recalcitrant compound with a slow degradation rate (Knorr et al. 2005). Many studies have demonstrated that N fertilization tended to decrease the activity of lignolytic enzymes and lignin decay (Waldrop et al. 2004, Talbot and Treseder 2012, Gong et al. 2015). Our results did not agree with previous studies. However, when decomposed litter was divided into short (<3 yr) and medium (3–10 yr) treatment duration, we found litter lignin significantly increased after short-term N addition treatment, and this effect disappeared when treatment duration got longer (Fig. 3a). Therefore, microbes related to lignin decomposition may get acclimated to N addition. We also found that lignin of decomposed herbaceous litter increased significantly under N addition, whereas lignin of decomposed woody litter decreased instead (Fig. 3a). Plant lignin in trees also decreased under N addition (Fig. 2a), which was also contrary to the results of herbs. Therefore, N addition effect on lignin in decomposed litter of trees was similar to that on lignin in trees although the litter had undergone decomposition processes.

We found decomposed litter cellulose statistically decreased by 13.97% under N addition (Fig. 1b). The decrease of litter cellulose was most likely caused by enhanced decomposition of cellulose. Carreiro et al. (2000) found that the activity of cellulase was stimulated by N addition, increasing cellulose decomposition rate. Alster et al. (2013) also found that the activity of  $\beta$ -glucosidase and cellobiohydrolase related to cellulose degradation was significantly increased by N addition. With the increase of N addition rate, the effect size of N addition on decomposed litter cellulose got more negative (Table 1), suggesting better decomposition of litter cellulose under high N addition rate.

It should be noted that the effects of N addition on litter chemistry varied at different decay stages (Talbot and Treseder 2012). However, the experimental subjects, decomposition duration, N addition rates, and ambient environment were greatly different among individual

studies and it was difficult to standardize the litter decay stage. Therefore, the effects of N addition on the chemical compounds of litter at different decay stages were not assessed in the meta-analysis. It would be interesting to investigate more on this aspect to better understand how decomposition affects different C compounds of litter.

Similarly, soil C chemical composition is also affected by both the input and the output processes. Our results indicated that N addition significantly increased soil lignin content by 7.30% (Fig. 1c) and the effect was quite consistent among different ecosystems or under different kinds of treatments of N addition (Fig. 3c). Soil lignin may be affected by plant lignin inputs, which have been found to increase under N addition (Li et al. 2015). N addition has also been found to change soil microbial community structure and decrease microbial biomass (Frey et al. 2004, Treseder 2008). It has been reported that N addition can inhibit the decomposition of lignin by suppressing the synthesis of lignolytic enzymes (Fog 1988, Carreiro et al. 2000, Waldrop et al. 2004, Gong et al. 2015). These all could have contributed to the increase of soil lignin under N addition.

Lignin and its degradation products (e.g., phenolic compounds, quinonoid compounds, and aliphatic compounds) are precursors for humus formation (Fustec et al. 1989, Yavmetdinov et al. 2003). Therefore, increasing N deposition could increase humus accumulation and soil C stability (Magill and Aber 1998, Waldrop et al. 2004), which may attenuate global climate change. Besides these chemical properties, other factors such as physical protection of labile materials and stabilization of microbial products could also contribute to soil C accrual. For instance, a few recent studies suggested that soil C storage increased under N addition and the major driving factor was the increase of mineral-associated or aggregate-occluded C pool, despite strong declines in the mass of labile C fractions (Cusack et al. 2011, Riggs et al. 2015). A study also showed that N deposition significantly increased the amount of new fungal-derived residues (glucosamine) in bulk soil and decreased the decomposition of old microbial residues associated with soil minerals, which was beneficial to C stabilization and long-term C sequestration (Griepentrog et al. 2014).

It should be noted that N addition cannot fully simulate increasing N deposition mainly due to its high application rates and low application frequency. In our meta-analysis, the N addition rates ranged from 9 to 500 kg N·ha<sup>-1</sup>·yr<sup>-1</sup> and most experiments applied N treatment once a year. Very high rates of fertilization used in some studies cannot reflect realistic effects under current or even future N deposition levels (Knorr et al. 2005). We also found that when the N addition rate exceeded 200 kg N·ha<sup>-1</sup>·yr<sup>-1</sup>, the effects on C chemical composition was greatly different from the effects of N addition rate at 0–100 and 100–200 kg N·ha<sup>-1</sup>·yr<sup>-1</sup>. Results from 0–200 kg N·ha<sup>-1</sup>·yr<sup>-1</sup> N addition rates may be more applicable to the issue of increasing N deposition. More long-term studies under naturally increasing N deposition should be carried out to better understand the issue of N deposition.

It is also interesting to find that the effects of N addition on plant chemistry were similar to the effects of N<sub>2</sub>-fixation. N<sub>2</sub>-fixing plants had higher protein content compared to non-N<sub>2</sub>-fixing plants, which agreed with the increase of protein under N addition treatment (Table 2). Fiber contents was not different between N<sub>2</sub>-fixing and non-N<sub>2</sub>-fixing plants, which also agreed with the effect of N addition on this chemical compound (Table 2). We understand that it was only a rough comparison between N<sub>2</sub>-fixing alfalfa and non-N<sub>2</sub>-fixing plants because data were from different studies and the environmental variables were different among different studies. However, previous studies on alfalfa and its neighboring grasses under similar environmental conditions also suggested that alfalfa had higher protein and lignin contents and similar fiber and cellulose contents compared to its neighboring non-N<sub>2</sub>-fixing grasses (Janicki and Stallings 1988, Sunvold and Cochran 1991, Balde et al. 1993, Dien et al. 2006). Therefore, our results at least point to a potential convergence of the plant chemistry between N<sub>2</sub>-fixing plants and non-N<sub>2</sub>-fixing plants exposed to added N.

## CONCLUSIONS

Our results suggested that most ecosystem C chemical components were sensitive to N addition. The increase of plant lignin, plant cellulose, and plant protein under N addition may be beneficial for plant growth and C sequestration. However, the decrease of plant hemicelluloses may be detrimental to mechanical support. Enhanced fresh litter and soil lignin accumulation under N addition would be advantageous to soil C stability, while cellulose of decomposed litter decreased instead, mainly due to stimulated cellulose decomposition by N addition. The influences of N addition on C chemical composition varied among different ecosystems, and our results provided a basic framework for future studies in forest, grassland, and cropland. Except for decomposed litter lignin and soil lignin, seven examined variables showed correlation with N addition rates, suggesting more severe effect in the future with higher N deposition rate. The weak relationships between the treatment duration and the effect size of N addition indicated that the effect of N addition may not exacerbate with increasing treatment time. However, more long-term studies are needed to understand the effects of constantly increasing N deposition. Our study provided a comprehensive picture of the effects of N addition on different C components in terrestrial ecosystems and could help with better evaluation of plant adaption, biofuel production, and terrestrial C sequestration under the context of increasing N deposition.

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## SUPPORTING INFORMATION

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