Global Change Biology

Global Change Biology (2017) **23**, 1328–1337, doi: 10.1111/gcb.13402

Costimulation of soil glycosidase activity and soil respiration by nitrogen addition

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Abstract

Unprecedented levels of nitrogen (N) have been deposited in ecosystems over the past century, which is expected to have cascading effects on microbially mediated soil respiration (SR). Extracellular enzymes play critical roles on the degradation of soil organic matter, and measurements of their activities are potentially useful indicators of SR. The links between soil extracellular enzymatic activities (EEAs) and SR under N addition, however, have not been established. We therefore conducted a meta-analysis from 62 publications to synthesize the responses of soil EEAs and SR to elevated N. Nitrogen addition significantly increased glycosidase activity (GA) by 13.0%, α-1,4-glucosidase (AG) by 19.6%, β-1,4-glucosidase (BG) by 11.1%, β-1,4-xylosidase (BX) by 21.9% and β-D-cellobiosidase (CBH) by 12.6%. Increases in GA were more evident for long duration, high rate, organic and mixed N addition (combination of organic and inorganic N addition), as well as for studies from farmland. The response ratios (RRs) of GA were positively correlated with the SR-RRs, even when evaluated individually for AG, BG, BX and CBH. This positive correlation between GA-RR and SR-RR was maintained for most types of vegetation and soil as well as for different methods of N addition. Our results provide the first evidence that GA is linked to SR under N addition over a range of ecosystems and highlight the need for further studies on the response of other soil EEAs to various global change factors and their implications for ecosystem functions.

Keywords: glycosidase activity, meta-analysis, nitrogen addition, soil respiration, soil type, vegetation type

Received 30 July 2015 and accepted 3 June 2016

Introduction

Atmospheric nitrogen (N) deposition has already increased by three- to five-fold compared with pre-industrial levels, and future global N deposition rates are expected to increase by a factor of 2.5 over the next century (Vitousek *et al.*, 1997; Galloway *et al.*, 2008; Davidson, 2009). Additional N has been shown to stimulate plant growth and sequestration of atmospheric CO_2 (Lebauer & Treseder, 2008; Maaroufi *et al.*, 2015). However, large uncertainties still remain concerning belowground C cycles, as the dynamics of soil C are often mediated by many complicated processes, such as soil microbial activities and extracellular enzymatic activities (EEAs) (Fog, 1988; Knorr *et al.*, 2005; Lu *et al.*, 2011; Zhou *et al.*, 2014; Rousk *et al.*, 2016). More than half of the C sequestered annually by terrestrial plants is ultimately allocated belowground, and soils contain

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about four times as much C as vegetation biomass (Vitousek *et al.*, 1997; Chen *et al.*, 2015b). Such large uncertainties concerning the effects of N addition on belowground C cycles would therefore constitute important challenges to quantifying and predicting the dynamics of terrestrial C, especially under the current scenarios of global climate change (Thornton *et al.*, 2007; Gruber & Galloway, 2008; Thomas *et al.*, 2015). To advance our understanding of C–climate feedbacks, more information of N regulation of terrestrial C dynamics is urgently needed.

Soil respiration (SR) represents the largest flux of C from soils to the atmosphere (Zhou *et al.*, 2007; Chen *et al.*, 2016a), but the effects of N addition on SR vary greatly for results from field observations (Eberwein *et al.*, 2015; Liu *et al.*, 2015), models (Magnani *et al.*, 2007; Devaraju *et al.*, 2016) and meta-analyses (Janssens *et al.*, 2010; Liu & Greaver, 2010; Lu *et al.*, 2011; Zhou *et al.*, 2014). The underlying mechanisms for these divergent responses remain largely unclear. Whatever the exact mechanisms, changes in microbial enzymatic activities are likely the fundamental drivers of the response of SR to N addition as more than half of SR is derived from enzyme-mediated decomposition of litter and soil organic matter (SOM) (Carreiro *et al.*, 2000; Waldrop *et al.*, 2004; Allison *et al.*, 2010b; Shahzad *et al.*, 2015; Chen *et al.*, 2016b). It is therefore likely that understanding how soil EEAs respond to N addition could provide novel ways to reconcile the divergent responses of SR to N addition (Allison *et al.*, 2008; Weedon *et al.*, 2011; Stone *et al.*, 2014), a concept also suggested by several recent reviews (Fog, 1988; Knorr *et al.*, 2005; Janssens *et al.*, 2010). Evidence from recent enzymatic kinetics-based modeling efforts also supported that inclusion of soil EEAs into models has greatly improved the estimates of SR and C–climate feedbacks (Allison *et al.*, 2010b; Ali *et al.*, 2015). Therefore, it is clear that our understanding of the effects of N addition on SR will greatly benefit from the study of soil EEAs.

Soil EEAs are commonly regarded as potential indicators of microbial nutrient requirements and depolymerization rates of SOM, yet their activities largely depend upon nutrient availability (Treseder, 2004; Waldrop *et al.*, 2004; Sinsabaugh *et al.*, 2008). Nitrogen limitation is widespread in terrestrial ecosystems (Lebauer & Treseder, 2008), and soil microorganisms and soil EEAs are therefore highly sensitive to increased levels of N (Allison *et al.*, 2008; Treseder, 2008). The effects of N addition on soil EEAs, however, are highly variable between individual studies (Currey *et al.*, 2010; Cusack *et al.*, 2010), which have greatly hindered our understanding of the possible mechanisms driving the response of soil EEAs following N addition. It has been reported that soil EEAs responded positively to N addition in farmland and grassland (Zeglin *et al.*, 2007; Nowinski *et al.*, 2009), but the responses were neutral or negative in temperate and boreal forests (Allison *et al.*, 2010a; Janssens *et al.*, 2010). The lack of consistency suggests the importance of evaluating the effects of N addition on EEAs and SR in specific types of ecosystems (Schmidt *et al.*, 2011). Addition of organic or combination of organic and inorganic N (mixed N addition) generally had positive effects on soil EEAs, while NH_4^+ or NO_3^- addition had negative impacts (Du *et al.*, 2014; Li *et al.*, 2014b). It stands to reason that the forms of added N should be reflected in soil N availability or pH and that each of these effects should influence soil EEAs and SR (Sinsabaugh *et al.*, 2008). Therefore, it is critically necessary to synthesize results from a variety of studies to accurately characterize the principle effects of N addition on soil EEAs and their possible impacts on SR.

We conducted a meta-analysis on the responses of soil EEAs and SR to N addition. Our goal was to focus more broadly on the links between soil EEAs and SR rather than evaluating them separately. The objectives of this study were (i) to test whether the response of SR to N addition could be linked to the responses of soil EEAs (ii) and, if so, to investigate the responses of these EEAs to the additional N in various types of ecosystems and the different methods of N addition and (iii) to further explore the factors affecting the possible links between soil EEAs and SR, such as substrate N availability and pH.

Materials and methods

Sources of data

We searched journal articles published before October 2015 using the Web of Science (http://apps.webofknowledge.com/). The keywords and phrases used for the literature search were (i) 'nitrogen deposition' OR 'nitrogen addition' OR 'nitrogen enrichment' OR 'nitrogen fertilizer' OR 'nitrogen amendment' OR 'nitrogen elevated', AND (ii) 'soil extracellular enzymes', AND (iii) 'terrestrial' OR 'soil' OR 'land'.

Articles were selected based on the following criteria. (i) Only field experiments were included, and we selected only studies with durations longer than 1 year. Control and N-addition treatments had to be at the same experimental site; that is, the microclimate and vegetation and soil types had to be similar between treatments. (ii) Standard deviations (SDs) and numbers of replicates were reported. If standard errors (SEs) were reported, the following equation was used to estimate SDs:

$$\mathrm{SD} = \mathrm{SE} \times \sqrt{n}, \quad (1)$$

where n is the number of replicates. (iii) The methods of N addition (rate, frequency, form and duration) were clearly described.

(iv) If more than one field manipulation experiment was reported in the same article but for different environmental variables or types of ecosystem (e.g. experiments conducted under various types of vegetation and soil), each experiment was treated as an independent study. (v) If parameters were measured more than once in the same year, we chose only the last set of measurements. (vi) If the results were reported for different soil layers, we included only the uppermost soil layer (García-Palacios *et al.*, 2014). We selected a total of 62 publications based on these criteria (Appendix S1 and Fig. S1).

Data acquisition

For each of the selected studies, we recorded the study site, location, vegetation type, soil type (http://www.fao.org/about/en/), N-addition rate (low <5 g N m^{-2} yr^{-1}, medium 5–15 g N m^{-2} yr^{-1} and high >15 g N m^{-2} yr^{-1}), N-addition duration (short <5 year, medium 5–10 year and long >10 year), N-addition frequency (low <4 per year, medium 4–12 per year and high >12 per year), N-addition form (NH_4^+, NO_3^-, NH_4NO_3, organic N or mixed N) and soil EEAs. Wherever possible, we also recorded SR, soil pH, soil total N, soil dissolved organic N (DON), substrate C: N ratios and aboveground biomass. We defined SR as the amount of soil CO_2 measured by soil chambers in the field studies or during laboratory incubations (Treseder, 2008; Chen *et al.*, 2015a). If the results were presented graphically, we used Engauge Digitizer 4.1 (http://digitizer.sourceforge.net) to digitize and extract the data. The authors were contacted when critical information could not be directly acquired from the selected articles or their references.

Data analysis

The effects of N addition were evaluated using the response ratio (RR), which was calculated as:

$$\mathrm{RR} = \ln\left(\frac{\overline{X_N}}{\overline{X_C}}\right) = \ln\left(\overline{X_N}\right) - \ln\left(\overline{X_C}\right), \tag{2}$$

where $\overline{X_N}$ and $\overline{X_C}$ are the arithmetic mean concentrations of the soil EEAs in the experimental (N addition) and control treatments, respectively. The statistical distribution of the RRs calculated in this way was found to be nearly normally distributed, and only minor biases were detected (Hedges *et al.*, 1999). The variances (v) were calculated by:

$$v = \frac{s_N^2}{n_N \overline{X_N^2}} + \frac{s_C^2}{n_C \overline{X_C^2}}, \tag{3}$$

where n_N and n_C are the replicate numbers of experimental and control treatments, respectively, and S_N and S_C are the SDs for the experimental and control treatments, respectively. The reciprocal of the variance was used as the weight (w) for each RR. The overall mean response ratio (RR_{++}) was calculated from the individual RRs for the experimental and control treatments:

$$\mathrm{RR}_{++} = \frac{\sum_{i=1}^{m}\sum_{j=1}^{k} w_{ij}\mathrm{RR}_{ij}}{\sum_{i=1}^{m}\sum_{j=1}^{k} w_{ij}}, \tag{4}$$

where m is the number of compared groups and k is the number of comparisons in the corresponding groups. The SE of RR_{++} was estimated by:

$$\mathrm{SE}(\mathrm{RR}_{++}) = \sqrt{\frac{1}{\sum_{i=1}^{m}\sum_{j=1}^{k} w_{ij}}}. \tag{5}$$

To determine whether N addition had a significant effect, we applied a random model using MetaWin (Sinauer Associates Inc., Sunderland, MA, USA), in which bootstrap 95% confidence intervals (CIs) were calculated for each categorical group. The effect of a treatment was deemed significant if the bootstrap CI did not overlap with zero. Changes induced by N addition were calculated by:

$$[\exp(\mathrm{RR}_{++}) - 1] \times 100\%. \tag{6}$$

Pearson correlation analyses were used to evaluate the relationships between the RRs for the soil EEAs and (i) environmental variables, (ii) N-addition methods and (iii) substrate N concentrations.

The total heterogeneity (Q_T) for each categorical group was divided into heterogeneities among groups (Q_M) and between groups (Q_B). The chi-square distribution for these Q statistics was approximately normal in previous studies (Treseder, 2008; Liu & Greaver, 2009), which allowed us to test our null hypothesis that all RRs were equal within a categorical group. The statistical confidence level was set at $P < 0.05$.

The frequency distribution of the individual RRs of each enzyme was tested by:

$$y = \alpha\exp\left[-\frac{(x-\mu)^2}{2\sigma^2}\right], \tag{7}$$

where y is the frequency, x is the RR of each enzyme, α is a coefficient for the expected number of RR values at $x = \mu$, and μ and σ are the mean and variance of the frequency distributions of RR, respectively. The frequency distributions are shown in Fig. S2.

Due to the preferential publication of larger over smaller effects, we used a series of statistical measures to evaluate publication bias within each group (Table S1). Kendall's tau rank correlation and Spearman's rank correlation were used to test the rank correlations between replicate numbers of each study and the standardized effect size (Begg, 1994; Rosenberg *et al.*, 2000). A fail-safe number (Rosenthal's method at $\alpha = 0.05$) was next calculated to determine the number of nonsignificant, unpublished studies that would be needed to change the summary of results from significant to nonsignificant (Rosenthal, 1979; Lebauer & Treseder, 2008).

Results

Effects of N addition on glycosidase activity and SR

Our preliminary analysis of 12 kinds of soil extracellular enzymes showed that SR-RRs were only positively correlated with RRs for α-1,4-glucosidase (AG), β-1,4-glucosidase (BG), β-1,4-xylosidase (BX) and β-D-cellobiosidase (CBH) activities (Table S2 and Fig. S3), but no

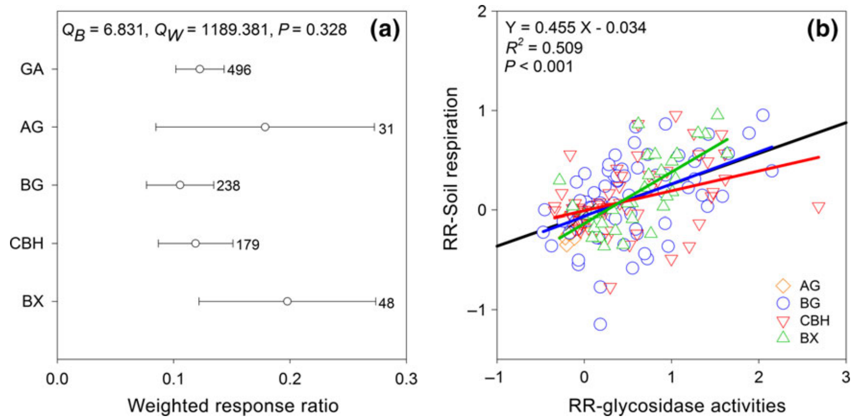

Fig. 1 The effects of N addition on soil glycosidase activity (a, GA) and the relationships between the response ratio (RR) for soil respiration and the RR for glycosidase activity following N addition (b). Error bars represent bootstrap 95% confidence intervals (CIs). The effect of N addition was considered significant if the CI of the effect size did not overlap with zero. The sample size for each variable is shown next to the CI. Q_B and Q_w are defined in the Materials and methods section. The RRs for of the activities of the following enzymes were positively correlated with RR-soil respiration: α-1,4-glucosidase (AG, Y = 0.763X - 0.037, $R^2 = 0.423$, $P < 0.001$), β-1,4-glucosidase (BG, Y = 0.323X - 0.063, $R^2 = 0.255$, $P < 0.001$), β-D-cellobiosidase (CBH, Y = 0.2X - 0.009, $R^2 = 0.145$, $P = 0.003$) and β-1,4-xylosidase (BX, Y = 0.515X - 0.134, $R^2 = 0.473$, $P < 0.001$).

correlation was found for the other 8 kinds of EEAs. In addition, there was no between-group heterogeneity among the four kinds of glycosidase and RRs for glycosidase activity (GA-RRs) did not vary with sample size (Figs 1 and S4). We therefore focused on the responses of GA to N addition and the factors affecting the relationships between the SR-RRs and GA-RRs.

Nitrogen addition significantly increased GA by an average of 13.0% (Fig. 1). In detail, N addition profoundly enhanced AG, BG, BX and CBH activities by 19.6%, 11.1%, 21.9% and 12.6%, respectively. GA-RRs were positively correlated with SR-RRs (Fig. 1), and this relationship was independent of SR measurement methods (Fig. S5).

Methods of N addition

NH_4NO_3, organic and mixed N addition significantly increased GA by 13.2%, 18.8% and 16.5%, respectively, but the addition of NH_4^+ or NO_3^- had no effects on GA (Fig. 2). Positive correlations between GA-RRs and SR-RRs were consistently identified when N was added as NH_4NO_3, organic or mixed forms (Fig. 3).

We found a significant positive linear relationship between GA-RRs and duration of N addition (Fig. S4). Specifically, short, medium and long durations of N addition significantly increased GA by 6.5%, 11.2% and 46.6%, respectively (Fig. 2). Similarly, the positive correlations between GA-RRs and SR-RRs were maintained for all durations of N addition (Fig. 3).

Low, medium and high rates of N addition increased GA by 8.0%, 11.7% and 20.8%, respectively (Fig. 2), and GA-RRs were positively correlated with N-addition rates (Fig. S4). The positive correlations between GA-RRs and SR-RRs held true for medium and high rates but not for a low rate of N addition (Fig. 3).

Low, medium and high frequencies of N addition increased GA by 13.2%, 15.0% and 1.6%, respectively. No between-group heterogeneity was found when

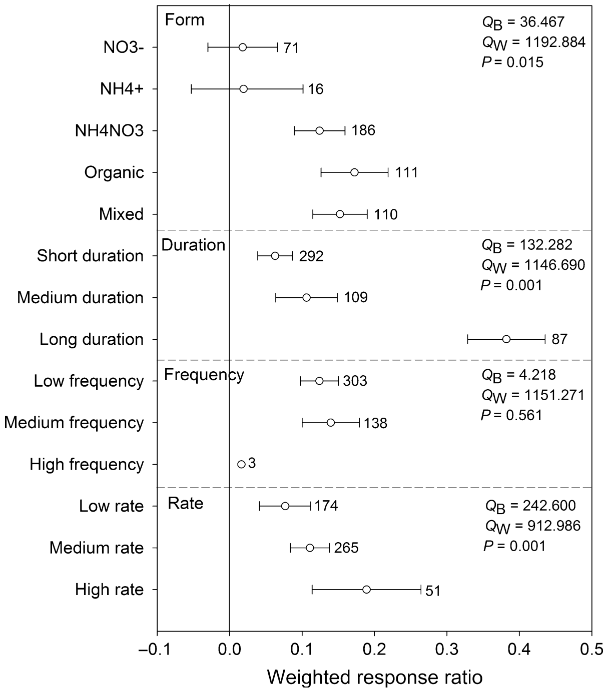

Fig. 2 The effects of nitrogen addition on soil glycosidase activity for various methods of nitrogen addition. See Fig. 1 for detailed information.

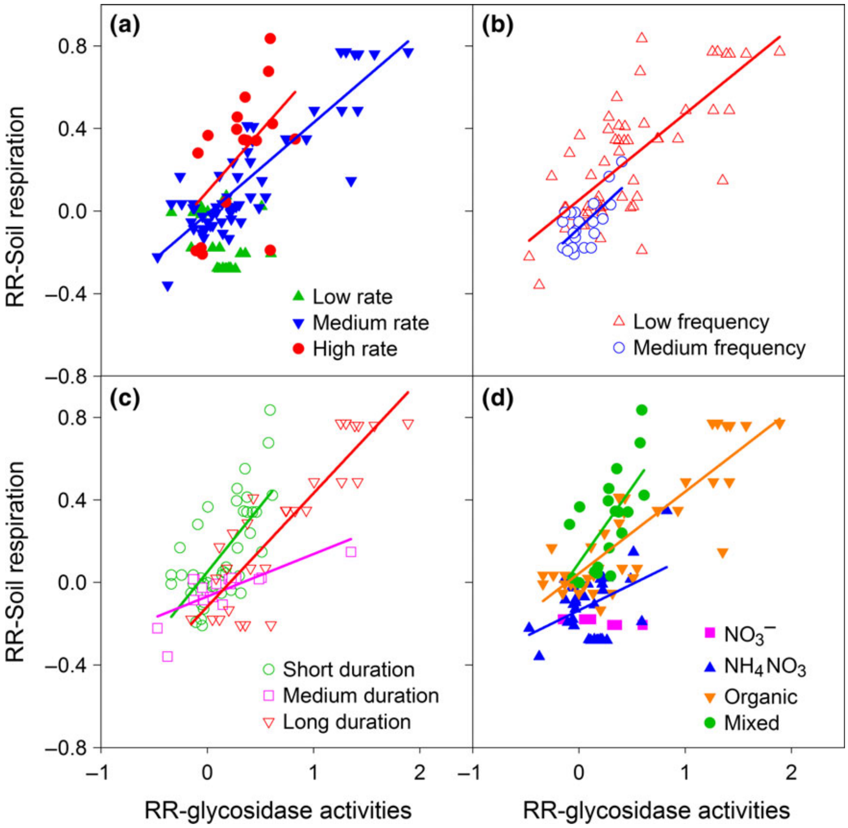

Fig. 3 Relationships between the response ratios (RRs) for soil respiration and the RRs for soil glycosidase activity for the methods of nitrogen addition. Relationships between the RRs for glycosidase activity and RRs for soil respiration for (a) N-addition rate (medium: $Y = 0.442X - 0.014$, $R^2 = 0.760$, $P < 0.001$; high: $Y = 0.583X + 0.095$, $R^2 = 0.286$, $P = 0.027$), (b) N-addition frequency (low: $Y = 0.418X + 0.053$, $R^2 = 0.548$, $P < 0.001$; medium: $Y = 0.480X + 0.083$, $R^2 = 0.430$, $P = 0.001$), (c) N-addition duration (short: $Y = 0.641X + 0.051$, $R^2 = 0.473$, $P < 0.001$; medium: $Y = 0.206X - 0.068$, $R^2 = 0.552$, $P < 0.001$; long: $Y = 0.550X - 0.118$, $R^2 = 0.764$, $P < 0.001$) and (d) N-addition form (NH_4NO_3: $Y = 0.253X - 0.135$, $R^2 = 0.185$, $P < 0.001$; organic: $Y = 0.396X + 0.044$, $R^2 = 0.727$, $P < 0.001$; mixed: $Y = 0.733X + 0.095$, $R^2 = 0.436$, $P = 0.002$).

studies were grouped by the frequency of N addition, and no clear relationship was detected between GA-RRs and frequency of N addition (Figs 2 and S4). GA-RRs were positively correlated with SR-RRs for low and medium frequencies of N addition, but this analysis was limited by the paucity of data for treatments with a high frequency of N addition (Fig. 3).

Vegetation and soil types

The responses of GA to N addition varied significantly among the types of vegetation and soil (Fig. 4). Nitrogen addition significantly increased GA by 34.7%, 6.4%, 5.5% and 19.9% for farmland, forest, grassland and shrubland, respectively, but N addition had no effect on GA in wetland. GA-RRs were positively correlated with SR-RRs for grassland and farmland but not for forest (Fig. 5). No SR data were available for wetland and shrubland, so we could not evaluate the links between GA and SR for those systems.

Nitrogen addition significantly increased GA in chernozems, cambisols, gleysols, entisols, histosols, podzols, lithosols and luvisols by 38.4%, 13.8%, 46.4%, 8.6%, 17.5%, 10.2%, 15.7% and 27.2%, respectively. In contrast, N addition decreased GA by 5.2% in ferralsols and had no effects on GA in solonchaks and arenosols (Fig. 4). Significant positive relationships between the GA-RRs and SR-RRs were found for cambisols and luvisols, but the relationship was negative for podzols (Fig. 5). The small numbers of studies of solonchaks, entisols and ferralsols, however, limited the statistical power of the analysis.

Discussion

This meta-analysis is among the first global syntheses of the effects of N addition on soil EEAs and their impacts on SR. The objectives herein go beyond separate assessments of soil EEAs and SR; rather, we address the broader question of whether the effects of

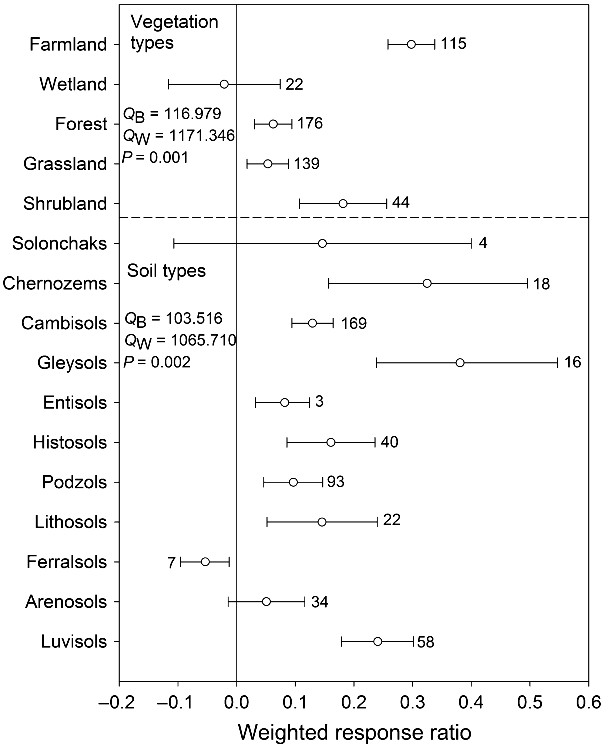

Fig. 4 The effects of nitrogen addition on soil glycosidase activity for various types of vegetation and soil. See Fig. 1 for detailed information.

N addition on soil EEAs can help to explain the enhanced SR reported in previous meta-analyses (Fig. S6) (Lu *et al.*, 2011; Zhou *et al.*, 2014). Our results show that SR-RRs are closely correlated with GA-RRs, and this correlation still holds true when evaluated for most types of vegetation and soil, as well as for different methods of N addition. These results suggest that the effects of N addition on four kinds of glycosidase are likely the effective proximate agents of the effects of N addition on SR (Fig. 1). But, what is the basis for linking the four kinds of glycosidase with SR under N addition? Preliminary analysis of 12 kinds of soil EEAs that were frequently investigated in previous studies (Table S2) indicates that only the RRs for AG, BG, BX and CBH are closely correlated with SR-RRs (Fig. S3). These four kinds of glycosidase are all classified as hydrolysis C-targeting enzymes. Our results suggest that shifts in the activities of various kinds of soil extracellular enzymes likely account for the highly divergent responses of SR to N addition.

Costimulation of GA and SR by N addition

Nitrogen addition significantly increased GA, and the stimulatory effects were positively correlated with SR-RRs (Fig. 1). The added N not only provides the building blocks for enzymatic production because enzymes are fundamentally N-rich molecules, but it also increases microbial C demands due to stoichiometry of microbial nutrients (Allison *et al.*, 2008; Weedon *et al.*, 2011; Sistla & Schimel, 2013). Increases in microbial C demands were expected to be alleviated by promoting the activities of C-degrading enzymes (Buchkowski *et al.*, 2015). This expectation is consistent with the typical economic strategy in which soil microbes would adjust themselves in physiology or community composition to produce enzymes needed for acquiring the most limited resources (Fig. S7) (Sinsabaugh *et al.*, 2008; Stone *et al.*, 2012). Despite the fact that various

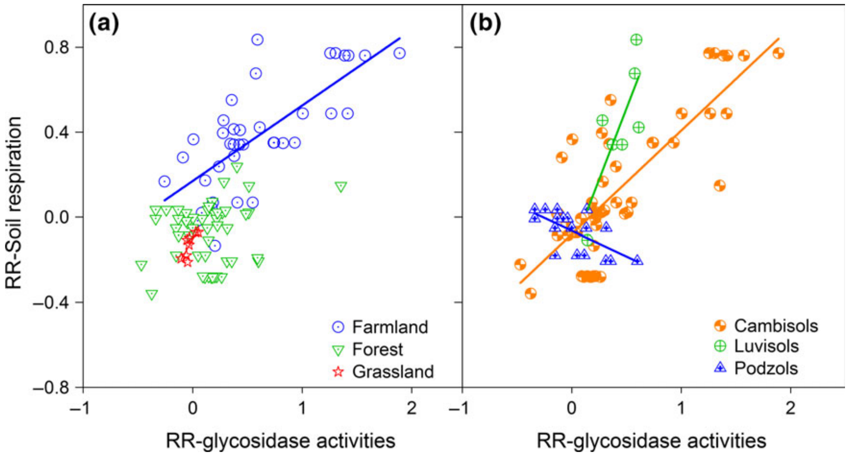

Fig. 5 Relationships between the response ratios (RRs) for soil respiration and the RRs for glycosidase activity for different types of (a) vegetation and (b) soil. Relationships between the RRs of glycosidase activity and RR-soil respiration within vegetation types (grassland: $Y = 0.885X - 0.103$, $R^2 = 0.626$, $P = 0.019$; farmland: $Y = 0.355X + 0.170$, $R^2 = 0.532$, $P < 0.001$) and soil types (cambisols: $Y = 0.487X - 0.079$, $R^2 = 0.640$, $P < 0.001$; luvisols: $Y = 1.340X - 0.158$, $R^2 = 0.638$, $P = 0.031$; podzols: $Y = -0.295X + 0.013$, $R^2 = 0.420$, $P = 0.004$).

kinds of C-degrading enzymes that can contribute to alleviating microbial C demands, N addition had negative effects on the oxidative lignin-degrading enzymes (Fig. S8). Therefore, C requirements of microbes may be mainly fulfilled by synthesizing glycosidase (Fig. 1), which would accelerate the hydrolysis of cellulolytic compounds.

Factors that affect the relationships between GA and SR

Higher substrate N concentrations lead to a more pronounced positive response of GA (Fig. S9), suggesting that high N availability enhances microbial C demands (Treseder, 2008; Nguyen *et al.*, 2016). Longer durations and higher rates of N addition can produce high and stable N concentrations (Bragazza *et al.*, 2012), so the positive responses of GA and the steeper slopes between GA-RRs and SR-RRs under these two conditions were not surprising (Figs 2 and S4). In farmland, N is often added at high rates through anthropogenic fertilization (Chang *et al.*, 2007; Mcdaniel *et al.*, 2014). Cambisols and luvisols are among the most productive soils on Earth, and these soils make good agricultural lands given their high soil fertility (i.e., N content). Consistently, we also observed positive responses of GA and positive correlations between GA-RRs and SR-RRs in these systems (Figs 4 and 5).

We found a more pronounced increase for GA in farmland (34.7%) than the other types of vegetation and soil (Fig. 2). In addition to the higher N rates discussed above, another possible explanation might be related to the forms of N addition, because 92% of the added N in farmland was in organic or mixed N forms (Table S3). Organic and mixed N must be transformed or converted by enzymes and so they had positive effects on GA and SR (Figs 2 and 3) (Ajwa *et al.*, 1999; Hawkins *et al.*, 2000). Considering the current ongoing intensified anthropogenic fertilization with organic and mixed N (Mcdaniel *et al.*, 2014), our results indicate that there might be a stronger positive response of SR in farmland under future N fertilization.

We did not find significant responses of GA for ferralsols, solonchaks, arenosols and wetlands. These types of soils have relatively low pHs due to the high concentrations of iron and aluminum (Chesworth, 2008). Soil nutrient availability and microbial activities may thus be constrained by the low pH or possibly other chemical properties (Evans *et al.*, 2008; Tian & Niu, 2015). This explanation was supported by our regression analysis, which indicates that GA-RRs are closely correlated with soil pH (Fig. S10). A comprehensive assessment of the relationships between GA-RRs and SR-RRs under these conditions is currently not feasible due to the small data set, but other studies have shown that N addition had negative or no effects on SR under these conditions (Tao *et al.*, 2013; Wang *et al.*, 2013; Zhou *et al.*, 2014). Our results suggest that soil pH should be considered when implementing GA-RRs as the proximate agents of SR-RRs.

GA-RRs were not correlated with SR-RRs for forests, podzols or low rates of N addition, even though GA did respond positively to N addition in these cases (Figs 2–5). This finding for forests was consistent with two recent meta-analyses indicating that N addition repressed SR (Janssens *et al.*, 2010; Zhou *et al.*, 2014), perhaps because degradation of the low-quality forest litter responded negatively to the additional N (Fog, 1988; Knorr *et al.*, 2005). Podzols are typical in coniferous and temperate forests, and most podzols are not suitable for agriculture due to their low water-retention capacity and low pH (Chesworth, 2008). These two characteristics of podzols were likely responsible for the negative effects of N addition on SR. The lack of correlation between GA-RRs and SR-RRs with a low rate of N addition may be due in part to undetectable effects caused by the small amount of additional N (Bowden *et al.*, 2004; Knorr *et al.*, 2005). These results would therefore provide valuable information for Earth system models when models are to be applied to various ecosystem types.

Uncertainties and implications

The GA-RRs were on average 2.5 times higher than SR-RRs (Fig. 1), suggesting that N-induced changes in SR might not be the direct result of changes in microbial growth. Assuming that enzyme expression is directly related to production rate, these results imply that N addition increases microbial carbon-use efficiency (CUE) (Allison *et al.*, 2010b). Alternatively, microbial growth and death rates could respond differentially to the elevated N (Treseder, 2008; Hagerty *et al.*, 2014). Nonetheless, the responses of microbial CUE and growth and death rates to N addition are currently unclear. Major limitations also may stem from the paucity of data for the various types of vegetation and soil, such as those in arid and semiarid regions and wetlands (Table S3). Future field experiments should be conducted with a wide range of variables to clarify the underlying principles.

The highly consistent responses of GA and SR to N addition identified here support the utility of explicitly incorporating microbial activities and soil EEAs into models for predicting the dynamics of soil C under various global change scenarios (Allison *et al.*, 2010b; Wieder *et al.*, 2013). Actually, several recent modeling efforts have confirmed that incorporation of soil EEAs into enzymatic-kinetics models has substantially improved the projection of both the direction and magnitude of C–climate feedbacks (Allison *et al*., 2010b; Li *et al*., 2014a; Xu *et al*., 2014; Ali *et al*., 2015). Our results also have important implications for other global change factors, for example, climatic warming. Warming has globally increased substrate N concentrations, aboveground biomass, SR (Bai *et al*., 2013; Lu *et al*., 2013), and it is likely that warming will also lead to increases in GA, as discussed above. This extrapolation seems reasonable, but we are well aware that ecosystems are often highly heterogeneous and may respond in unexpected ways to environmental changes. Our meta-analysis is a first attempt at linking GA to SR under N addition, and future studies are clearly needed to determine how other soil EEAs and associated ecosystem functions respond to various global change factors.

Acknowledgements

This study was supported by the Ministry of Science and Technology (2012BAH31B03) and the State Key Laboratory of Loess and Quaternary Geology, Institute of Earth Environment, Chinese Academy of Sciences (SKLLQG1303). Contributions from Dr. Luo's Eco-lab to this study were financially supported by Terrestrial Ecosystem Sciences grant DE SC0008270 from the U.S. Department of Energy and by the US National Science Foundation grants DBI 0850290, EPS0919466, DEB 0743778, DEB 0840964 and EF 1137293. Contributions from Dr. Jianwei Li's laboratory were financially supported by USDA National Institute of Food and Agriculture – Evans-Allen project No. 1005761. Contributions from Dr. Xuhui Zhou's laboratory were financially supported by the National Natural Science Foundation of China (31290221, 31070407 and 31370489), the National Basic Research Program (973 Program) of China (No. 2010CB833502), the Program for Professor of Special Appointment (Eastern Scholar) at Shanghai Institutions of Higher Learning and the 'Thousand Young Talents' Program in China. The authors also gratefully acknowledge financial support from the China Scholarship Council (an award for one year of study abroad at the University of Oklahoma).

Competing financial interests

The authors declare no competing financial interests.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Supplementary notes.
Table S1 Results for publication bias.
Table S2 Description of the 12 kinds of enzymes included in our preliminary analysis.
Table S3 Distribution of the methods of nitrogen addition for the various types of vegetation and soil.
Figure S1 Global distribution of the nitrogen-addition experiments selected in this meta-analysis. The map was created with ArcGIS.
Figure S2 Frequency distributions of the response ratios (RR) of (a) α-1,4-glucosidase (AG), (b) β-1,4-glucosidase (BG), (c) β-D-cellobiosidase (CBH) and (d) β-1,4-xylosidase (BX).
Figure S3 Relationships between the response ratio (RR) of soil respiration (SR) and the RRs of (a) α-1,4-glucosidase (AG), (b) β-1,4-glucosidase (BG), (c) β-D-cellobiosidase (CBH), (d) β-1,4-xylosidase (BX), (e) phenol oxidase (PO), (f) polyphenol oxidase (PHO), (g) invertase, (h) urease, (i) peroxidase (PER), (j) β-1,4-N-acetylglucosaminidase (NAG), (k) acid (alkaline) phosphatase (AP) and (l) leucine amino peptidase (LAP).
Figure S4 Relationships between the response ratio (RR) of soil glycosidase activity and (a) N-addition rate, (b) N-addition duration, (c) N-addition frequency and (d) sample size.
Figure S5 Relationships between the response ratio (RR) of glycosidase activity and the RR of soil respiration (SR) for the different methods of SR measurement.
Figure S6 The effects of N addition on soil respiration from previous meta-analyses. Error bars represent bootstrap 95% confidence intervals (CIs). The effect of N addition was considered significant if the CI of the effect size did not overlap zero. The sample size for each variable is shown next to the CI. This figure was redrawn from previous meta-analyses published by (a, b and c) Zhou *et al.* 2014, (d) Liu *et al.* 2010, (e) Lu *et al.* 2011 and (f) Janssens *et al.* 2010. Ra, autotrophic respiration; Rh, heterotrophic respiration; SR, soil respiration.
Figure S7 Relationships between the possible changes in microbial communities and physiology and the response ratios (RR) of glycosidase activity of (a) microbial abundance, (b) bacterial abundance, (c) fungal abundance, (d) fungi/bacteria, (e) microbial biomass carbon (MBC), (f) microbial biomass nitrogen (MBN) and (g) MBC/MBN. The relationships between the changes in microbial communities and physiology induced by N addition and their links with the corresponding changes in soil respiration were synthesized by Treseder *et al.* (2008).
Figure S8 (a) The effects of N addition on the activities of soil oxidative C-acquiring enzymes. Frequency distributions of the response ratios (RR) of (b) oxidative enzymes, (c) phenol oxidase (PO), (d) peroxidase (PER) and (e) polyphenol oxidase (PHO). Error bars represent bootstrap 95% confidence intervals (CIs). The effect of N addition was considered significant if the CI of the effect size did not overlap zero. The sample size for each variable is shown next to the CI. Q_B and Q_w are defined in the Materials and methods section.
Figure S9 Relationships between the response ratio (RR) of glycosidase activity and the (a) RR of soil total nitrogen (STN), (b) RR of dissolved organic nitrogen (DON), (c) RR of the substrate C:N ratio and (d) substrate C:N ratio.
Figure S10 Relationships between the response ratio (RR) of glycosidase activity and (a) the substrate pH and (b) the RR of the substrate pH.