Positive responses of belowground C dynamics to nitrogen enrichment in China

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HIGHLIGHTS
- Positive effects of N enrichment on belowground C cycles was found in China.
- N enrichment promoted C inputs into soil mainly by increasing litter and root inputs.
- Climate played a greater role than N deposition in affecting ecosystem C cycle.
- Belowground C cycle were determined by interactions of land use type, N and climate.

GRAPHICAL ABSTRACT

ABSTRACT
Determining how nitrogen (N) impacts ecosystem carbon (C) cycling is critical to using C sequestration to offset anthropogenic CO2 emissions. The N deposition rate in China is higher than the global average; however, many results of N enrichment experiments in China have not been included in global syntheses. In this study, we assembled a large dataset that comprised 124 published studies concerning N addition experiments, including 570 observations at 127 sites across China, to quantify the responses of belowground C dynamics to N enrichment in terrestrial ecosystems in China by a meta-analysis. The results showed that overall soil organic C, dissolved organic C (DOC) and soil microbial biomass C (MBC) increased by 1.8, 7.4, and 8.8%, respectively (P < 0.05), in response to N enrichment; belowground biomass and litter increased by 14.6 and 24.4%, respectively (P < 0.05); and soil respiration increased by 6.1% (P < 0.05). N enrichment promoted C inputs into the soil mainly by increasing litter and belowground biomass inputs. Additionally, N enrichment increased C output by increasing soil respiration. Land use type and N addition level had different impacts on the soil C pool and on soil respiration. DOC, MBC, and litter exhibited more positive responses to N deposition in cooler and more arid regions than in other regions. The meta-analysis indicated that N enrichment had a positive impact on belowground C cycles in China. Climate played a greater role than did N deposition level in affecting processes of ecosystem C cycling. Moreover, belowground C cycle processes are determined by complicated interactions among land use type, N enrichment, and climate.

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1. Introduction

Global climatic changes have greatly impacted ecosystem services and function (Rockstrom et al., 2009). Atmospheric nitrogen (N) deposition is a primary component of global climatic change (Liu and Greaver, 2009; IPCC, 2013). The annual input of N into terrestrial ecosystems has increased >10 times in the past 150 years, mainly due to intensive N fertilizer applications and fossil fuel combustion (Galloway and Cowling, 2002). Ecosystem carbon (C) cycling is projected to affect future climate change (Le Quere et al., 2009; Luo et al., 2009; Arora et al., 2013; Shao et al., 2014; Deng et al., 2017a), which has been greatly impacted by N enrichment. Thus, knowledge of how N enrichment impacts ecosystem C cycling is important for understanding whether increasing N input would influence global climatic changes.

To better understand N enrichment effects on the C cycling of terrestrial ecosystems, many N addition experiments have been conducted across the world (Hillebrand and Gurevitch, 2013). Considering both above- and belowground C dynamics is important to understand the effects of N on C cycling within terrestrial ecosystems (Pregitzer et al., 2008; Liu and Greaver, 2010; Fu et al., 2015). Previous studies have reported that increased N enrichment could improve C stocks in terrestrial ecosystems (Lu et al., 2011; Chang et al., 2016; Carvalho et al., 2017; Zhong et al., 2017). This phenomenon has been confirmed by eddy flux and biomass accumulation data in temperate and boreal forests (Magnani et al., 2007); however, the findings were based on the changes in aboveground C cycling. Although belowground C pools had significant correlations with aboveground vegetation, this does not mean aboveground C cycling would always reflect belowground C cycling. For example, Sullivan et al. (2007) reported that increased inputs of aboveground litter did not cause marked increases in soil C pools in N addition experiments. The uncoupled aboveground–belowground processes suggested that extrapolating belowground C dynamics from aboveground C dynamics during N enrichment was not feasible (Liu and Greaver, 2010).

The addition of N usually can stimulate plant growth, leading to plant C stock increases in most terrestrial ecosystems (LeBauer and Treseder, 2008; Deng et al., 2017b). However, whether this increases C stocks in the soil is still disputed (Chapin et al., 2009; Lu et al., 2011). In general, N addition significantly stimulates soil C sequestration in some terrestrial ecosystems (Högberg, 2007; Lu et al., 2011), but there are clear losses in other terrestrial ecosystems (Khan et al., 2017; Zhu et al., 2009). N addition can also change the dynamics of dissolved organic C (DOC). Some studies have reported that DOC significantly increased not only at experimental sites under chronic N addition but also in natural ecosystems that have high N deposition rates (Freschl et al., 2008; Wang et al., 2008). However, one N addition experiment in a mature tropical forest involved a negative response of DOC to N addition (Mo et al., 2008). Thus, individual studies are unlikely to effectively resolve the controversy because of complex interactions and diverse spatial heterogeneity within complicated ecological processes (Lu et al., 2011). A synthesized analysis is necessary to reveal a common tendency and identify broad-scale patterns of soil C pool changes due to N enrichment.

Belowground C increase via litter and fine root inputs are two important processes in C cycles of terrestrial ecosystems (Pregitzer et al., 2008; Yuan and Chen, 2012; Li et al., 2015; Song et al., 2017). Increased soil N enrichment can alter plant community composition by its effects on nutrient cycling and stocks in plants (Li et al., 2016), thus altering plant productivity, litter and fine root production (Liu and Greaver, 2010; Cleveland et al., 2011). Sullivan et al. (2007) reported that aboveground plant biomass (i.e., leaves) was a key contributor to soil C pools. Moreover, increased N enrichment also has a strong effect on plant root structure and function (Li et al., 2015). However, N enrichment can have positive (Knorr et al., 2005), negative (Mo et al., 2007), or no effects (Yuan and Chen, 2012) on litter accumulation and fine root production in different ecosystems in various regions. Therefore, additional studies focused on how N enrichment affects litter and fine root production on a broad scale are crucial to better explore the contribution of N enrichment to ecosystem C cycling.

In addition, N enrichment has potential key effects on microbially mediated belowground C dynamics of various ecosystems (Ramirez et al., 2012). N enrichment not only influences microbial diversity (Bates et al., 2011) but also can strongly impact microbial biomass C (MBC) dynamics (Fu et al., 2015). Diversity and biomass of soil microbes are closely related to soil microbial respiration ($R_{soil}$) (Sun et al., 2014), and $R_{soil}$ is an important component of soil respiration ($R_s$) (Sun et al., 2014). Janssens et al. (2010) reported that N enrichment can alter plant and microbial activities that directly regulate the process of $R_s$. However, it is still unclear whether changes in soil microbe and $R_s$ dynamics are consistent across ecosystems, because few N enrichment studies have explored the responses across a broad range of soil and ecosystem types. Moreover, with inconsistent results from field studies, understanding how soil C output via $R_s$ responses to N enrichment affects terrestrial ecosystems is increasingly important in relation to the terrestrial C budget.

China has wide spatial variation of climatic conditions and complex and diverse soil and ecosystem types as well as large-scale ongoing and expected N deposition, providing an ideal platform for studying ecosystem C cycle responses to N enrichment at the regional scale (Fu et al., 2015). Although many N enrichment experiments have been conducted in China, few experiments have been included in global synthesis studies (Liu and Greaver, 2010; Appendix Fig. S1). Thus, further study is needed to determine how N enrichment affects belowground C dynamics in China and to analyze distributions across terrestrial ecosystems. Specifically, this study addressed the following two questions: (i) To what extent has belowground C (SOC, DOC, MBC, litter, belowground biomass flux [root], $R_{soil}$, and $R_s$) dynamics been altered by N enrichment in China? (ii) What are the potential mechanisms for belowground C dynamics in responding to N enrichment in China? To clearly understand the central topic and framework of the study, here, we have provided a conceptual diagram of N enrichment effects on aboveground and belowground C cycles and components (Fig. 1). The influence, direction, extent and mechanism of N enrichment effects on belowground C dynamics were characterized by a meta-analysis. The dataset comprised 124 studies, including 570 observations at 127 study sites across China.

2. Materials and methods

2.1. Data compilation

In this study, all of the available peer-reviewed publications before 2017 were searched from the China Knowledge Resource Integrated Database (http://www.cnki.net/), Google Scholar and Web of Science. The publications were chosen because their keywords, titles, or abstracts were related to N addition/fertilization/enrichment experiments in China. To avoid bias in selection, the following considerations were used to select publications for analysis:

(i) Only studies in which control and treatment sites under the same climatic, soil, and vegetation conditions were included, and these studies included at least one of our selected variables;

(ii) Only field experiments conducted in situ with no other management practices (e.g., irrigation, warming, and phosphate potassium fertilizer) were included;

(iii) Only studies that had adequate replications and studies that had paired or chronological sequence sites with uniform soil types were included; and,

(iv) The means, standard deviations/errors, and replications of variables in the control and treatment groups could be extracted directly from the context, tables, or digitized graphs.

In addition, environmental variables, such as the latitude and longitude, climatic factors (mean annual temperature [MAT] and mean...
annual precipitation (MAP), land use types (cropland, grassland, forests, and wetlands), N addition level (kg ha$^{-1}$ yr$^{-1}$) and N chemical form ($\text{NH}_4\text{NO}_3$, urea, $\text{NH}_4^+$, and $\text{NO}_3^-$), were recorded directly from the papers. The reported litter, belowground biomass, DOC, MBC, Rs, and $R_m$ in the control and treatment groups were also collected. Studies that involved more than one N enrichment rate or vegetation type were treated as multiple observations. Finally, the dataset comprised a total of 124 studies (Appendix Word S1) that both concerned N fertilization addition experiments and satisfied our selection criteria for the meta-analysis; the 124 studies were selected from $\geq 2000$ publications.

The dataset included 570 observations across 127 sites in China (Fig. 2); 34 observations were in cropland, 158 were in grassland, 369 were in forests, and nine were in wetlands (Appendix Dataset S1). All the raw data were either obtained from tables or extracted by digitizing graphs using GetData Graph Digitizer (version 2.24, Moscow, Russia), and the collected dataset included only data within a soil depth of 0–30 cm. To explore more obvious trends of N enrichment effects on belowground C dynamics, the N addition levels were divided into three groups: $\leq 50$, 51–150, and $> 150$ kg ha$^{-1}$ yr$^{-1}$, corresponding to low N, medium N, and high N addition levels, respectively; this division was also used in a global N enrichment study by Liu and Greaver (2010).

### 2.2. Meta-analysis

We used the meta-analysis of Hedges et al. (1999) to evaluate how variables (SOC, DOC, MBC, belowground biomass, litter, $R_s$, and $R_m$) related to belowground C cycles respond to N enrichment in China. A response ratio ($RR = \ln rr$) was used to represent the magnitude of the effects of N enrichment; the bias of this ratio is small, and its sampling distribution is approximately normal (Hedges et al., 1999; Koricheva et al., 2013; Fu et al., 2015). $rr$ was calculated as the ratio of the mean value of a given variable in the treatment group ($X_e$) to that in the control group ($X_c$) (Eq. (1)):

$$RR = \ln rr = \ln \left(\frac{X_e}{X_c}\right) = \ln \left(\frac{\bar{X}_e}{\bar{X}_c}\right) - \ln \left(\frac{\bar{X}_c}{\bar{X}_e}\right)$$

(1)

The corresponding variance for each ln RR was calculated as follows:

$$v = \frac{1}{n_e} \times (\bar{S}_e/X_e)^2 + \frac{1}{n_c} \times (\bar{S}_c/X_c)^2$$

(2)

in which, $n_e$ and $n_c$ are sample numbers in the treatment and control groups, respectively, and $S_e$ and $S_c$ are the corresponding standard deviations.

The following equation was used as the weighting factor ($w_{ij}$) for each ln RR:

$$w_{ij} = \frac{1}{v}$$

(3)

In the meta-analysis, the weighted response ratio ($RR_{++}$) was calculated from the RR values of individual pairwise comparisons between the treatment and control groups, $RR_{ij}$ ($i = 1, 2, \ldots, m; j = 1, 2, \ldots, k$) as in Eq. (4). Here, $m$ is the number of groups (e.g., land use types,
N forms, N addition levels), and \( k \) is the number of comparisons in the \( i \)th group.

\[
RR^{++} = \frac{\sum_{i=1}^{m} \sum_{j=1}^{k} w_{ij} RR_{ij}}{\sum_{i=1}^{m} \sum_{j=1}^{k} w_{ij}}
\]  

(4)

The standard error of \( RR^{++} \) and the 95% CI were estimated by Eqs. (5) and (6), respectively.

\[
S(\text{RR}^{++}) = \sqrt{\frac{1}{\sum_{i=1}^{m} \sum_{j=1}^{k} w_{ij}}} \]  

(5)

\[
95\% \text{CI} = \text{RR}^{++} \pm 1.96 S(\text{RR}^{++})
\]  

(6)

The weighted response ratio (\( RR^{++} \)) and 95% confidence interval (CI) were calculated using METAWin 2.1 software (Sinauer Associates Inc., Sunderland, MA, USA). In this meta-analysis, we used the fixed-effect model, which has been adopted in other meta-analysis studies (Hedges et al., 1999; Koricheva et al., 2013). In addition, the effects were considered significant if the 95% CI did not overlap with zero (Hedges et al., 1999; Laganière et al., 2010; Liu and Greaver, 2010; Fu et al., 2015; Deng et al., 2016).

2.3. Statistical analysis

To address whether the response variables differed among different categorical variables (land use types, N forms, and N addition levels) in response to N addition, we used multiway analysis of variance (ANOVA) to test whether the interactions among multiple treatments were significant. Differences were evaluated at \( P < 0.05 \). When testing whether homogeneity of variance was passed and significance was observed at \( P < 0.05 \), a least significant difference test (LSD) was used for multiple comparisons. Pearson correlations were performed to explore relationships between MAP, MAT, N enrichment rate and the response variables. Multivariable linear regression analysis was conducted to quantify the contributions of relevant factors (MAP, MAT and N enrichment rate) to the response variation. Here, we used the standardized regression coefficient’s relative importance (%) among each independent variable to explain the relative contributions of each variable. All statistical analyses were performed using the software program SPSS ver. 17.0 (SPSS Inc., Chicago, IL, USA). Also, the N enrichment-induced belowground C dynamics factor (\( F \)) \( (\text{yr}^{-1}) \) was estimated as follows:

\[
F = \frac{(G_e - G_c)}{N}
\]  

(7)

in which \( G_e \) and \( G_c \) represent the belowground C (SOC, DOC, MBC, belowground biomass, litter, \( R_p \), and \( R_m \)) from the treatment and control, respectively, and \( N \) is the annual N enrichment rate \( \text{(kg N ha}^{-1} \text{yr}^{-1})} \).

3. Results

3.1. Response of C pools (SOC, DOC, and MBC) to N enrichment

Overall, N enrichment exhibited a significant positive effect on soil C pools (SOC, DOC, and MBC), with \( RR^{++} \) values for SOC, DOC, and MBC of +1.8%, +7.4%, and +8.8% (all \( P < 0.05 \)), respectively (Fig. 3). When the dataset was subdivided, land use type, N form, and N addition level all had different effects on the soil C pool (Fig. 4, Appendix Table S1). For different land use types, the SOC in cropland exhibited a significant positive response to N enrichment (+10.7%, \( P < 0.05 \)), but grassland and forests showed no significant changes \( (P > 0.05) \) (Fig. 4a). The DOC of grassland (+33.4%, \( P < 0.05 \)) and forests (+6.6%, \( P < 0.05 \)) had significantly improved responses to N enrichment (Fig. 4b); the RR for grassland was greater than that for forests \( (P < 0.05) \), but the \( RR^{++} \) of the MBC for forests (+9.3%) was greater than that for grassland (+1.7%) \( (P < 0.05) \) (Fig. 4c). Regarding different N addition forms, SOC and
DOC were significantly increased by NH4NO3 by 1.6 and 7.5% (both P < 0.05), respectively, but were not affected by urea (Fig. 3a and b), and MBC was significantly increased by three N forms: NH4NO3, urea, and NH4+ (Fig. 4c). The low level of N addition (≤ 50 kg ha⁻¹ yr⁻¹) significantly increased SOC (+7.7%), DOC (+5.1%) and MBC (+7.1%, all P < 0.05). The medium level of N addition (51–150 kg ha⁻¹ yr⁻¹) significantly increased DOC (+8.8%) and MBC (+11.4%, both P < 0.05) but did not significantly affect SOC (Fig. 4c). The high level of N addition (>150 kg ha⁻¹ yr⁻¹) did not significantly affect SOC or DOC but did significantly increase MBC (+7.2%, P < 0.05).

3.2. Response of C inputs (belowground biomass and litter) to N enrichment

Overall, belowground biomass and litter significantly responded to N enrichment (P < 0.05), with RR++ values of +14.6 and +24.4%, respectively (Fig. 3). Belowground biomass responses to N enrichment were not significant for all land use types; differences among land use types were found (Fig. 5). The four land use types - cropland, grassland, forests, and wetlands - significantly positively responded to N enrichment (P < 0.05), with belowground biomass RR++ values of 29.6, 11.5, 15.2, and 21.9%, respectively (Fig. 5a). The litter of grassland and forests significantly increased by 54.0 and 10.5% (P < 0.05), respectively (Fig. 5b). In the meta-analysis, NH4NO3, urea, NH4+ and NO3⁻ all had significant positive effects on belowground biomass (P < 0.05), but differences among the N forms were found (Fig. 5a). Moreover, three N addition levels also had significant positive effects on belowground biomass and litter (Fig. 5), and among the N levels, high N addition had the greatest effect on belowground biomass (+42.1%) and litter (+73.4%) (Fig. 5).

3.3. Response of C output (Rs) to N enrichment

Overall, Rs exhibited a significant positive response (+6.1%, P < 0.05) to N enrichment (Fig. 3). Land use type and N addition form resulted in

Fig. 3. The responses of belowground C variables to N addition in China. Note: SOC, soil organic C concentration; DOC, soil dissolved organic C concentration; MBC, soil microbial biomass C; Rs, soil respiration; Rm, microbial respiration. Red bars represent 95% confidence intervals.

Fig. 4. Effects of N addition on (a) soil organic C (SOC) concentration; (b) soil dissolved organic C (DOC) concentration; and (c) soil microbial biomass C (MBC). Note: LN, MN, and HN indicate N addition levels of ≤50, 51–150, and >150 kg ha⁻¹ yr⁻¹, respectively. Red bars represent 95% confidence intervals. The different letters above the bars indicate a significant difference at P < 0.05. No values indicate no data were entered into the meta-analysis.
significant differences in Rs but did not significantly differ among N addition levels (Fig. 6). For different land use types, wetlands had the highest RR++ (+39%) response to N addition, followed by cropland with +15%, forests with +8.0%, and grassland with +2.4% (Fig. 6). Regarding the different N addition forms, Rs significantly increased in response to NH4NO3, urea, and NH4+, with RR++ values of 6.0, 4.1, and 48.8%, respectively (Fig. 6). The low N, medium N, and high N addition levels significantly increased Rs by 11, 4.0, and 10%, respectively (Fig. 6), and N addition also significantly increased Rm by 52.8% (Fig. 3).

3.4. Factors affecting belowground C dynamics

N addition rate and climatic factors (MAP and MAT) have important effects on belowground C cycling. N addition rate showed a positive effect on belowground biomass (P < 0.05) but did not significantly affect SOC, DOC, MBC, litter, Rs, or Rm (P > 0.05) (Table 1). The relationships of RR vs. N addition rate of each response variable with MAT in the meta-analysis showed that MAT had a significant positive effect on DOC, MBC, belowground biomass, and litter (all P < 0.05) but not on SOC or Rs, (both P > 0.05) (Table 1). In addition, MAP had a significant negative effect on DOC, MBC, and litter (all P < 0.05) but not on SOC, belowground biomass, or Rs (all P > 0.05) (Table 1).

4. Discussion

4.1. Effects of N enrichment on belowground C pools

Our meta-analysis showed that N enrichment had a significant positive effect on SOC; similar results have been reported in global syntheses of N enrichment (Liu and Greaver, 2010; Lu et al., 2011). Similar N application forms, experimental methods, and the latitudinal distribution of N addition experiments in China were consistent with those in global syntheses (Fu et al., 2015). Moreover, China has diverse climatic zones and vegetation types resulting from its high geographic heterogeneity and complex topography. These zones and vegetation types easily give rise to similar responses of ecosystem C cycling to global climate change as those in other regions in the world (Fu et al., 2015).

In addition, the results of our meta-analysis showed that the DOC increased by 7.4% under N enrichment. This value was lower than that at the global scale (18%; Liu and Greaver, 2010). Monteith et al. (2007) reported that decreased soil pH resulting from N enrichment was the main influence factor on DOC accumulation. However, in their global meta-analysis, Liu and Greaver (2010) reported that N enrichment reduced MBC by 20%. In our meta-analysis, N addition generally increased MBC in the ecosystems of China by 8.8%. Usually, N enrichment increases aboveground litter input (Fu et al., 2015), and increasing fresh litter inputs to the soil surface generally increase MBC accumulation in soils (Sylvia et al., 2004), presumably because of increasing labile
organic C (LOC) input from the litter. Moreover, the RR of belowground biomass was significantly positively correlated with the RR of the MBC ($P < 0.01$, Fig. 7), indicating that root production increased soil microbial production with N addition. However, in the meta-analysis of Liu and Greaver (2010), no correlations were found between the RRs of MBC and aboveground litter or fine root production, as MBC varied more than litter and fine root responses did to climate and micromosaic factors. Additionally, Lu et al. (2011) reported that N addition decreased MBC but increased belowground biomass in a global study.

4.2. Effects of N enrichment on belowground C input

It is well documented that N fertilization positively affects plant growth. This phenomenon has been consistently reported by recent meta-analyses, with the result that aboveground net primary productivity (ANPP) and belowground biomass both increased in response to N enrichment (Liu and Greaver, 2010; Tang et al., 2017; Peng et al., 2017). Lu et al. (2011) also reported that C stocks in both above- and belowground biomass were significantly increased with N enrichment, leading to increased organic C inputs into the soil from the decomposition and turnover of litter and roots. In our meta-analysis, belowground biomass and litter significantly increased in the N enrichment treatments compared with the non-N addition treatments, with corresponding RRs of +14.6 and +24.4% (both $P < 0.05$), respectively (Fig. 7). Matsushima and Chang (2007) reported that N enrichment can stimulate more litter and fine root inputs and can improve litter quality (i.e., lower lower C:N ratio), thus influencing the processes of litter and root decomposition and, in turn, the accumulation of soil C stocks (Knorr et al., 2005). Li et al. (2015) reported that simulated N deposition could stimulate C accumulation in root biomass and thus increase C and nutrient retention in the soil.

The positive responses of fine root production to N enrichment suggested that N nutrients are limited in China, adding new evidence of N limitation to global terrestrial ecosystems (Yuan and Chen, 2012). In soils with enriched N, plants allocate less C to the roots, leading to a lower root:shoot ratio (Li et al., 2015). However, belowground NPP could still be higher because much higher ANPP exists in N-enriched soils than in soils without N addition. Similarly, this could also occur for plant litter and fine root production. In our meta-analysis, the litter response to N enrichment was higher (24.4%) than the belowground biomass response (14.6%) (Fig. 3). Therefore, although N enrichment increased fine root production and likely belowground net primary productivity (BNPP), N enrichment probably reduced the proportion of root systems accounting for total NPP, which in turn altered C allocation patterns (Yuan and Chen, 2012).

4.3. Effects of N enrichment on belowground C output

In our meta-analysis, N enrichment increased $R_s$ by 6.1% and $R_m$ by 52.8% (Fig. 3). Because soil microbes are often C limited (Sylvia et al., 2004), it is possible that increasing litter inputs could lead to higher $R_m$ rates, which would cause greater soil C output (Liu and Greaver, 2010). We found that the RR of $R_s$ was positively correlated with that of both MBC and belowground biomass (Fig. 7). The $R_s$ results mainly included root respiration and soil microbial respiration associated with organic matter decomposition (Schindlbacher et al., 2009). Due to data limitations in our meta-analysis, we did not directly explore how N enrichment impacted root respiration. However, it is well documented that root dynamics can influence the process of CO$_2$ production/emissions. Silver et al. (2005) reported that living roots can release CO$_2$, and both living and dead roots contribute LOC and N to provide fuel for CO$_2$ production by soil microbes. More inputs of litter and fine roots and higher rates of litter and fine root decomposition often influence the process of soil CO$_2$ emissions (Silver et al., 2005). Högborg et al. (2002) reported that high root biomass and production usually resulted in high rates of root respiration and $R_m$. High NPP, large microbial

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**Table 1**

Pearson correlations of each response variable with mean annual precipitation (MAP), mean annual temperature (MAT) and N addition rate in the meta-analysis. Note: SOC, soil organic C; DOC, soil dissolved organic C; MBC, soil microbial biomass C; $R_s$, soil respiration; $R_m$, microbial respiration. ns, non-significant ($P > 0.05$); *, significant at $P < 0.05$; ***, significant at $P < 0.001$. The relationships of ln RR vs. N addition rate of $R_m$ with MAP and MAT are not shown because observations were collected at only one site.

<table>
<thead>
<tr>
<th>Response variable</th>
<th>MAP</th>
<th>MAT</th>
<th>N addition rate</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOC</td>
<td>0.14 ns</td>
<td>0.16 ns</td>
<td>0.22 ns</td>
<td>60</td>
</tr>
<tr>
<td>DOC</td>
<td>0.79**</td>
<td>0.83**</td>
<td>0.19 ns</td>
<td>19</td>
</tr>
<tr>
<td>MBC</td>
<td>0.45**</td>
<td>0.41**</td>
<td>0.19 ns</td>
<td>92</td>
</tr>
<tr>
<td>Belowground biomass</td>
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<td>0.34'</td>
<td>0.20'</td>
<td>133</td>
</tr>
<tr>
<td>Litter</td>
<td>0.58'</td>
<td>0.58'</td>
<td>0.35 ns</td>
<td>13</td>
</tr>
<tr>
<td>$R_s$</td>
<td>0.08 ns</td>
<td>0.03 ns</td>
<td>0.03 ns</td>
<td>323</td>
</tr>
<tr>
<td>$R_m$</td>
<td>–</td>
<td>–</td>
<td>0.92 ns</td>
<td>3</td>
</tr>
</tbody>
</table>

* Indicates the correlations between response ratios (ln RR) vs. N addition rate of each response variable and MAP and MAT.

** Indicates the correlations between the response ratios (ln RR) of each response variable and N addition rate.

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**Fig. 6.** Effects of (a) land use type, (b) N form, and (c) N addition level on soil respiration ($R_s$) with N addition. Note: LN, MN, and HN indicate N addition levels of ≤50, 51–150, and >150 kg ha$^{-1}$ yr$^{-1}$, respectively. Or they addition was used in wetland in the meta-analysis (Appendix Dataset S1). Red bars represent 95% confidence intervals. The different letters above the bars indicate a significant difference at $P < 0.05$. 

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biodiversity, and rapid rates of soil C decomposition and turnover all support high rates of \( R_m \) (Silver et al., 2005). However, some global meta-analyses found no significant relationship across all land use types due to the large heterogeneity among different land uses (Liu and Greaver, 2010; Zhong et al., 2016).

### 4.4. Factors affecting belowground C under N enrichment

Land use type was an important factor influencing SOC, DOC, MBC, belowground biomass, litter, and \( R_m \) in the meta-analysis (Figs. 3–5, Appendix Table S1, Appendix Fig. S2). However, land use type did not have a significant impact on SOC or belowground biomass under N enrichment (Figs. 4 and 5). Our meta-analysis indicated that SOC has not significantly changed in forests and grasslands in response to N enrichment, but several previous reviews and meta-analyses with limited sample sizes showed that N enrichment significantly increased the SOC in forests (Pregitzer et al., 2008; Janssens et al., 2010). Our results showed that the SOC in cropland increased by 10.7% (Fig. 4a), which was higher than one result (3.3%) of a global synthesis (Lu et al., 2011). In our meta-analysis, N enrichment improved the DOC in grassland (+33.4%, \( P < 0.05 \)) more than it did in forests (+6.6%, \( P < 0.05 \)) (Fig. 4b); this phenomenon may be related to the much greater increase in litter in grassland (+54%) than in forests (+10.5%) (Fig. 4b). As previously discussed (Liu and Greaver, 2010), one potential reason is that N enrichment increased the production and decomposition of litter and roots and thus increased the C available for leaching in the soil. However, N addition stimulated soil microbial production and activity in forests (+9.3% of MBC) more than in grassland (+1.7% of MBC) (Fig. 4c); this phenomenon may constitute another reason, because higher soil microbial activity consumes more DOC due to the requirement for microbes to maintain their own metabolism. Overall, N addition could have a positive impact on DOC concentration by increasing organic C pools and decreasing microbial activity (Liu and Greaver, 2010). Further research is needed to distinguish the driving mechanism behind changes in DOC.

In general, forests have more complex community structure and composition than does grassland. This complexity can provide many more kinds of C sources for utilization by soil microbes, thus increasing microbial diversity (Zak et al., 2001). This finding may be the reason for higher RR values of MBC in the forests than in the grassland in our meta-analysis (Fig. 4c). Ramirez et al. (2012) also reported that N addition may affect microbial diversity by altering the structure and composition of plant communities as well as the quantity and quality of plant C. Regarding the \( R_m \) in our meta-analysis, wetlands had the highest RR for N enrichment, followed by cropland with +15%, forests with +8.0%, and grassland with +2.4% (Fig. 6). The result in which the \( R_m \) of wetlands had a significant positive response to N enrichment was in agreement with previous results (Zhong et al., 2016), in which N enrichment significantly enhanced the root biomass of plants and active C fractions (Figs. 4 and 5), thus increasing the \( R_m \) in wetlands. Cropland with a higher \( R_m \) mainly due to N enrichment, presented significantly increased belowground biomass (Fig. 5) and thus increased root respiration. The greater increase in \( R_m \) for forests with N enrichment compared to grassland may be related to N enrichment enhancing MBC more for forests than for grassland (Fig. 4), resulting in significant increases in \( R_m \) in forests. Our result is consistent with that of Lu et al. (2011), who reported that N enrichment increased the \( R_m \) by 16.1% in an agricultural ecosystem and by 22.2% in both forests and grassland, but not with some global syntheses that showed no significant differences among cropland, grassland, and forests (Liu and Greaver, 2010; Zhong et al., 2016).

In our meta-analysis, N fertilizer form (NH4NO3 and urea) had no significant effect on SOC, DOC, MBC, belowground biomass, litter, and \( R_m \) (Figs. 4–6). Although NH4+ addition significantly enhanced \( R_m \) (Fig. 6), this phenomenon was mainly due to the effect of NH4+ only in wetlands (Appendix Dataset S1), indicating the role of wetlands rather than N form. In addition, N addition level significantly affected SOC (Fig. 4). In our meta-analysis, low N addition significantly increased SOC by 77%, but medium and high N additions did not have significant effects (Fig. 4a). Other studies also reported that low N addition (+50 kg ha\(^{-1}\) yr\(^{-1}\)) could increase soil C sequestration (Liu and Greaver, 2010); however, King et al. (2005) reported that the level of N addition did not have an effect on SOC because N addition increased aboveground plant productivity but did not increase root inputs into the soil C pool. This phenomenon is also supported by our meta-analysis, which showed that belowground biomass did not differ among different N addition levels (Fig. 5a). Moreover, the medium-level N addition had the lowest positive effect on belowground biomass (+7.1%) compared with the low N (+11.5%) and high N levels (+42.1%) (\( P < 0.05 \)) (Fig. 5a); this phenomenon may explain why \( R_m \) was lowest for the medium N level. Overall, belowground biomass increased with N addition rate (\( P < 0.05 \)) (Table 1), indicating that N enrichment could stimulate root biomass accumulation and thus increase C and nutrient retention in the soil.

In our meta-analysis, MAT and MAP exhibited non-significant effects on SOC and \( R_m \) response to N addition, but DOC, MBC, and litter responses to N addition were smaller in regions with high MAT and

**Fig. 7.** Relationships of belowground biomass and microbial biomass C (MBC), MBC and soil respiration (\( R_m \)), and belowground biomass and \( R_m \) **.

*Note: Figure text likely contains various symbols and equations not visible in the image.*
MAP (Table 1). This finding indicated that DOC, MBC, and litter had more sensitive responses to N deposition in colder and more arid regions. In these regions, the N content in the soil is lower; thus, plant growth and soil development are often N limited (Deng and Shangguan, 2017). In contrast, N in the soil is relatively abundant in areas of high temperature and humidity (Deng and Shangguan, 2017); therefore, plant and soil systems have less sensitive responses compared with those in colder and more arid areas. In addition, from the relative contributions of MAP, MAT, and N addition rate to SOC, DOC, MBC, belowground biomass, litter, and Rs, our results showed that climatic factors (i.e., MAT and MAP) played a greater role than did N enrichment in affecting these response variables (Fig. 8). This finding indicated that in China, with its large spatial heterogeneity in climatic conditions and ecological types, the climatic conditions of regions played a greater role than did N deposition level in affecting processes of ecosystem C cycling. This finding may explain why most response variables showed non-significant relationships with N addition rates in our meta-analysis (Table 1).

4.5. Implications for ecosystem C balance

Ecosystem C balance is determined by three aspects: C assimilation via photosynthesis, C loss via ecosystem respiration, and non-respiratory loss such as DOC leaching (De Deyn et al., 2008). The meta-analysis results suggested that N enrichment both increased C inputs to soil mainly by increasing litter and belowground biomass inputs (Table 2) and increased C output by increasing Rs (Table 2). Overall, our meta-analysis showed that N enrichment had a positive effect on ecosystem C cycles in China, and we found that N enrichment (kg ha$^{-1}$ yr$^{-1}$) increased belowground C pool accumulation (Table 2), indicating that N enrichment may increase ecosystem C sequestration under N deposition. However, large variation in the RR values (Fig. 3–6, Appendix Fig. S2) indicates that the effects vary greatly across climatic conditions and vegetation types. A more adequate comparison of responses across the heterogeneous landscapes and vegetation types of China would require a more comprehensive suite of experimental studies (Fu et al., 2015). Moreover, it is difficult to predict whether N addition will increase long-term belowground C sequestration based on the short-term N addition studies included in our meta-analysis.

In addition, our meta-analysis found that N enrichment significantly altered several C cycle components: SOC, DOC, MBC, litter, Rs, and RM (Fig. 3). However, we found no relationship between the amount of N addition and the response magnitude of these C cycling components (Table 1). C-cycling processes are impacted by complicated interactions among such factors as land use type, N addition, soil type, and climate.

The variation caused by these interactions makes it very difficult to detect any dose-response relationship in our meta-analysis and in other such synthesis studies (Liu and Greaver, 2010; Lu et al., 2011).

Despite our meta-analysis focusing on China, our results have implications for global climate change studies throughout the world. Although some global synthesis studies have already explored the effects of N enrichment on belowground C dynamics (Liu and Greaver, 2010; Lu et al., 2011), few data from China were included in those syntheses. Ecosystems in China have wide spatial heterogeneity and diverse biogeography, and many N enrichment experiments have been conducted in China. The findings of our meta-analysis are a beneficial complement to current global syntheses, adding the knowledge gained from the wide range of N enrichment experiments conducted in China in recent years and providing new insight into regional responses to global N deposition.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2017.10.215.

References


Table 2

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>Standard error</th>
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<tbody>
<tr>
<td>SOC (g kg$^{-1}$)</td>
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<td>0.01</td>
<td>60</td>
</tr>
<tr>
<td>DOC (mg kg$^{-1}$)</td>
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<tr>
<td>MBC (mg kg$^{-1}$)</td>
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<tr>
<td>Belowground biomass (kg ha$^{-1}$)</td>
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<tr>
<td>Litter (kg ha$^{-1}$)</td>
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<td>13</td>
</tr>
<tr>
<td>Rs (kg ha$^{-1}$)</td>
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<td>0.55</td>
<td>132</td>
</tr>
<tr>
<td>Rm (kg ha$^{-1}$)</td>
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<td>1.83</td>
<td>3</td>
</tr>
</tbody>
</table>

Fig. 8. Relative contributions of MAP, MAT, and N addition rate to response variables, estimated using a Multivariable linear regression analysis. Note: SOC, soil organic C concentration; DOC, soil dissolved organic C concentration; BGB, belowground biomass; LB, litter; MBC, soil microbial biomass C; Rs, soil respiration.


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