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Soil Extracellular Enzyme Activities, Soil Carbon and Nitrogen Storage under Nitrogen Fertilization: A Meta-analysis

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Abstract. Nitrogen (N) fertilization affects the rate of soil organic carbon (SOC) decomposition by regulating extracellular enzyme activities (EEA). Extracellular enzymes have not been represented in global biogeochemical models. Understanding the relationships among EEA and SOC, soil N (TN), and soil microbial biomass carbon (MBC) under N fertilization would enable modeling of the influence of EEA on SOC decomposition. Based on 65 published studies, we synthesized the activities of α-1,4-glucosidase (AG), β-1,4-glucosidase (BG), β-D-cellobiosidase (CBH), β-1,4-xylosidase (BX, β-1,4-N-acetyl-glucosaminidase (NAG), leucineamino peptidase (LAP), urease (UREA), acid phosphatase (AP), phenol oxidase (PHO), and peroxidase (PEO) in response to N fertilization. The proxy variables for hydrolytic C acquisition enzymes (*C-acq*), N acquisition (*N-acq*), and oxidative decomposition (*OX*) were calculated as the sum of AG, BG, CBH and BX; AG and LAP; PHO and PEO, respectively. The relationships between response ratios (RRs) of EEA and SOC, TN, or MBC were explored when they were reported simultaneously. Results showed that N fertilization significantly increased CBH, *C-acq*, AP, BX, BG, AG, and UREA activities by 6.4, 9.1, 10.6, 11.0, 11.2, 12.0, and 18.6%, but decreased PEO, *OX* and PHO by 6.1, 7.9 and 11.1%, respectively. N fertilization enhanced SOC and TN by 7.6% and 15.3%, respectively, but inhibited MBC by 9.5%. Significant positive correlations were found only between the RRs of *C-acq* and MBC, suggesting that changes in combined hydrolase activities might act as a proxy for MBC under N fertilization. In contrast with other variables, the RRs of AP, MBC, and TN showed unidirectional trends under different edaphic, environmental, and physiological

conditions. Our results provide the first comprehensive set of evidence of how hydrolase and oxidase activities respond to N fertilization in various ecosystems. Future large-scale model projections could incorporate the observed relationship between hydrolases and microbial biomass as a proxy for C acquisition under global N enrichment scenarios in different ecosystems.

Key words: Nitrogen fertilization; extracellular enzyme activities (EEA); soil organic carbon (SOC); microbial biomass carbon (MBC); meta-analysis

1. Introduction

Nitrogen (N) fertilization is the major contributor to global reactive nitrogen inputs, which are projected to increase from 86 Tg N in 1995 to 135 Tg N in 2050 (Galloway et al., 2008; Fowler et al., 2013). This enhanced N availability can alter the formation and decomposition of soil organic matter (SOM) due to the essential coupling of carbon (C) and N cycling in terrestrial ecosystems (Vitousek et al., 1997; Thornton et al., 2007; Galloway et al., 2008; Schlesinger, 2009). Because soils contain the largest reservoir of terrestrial organic C in the biosphere [i.e., 2344 Pg C in the top 3 meters of soil (Jobbágy and Jackson, 2000)], elevated N bioavailability could alter soil C turnover and exert strong feedbacks on global climate change (Federle et al., 1986; Davidson and Janssens, 2006; Friedlingstein et al., 2006; Billings and Ziegler, 2008; Schimel, 2013; Li et al., 2014). Extracellular enzyme activities (EEA) are good indicators of soil C decomposition (Sinsabaugh, 1994; Sinsabaugh et al., 2008), therefore N fertilization could affect observed EEA (or EEAs). In spite of the increasing number of field and laboratory studies on this topic,

only one synthesis paper has explored N fertilization effects on EEA, and the study was limited to agricultural ecosystems (Geisseler and Scow, 2014).

A wide range of EEAs have been associated with C and N turnover (Burns, 1982; Dick, 1994; Wallenstein and Burns, 2011; Burns et al., 2013; Henry, 2013). In general, soil extracellular enzymes include hydrolases and oxidases that decompose substrates of varying composition and complexity (Sinsabaugh, 2010; Sinsabaugh and Follstad Shah, 2012). Cellulases are a group of hydrolytic enzymes that soil microbes produce to decompose polysaccharides; they include α-1,4-glucosidase (AG); β-1,4-glucosidase (BG); β-D-cellobiosidase (CBH); and β-1,4-xylosidase (BX) (Deng and Tabatabai, 1994). The enzymes associated with microbial N acquisition include β-1,4-N-acetyl-glucosaminidase (NAG); leucine amino peptidase (LAP); and urease (UREA), which target chitin, protein, and urea, respectively (Tabatabai and Bremner, 1972). The enzymes associated with P acquisition cleave PO_4^{3-} from P-containing organic compounds; they include acidic phosphatase (AP) and alkaline phosphatase (Tabatabai and Bremner, 1972; Eivazi and Tabatabai, 1977; Hui et al., 2013). The production of oxidative enzymes incurs high energy costs; they are produced by microbes specifically to decompose substrates which must be oxidized (i.e., lignin). Phenol oxidase (PHO) and peroxidase (PEO) are the two most frequently assayed oxidases (Sinsabaugh, 2010; Wang et al., 2012).

The responses of EEA under N fertilization have been studied for decades and generally showed variations in both direction and magnitude across studies (Burns et al., 2013; Henry, 2013; Geisseler and Scow, 2014; Sinsabaugh et al., 2014). BG

activities increased (Saiya-Cork et al., 2002; Waldrop et al., 2004a; Sinsabaugh et al., 2005), remained constant (Zeglin et al., 2007), or decreased as a result of N fertilization (Ramirez et al., 2012). NAG activities were stimulated by 14% or suppressed by 24% as a result of N fertilization across different sites (Saiya-Cork et al., 2002; Billings and Ziegler, 2008). Stimulation of AP activities under N fertilization has been widely observed across studies (Marklein and Houlton, 2012). The ligninolytic enzyme activities were suppressed under N fertilization (Carreiro et al., 2000; Waldrop et al., 2004b; Sinsabaugh, 2010), but PHO was both stimulated and remained constant in other sites (Allison et al., 2008; Sinsabaugh, 2010; Li et al., 2013). A meta-analysis based on 8 to 26 agricultural sites revealed that N fertilization significantly increased BG but had no significant effect on protease, AP, and urease (Geisseler and Scow, 2014).

N fertilization also affected microbial growth and activities, which directly altered soil organic carbon (SOC) turnover and subsequently led to changes in C and N pool sizes. N fertilization caused reductions of 8%~11% in microbial respiration (Treseder, 2008; Liu and Greaver, 2010) and of 15%~35% in microbial biomass carbon (MBC) (Treseder, 2008; Liu and Greaver, 2010; Ramirez et al., 2010). However, a recent meta-analysis reported that N fertilization increased MBC by 15% in agricultural soils, which was attributed to higher crop production (Geisseler and Scow, 2014). It was also pointed out that MBC may decrease due to N fertilization reducing the pH of the soil (Geisseler and Scow, 2014). The change of MBC under N fertilization was observed to be regulated by the net effect of increased relative

abundance of *Actinobacteria* and *Firmicutes* and decreased relative abundance of *Acidobacteria* and *Verrucomicrobia* (Ramirez et al., 2012). Similar to the large variations in effects of MBC, N fertilization can enhance, decrease, or have no effect on SOC stocks (Neff et al., 2002; Mack et al., 2004; Hyvonen et al., 2008; Pregitzer et al., 2008; Lu et al., 2011b); and the effects may vary in different ecosystems (Lu et al., 2011b). Recent reviews and meta-analyses also showed that N fertilization generally increased N stock in bulk soil and in different soil N pools (Liu and Greaver, 2010; Lu et al., 2011a; Lu et al., 2011b; Lu et al., 2013; García-Palacios et al., 2015).

Because of the increasing availability of soil EEA measurements in the last decade, it has become possible to use a meta-analysis approach to synthesize various EEA responses to N fertilization (Gurevitch and Hedges, 1999; Hedges et al., 1999; Luo et al., 2006; Lu et al., 2013). In this study, we collected and synthesized 65 independent studies to elucidate the impact of N fertilization on EEA associated with soil C, N and P acquisition, SOC stock, soil N (TN), and MBC pool sizes. We hypothesized that (1) N fertilization will significantly increase EEA associated with C and P acquisitions but depress EEA associated with N and oxidative C acquisitions, 2) N fertilization will increase SOC and TN but decrease MBC, (3) SOC and *OX* or MBC and *C-acq* will be positively correlated. We further explored these patterns across different edaphic, environmental, and physiological conditions. This study summarizes the increasing N inputs in terrestrial ecosystems, important microbial extracellular enzyme changes, and the impact of EEA on soil C and N dynamics.

2. Materials and methods

2.1 Data collection

We used the search engine Web of Science to locate published journal articles, using the combinations of key words that included "soil, "extracellular enzyme", "exoenzyme" and either "nitrogen fertilization", "nitrogen deposition", "chronic nitrogen fertilization", "nitrogen enrichment", or "nitrogen addition". We found 65 published papers that reported at least one of our targeted variables either in absolute values or in figures. If only relative changes of enzyme activities were reported, we contacted the corresponding authors; some of the absolute values from their replies have been included. Data were extracted according to the following criteria: (1) if data were only reported in graphs and figures, the means and standard deviations (SDs) were extracted using GetData Graph Digitizer 2.26 (http://www.getdata-graph-digitizer.com/index.php). If replicate numbers (n) and standard errors (SEs) were reported, they were converted to SDs using $SD = SE \times \sqrt{n}$. (2) If one article reported multiple independent manipulative experiments (e.g., two experiments at separate locations), each of them was considered as an independent study and incorporated into our dataset (García-Palacios et al., 2015). (3) For studies with multiple global changes or ecological factors being manipulated (i.e., altered temperature, carbon dioxide concentration, or precipitation regime), we only extracted data from control plots and N fertilization plots (García-Palacios et al., 2015). (4) If one article contained results from multiple sampling dates and soil depths, we used the measurement of the latest sampling time and the uppermost soil layer. The

complete dataset and 65 publications are attached in the supplementary material.

In total, data describing ten different extracellular enzymes were collected (Table 1). We further integrated individual EEA into combined EEA to represent proxies targeting specific substrate or nutrient acquisitions – hydrolytic, oxidative, N, or P acquisition. The combined EEA was calculated as the average of multiple individual enzyme activities measured in each study by assuming that the absolute values from potential assays correspond to meaningful differences in functional rates (Li et al., 2012; Li et al., 2013). The C acquiring enzymes (*C-acq*) denote the average enzyme activity of AG, BG, BX and CBH; N acquiring enzymes (*N-acq*) denote the average enzyme activity of NAG and LAP; and oxidative enzymes (*OX*) denote the average enzyme activity of PHO and PEO. A ratio of enzymatic C over N acquisition (*C:N-acq*) was obtained by the *C-acq* divided by *N-acq*. We also collected SOC, TN, and MBC from studies that reported EEA simultaneously; this data allowed us to explore the relationship between EEA and these C or N pool sizes.

For each site, we also collected edaphic, climatic and experimental information. The edaphic properties included soil type, soil texture, soil depth, and ecosystem type; climatic properties included mean annual temperature (MAT) and mean annual precipitation (MAP); experimental properties included the type of experiment, the duration of the experiment, and the quantity and form of N fertilization. Soil type and soil texture records were extracted from the original publications, or they were determined from soil characteristics following USDA soil taxonomy (Soil Survey Staff, 1999). Given the fact that N fertilization was carried out

in the field or during lab incubation, the experiment type was categorized as either "field" or "lab". We further divided continuous variables (i.e., MAT, MAP, duration of the experiment, and N fertilization rate) into categorical variables to conduct group meta-analysis. Different schemes of categorical groups and multiple tests were conducted given the number of observations in each category and the outputs of each test. The following categorical groups were established in this study. MAT was divided into ≤5 °C, 5-10 °C, 10-20 °C, and >20 °C, while MAP was divided into ≤ 250 mm, 250-1000 mm, and >1000 mm. Experiment duration was categorized into ≤1 year, 1-10 years, and >10 years. N fertilizer quantity was grouped into ≤50 kgN/ha/yr, 50-150 kgN/ha/yr, and >150 kgN/ha/yr, while the form of N fertilizer was grouped into NO_3^-, NH_4^+, NH_4^+ and NO_3^-, urea, organic N, and organic N and inorganic N (ON & IN).

2.2 Meta-data analysis

The response ratio (RR) was calculated by the natural log of the ratio between a given variable in the treatment group ($\bar{x}_t$) to that in the control group ($\bar{x}_c$):

$$RR = \ln\left(\frac{\bar{x}_t}{\bar{x}_c}\right) = \ln(\bar{x}_t) - \ln(\bar{x}_c) \quad (1)$$

The variance of effect size (v) was calculated as below:

$$v = \frac{s_t^2}{n_t \bar{x}_t^2} + \frac{s_c^2}{n_c \bar{x}_c^2} \quad (2)$$

where s_t, s_c, n_t and n_c represent standard deviation of treatment groups and control groups, and replicate numbers of treatment and control groups, respectively. In order to derive the overall response effect of treatment group relative to control group, we used the weighted (or average) response ratio (RR_{++}), defined as (Hedges et al., 1999;

Luo et al., 2006; Lu et al., 2013):

$$\mathrm{RR}_{++} = \frac{\sum_{i=1}^{m}\sum_{j=1}^{k} w_{ij} RR_{ij}}{\sum_{i=1}^{m}\sum_{j=1}^{k} w_{ij}} \quad (3)$$

The standard error of RR_{++} was calculated by:

$$s(RR_{++}) = \sqrt{\frac{1}{\sum_{i=1}^{m}\sum_{j=1}^{k} w_{ij}}} \quad (4)$$

The *w* in equations (3) and (4) is the weighting factor, the inverse of the pooled variance (w_{ij} = 1/*v*); *m* is the number of compared groups; and *k* is the number of comparisons in the corresponding groups.

A 95% confidence interval (95% CI) for the RR_{++} was derived by the following equation:

$$95\%\ \mathrm{CI} = RR_{++} \pm 1.96 \times s(RR_{++}) \quad (5)$$

When a 95% CI value of the response valuable did not overlap with 0, we considered the effect of nitrogen fertilization on the variable to be significantly different for the control and treatment groups. A transformation from average response ratio to percentage change was conducted in order to evaluate the effect directly using the equation below:

$$\text{Percentage change} = [\exp(RR_{++}) - 1] \times 100\% \quad (6)$$

2.3 Data analysis

The meta-analysis method was used to calculate the mean effect size and 95% CI of the overall effect of N fertilization on EEA and C and N responses. We also explored the effect of N fertilization on each variable under different groups. In categorical group analysis, the heterogeneities within groups (Q_W) and between

groups (Q_B) were reported, and the chi-square test was applied to determine whether there was significant difference in heterogeneity between groups (i.e., Q_B) (Treseder, 2008; Bai et al., 2013). To elucidate publication bias, we plotted the number of studies against RR of EEA by removing one publication from a dataset each time and calculating the average RR and 95% CI (Deng et al., 2015). If the 95% CI without a specific publication was significantly different from the entire dataset's 95% CI, the observations in that publication were removed, and the rest of the dataset was reanalyzed. The meta-analysis was conducted by MetaWin 2.1 (SinauerAsSOMiates Inc., Sunderland, MA, USA) using random-effect models. A bootstrapping procedure was selected to meet the normal distribution requirement as most of these variables violate the normality assumption (Supplementary Table S1).

In addition, we conducted regression analyses and plotted RR versus the number of observations by randomly selecting a certain number of observations (starting from 20 and adding 5 each time until all observations were incorporated) and calculating the RR (Philibert et al., 2012; Loladze, 2014; Deng et al., 2015). Pearson-moment correlation coefficients were obtained between different RR of enzyme activities, SOC, MBC, and TN by R (R Core Team, 2015).

3. Results

3.1 N fertilization effects on EEA and soil C and N pools

N fertilization significantly increased CBH, AP, BX, BG, AG and UREA activities by 6.4%, 10.6%, 11.0%, 11.2%, 12.0%, and 18.6% ($P < 0.05$), but

significantly decreased the activities of PEO and PHO by 6.1% and 11.1%, respectively (Fig. 1). As for the enzyme proxies, N fertilization significantly increased *C-acq* by 9.1%, but decreased *OX* by 7.9%. AG, NAG, LAP, and *N-acq* were not significantly altered by N fertilization. The ratio of C:N acquisition enzyme activity did not change significantly in response to N fertilization. Based on studies reporting SOC, TN, and MBC with EEA simultaneously, N fertilization significantly increased SOC and TN contents by 7.6% and 15.3%, respectively, while significantly decreasing MBC content by 9.5% (Fig. 1). The publication bias and independence tests of our dataset satisfied the requirements for our meta-analysis (Supplementary Figures S1-S2).

3.2 Correlations between response ratios of EEA, soil C, and N pools

Significant positive correlations were found among any two of AG, BG, BX, and CBH, or between hydrolytic enzymes and NAG or AP (Table 2). Significant positive correlations were also observed between PHO and NAG, and between PEO and BG (Table 2). Significant correlations were also present between any two of the combined enzyme proxies (*C-acq*, *N-acq*, and *OX*). Significant positive correlations were found for the *C:N-acq* with *C-acq*, BG, and CBH, and significant negative correlations were present for the *C:N-acq* with *N-acq*, NAG, and LAP. Among EEA, SOC, TN, and MBC, significant correlations were found between MBC and *C-acq*, and between MBC and BG (Table 2).

To examine whether a linear or nonlinear relationship exists, further regression analysis revealed a linear relationship between the RR of soil

carbon-acquiring enzymes (*C-acq*) and RR of MBC (y=0.29*x-0.12 R^2=0.20, *P*<0.05). We further explore this relationship under different conditions (Fig. 2). Significant differences between the relationships (i.e., slopes in Fig. 2) were found only between forest and farmland (*P*<0.05).

3.3 N fertilization effects on *C-acq, N-acq, OX,* and *C:N_acq*

The most pronounced results we discovered are those regarding N fertilization on EEA and C and N pool sizes. The change of *C-acq* in response to N fertilization was significantly negative for Histosols and Aridsols, which contrasted with the significantly positive changes for other soil types (Fig. 3). There are either insignificant or significantly positive changes in all other edaphic, climatic, and physiological conditions with N fertilization (Fig. 3).

Changes of *N-acq* in response to N fertilization were significantly negative for Histosols, Gelisols, and Andisols, but significantly positive for Alfisol and Aridsols (Fig. 4). Changes with N fertilization were significantly negative when the N load was higher than 150 kg/ha/yr, when inorganic and organic N fertilizers were simultaneously applied, or when MAT is between 10^o C and 20^o C (Fig. 4). The amount of change in response to N fertilization was significantly positive for loamy soils. Changes of *OX* were either significantly negative or insignificant for any specific conditions (Fig. 5).

Changes of *C:N_acq* in response to N fertilization were significantly negative for Aridsoils, for grassland or farmland, and for NH_4^+ or urea-treated fertilization experiments. Changes of *C:N_acq* with N fertilization were significantly

positive for Gelisols, for organic N fertilizer input, for experiments longer than 10 years, for forest ecosystems, for sites with MAP less than 250 mm, and for N fertilizer input less than 50 or more than 150 kg/ha/yr (Fig. 6).

3.4 N fertilization effects on soil C and N pools under different conditions

Changes of SOC in response to N fertilization were significantly negative only for Oxisols. There were either insignificant or significantly positive changes in all other edaphic, climatic, and physiological conditions (Supplementary Fig. S3). Changes of MBC associated with N fertilization were either significantly negative or insignificant for specific conditions (Supplementary Fig. S4). Changes of TN were either significantly positive or insignificant under all conditions (Supplementary Fig. S5).

4. Discussion

4.1 N fertilization stimulated hydrolytic EEA but depressed oxidative EEA

The growing understanding of the role of extracellular enzymes in soil C dynamics and its feedback to climate change has drawn the attention of scientists to enhance the representation of mechanisms in models and therefore improve their predictive performance (Tang and Riley, 2015). Our study represents a comprehensive synthesis and strives to reveal the effect of N fertilization on soil EEA, as well as possible linkages between EEA and soil C and N dynamics.

In our first hypothesis, we speculated that N fertilization would decrease EEA associated with microbial N and oxidative C acquisitions, and would increase EEA

associated with hydrolytic C and P acquisitions. Results from this meta-analysis partially support the first hypothesis. All four EEAs associated with hydrolytic C acquisition increased significantly (Fig. 1). Meanwhile, AP increased significantly by 10.6%, which is lower than the 46% increase revealed in a former meta-analysis (Marklein and Houlton, 2012), possibly due to increasing the number of observations from 80 to 163. As a result of N fertilization, sufficient N supply appears to sustain soil microbes to produce more extracellular enzymes associated with hydrolytic C-acquisition, resulting in overall lower energy acquisition costs. This assertion is supported by the positive *C-acq* under N fertilization after the original *C-acq* was normalized by microbial biomass (Supplementary Fig. S6). These stimulated EEA responses suggest that soil microbial communities are likely constrained by C or P under N fertilization.

We did not observe significant changes in LAP, NAG, or their sum; thus, part of our first hypothesis was not supported. Zeglin et al. (2007) found responses of LAP and NAG to N fertilization were related with LAP to NAG ratio in grassland ecosystems. That is, when the LAP to NAG ratio was high, N fertilization reduced LAP activity and increased NAG; where the ratio was low, N fertilization increased LAP and depressed NAG. Thus, a proxy involving the sum of LAP and NAG could be insensitive to fertilization, suggesting that a more sophisticated proxy might be warranted. Sinsabaugh and Follstad Shah (2012) showed that at ecosystem scale, microbial N-acquiring EEAs did not have a simple relationship with N availability. These insignificant responses suggest that LAP and NAG attack different classes of

N- and C-containing substrates, the former on leucine and amino acids, and the latter on N-acetyl glucosamine and peptidoglycan-derived oligomers (Sinsabaugh et al., 2008).

Another interesting finding is that the ratio of C acquisition to N acquisition enzyme activities (C:N_acq) was not significantly affected by N fertilization, which is consistent with the well constrained stoichiometry of EEA across large-scale studies (Sinsabaugh et al., 2008). This finding reflects the intrinsic needs of microbial acquisition of C and nutrients even if the environment is below N saturation. A possible explanation is the linkages between nutrient availability to soil decay on the basis of microbial allocation of resources to extracellular enzyme production (Sinsabaugh et al., 1993; Sinsabaugh and Findlay, 1995).

However, PHO, PEO, and combined *OX* all significantly decreased under N fertilization (Fig. 1). Other studies have also observed inhibited PHO and PEO activities, especially in ecosystems with high lignin litter content (Waldrop et al., 2004b; Sinsabaugh et al., 2009; Hobbie et al., 2012). As Liu and Greaver (2010) reveal, N fertilization generally increases the aboveground litter production by 20%. Therefore, the increased lignin litter input to soils could provide one possible explanation for the depressed oxidase activity. There are other possibilities as well. For example, N fertilization can suppress fungi that produce these oxidative enzymes (Matocha et al., 2004; Allison et al., 2008). Another possibility is that N fertilization reduced the production of oxidase enzymes for lignin decomposition. Plentiful and readily bioavailable N would be more favorable for efficient production of hydrolytic

enzymes (Taylor et al., 1989; Hobbie et al., 2012; Talbot and Treseder, 2012). It is also possible that the high cost of oxidative enzyme production prohibited lignin decomposition. Hobbie (2008) pointed out that lignin-degrading enzymes are not always inhibited with N fertilization, and decreased activity could be a result of other processes such as oxygen availability, which can constrain phenol oxidase activity (Freeman et al., 2001).

4.2 N fertilization enhanced SOC but depressed MBC pool sizes

In our second hypothesis, we speculated that N fertilization will increase SOC and TN but decrease MBC. Consistent with our second hypothesis, SOC content significantly increased by 7.7% due to N fertilization; this is a little larger than the 3.2% increase reported by Lu et al. (2011b). In fact, increasing evidence supports the theory that N fertilization enhances SOC sequestration in terrestrial ecosystems (DeForest et al., 2004; Hyvonen et al., 2008; Pregitzer et al., 2008). One mechanism for explaining the phenomenon is that the lignin-rich and aromatic compounds may become preserved from decomposition due to depressed oxidative activities under N fertilization (Waldrop and Firestone, 2004). Our results indeed showed 11.8% and 6.4% decreases in phenol oxidase (PHO) and peroxidase (PEO) activities due to N fertilization, respectively. Waldrop and Firestone (2004) also explored relationships between response ratios of the SOC pool and phenol oxidase (PHO) and peroxidase (PEO); however, no significant correlations were detected which is confirmed by the present study. In another meta-analysis, lignin decomposition with high N: lignin ratio was inhibited by 18% under N fertilization, corroborating the strong influence of N

fertilization on SOC accumulation (Knorr et al., 2005). While the linkage between lignin-like substrate decomposition and oxidative enzyme activities is still missing, it is difficult to establish a relationship between oxidative EEA and SOC pools. For example, lignin-like substrates are thought to be associated with slower-cycling SOC pools, which could therefore be associated with reduced oxidative enzyme activities under N fertilization. However, data from multiple sites, and real analyses of SOC composition as a function of age, would be needed to establish the existence of so-called "slower-cycling" pools of SOC with oxidative enzymes under N fertilization.

In support of our second hypothesis, N fertilization significantly decreased MBC by 9.0%. In Treseder (2008), MBC declined by 15% based on 29 studies. The widely observed decrease in MBC under N fertilization has been attributed to microbial composition changes. Ramirez et al. (2012) found the relative abundance of *Acidobacteria* and *Verrucomicrobia* was decreased by 13.5% and 5%, respectively under N fertilization. Because *Acidobacteria* is abundant in the soil (Janssen, 2006; Jones et al., 2009), changes in its size and relative abundance may play a role in the overall microbial biomass change under N fertilization. *Verrucomicrobia* are important methylotrophs and live upon single carbon compounds derived from the demethylation of lignin and other secondary compounds (Dunfield et al., 2007; Islam et al., 2008; Chistoserdova et al., 2009), and N fertilization induced depression on *Verrucomicrobia* thus may contribute to the preservation of the lignin-like C pool in soils. Due to more diverse and abundant groups of microbes harbored in soils, the

microbial compositional changes require further scrutiny in order to predict microbial biomass change under N fertilization. Nevertheless, it remains unclear exactly why microbial biomass and soil respiration decreased under N fertilization (Treseder, 2008; Ramirez et al., 2010), while microbially-mediated activities in soil were concomitantly enhanced. In particular, the roles of the key bacterial and fungal groups still remain elusive in terrestrial ecosystems. Thus it is imperative to further study microbial functional group responses to N fertilization to elucidate their contributions to observed changes in microbial biomass.

4.3 Correlation between response ratios of *C-acq* and MBC

If a time-integrated variable can be linked with SOC pool size, it will make soil modeling and long-term prediction much easier. Some current soil microbial models include EEA as an independent C pool and catalyst for SOC decompositions (Allison et al., 2010; Wang et al., 2013; Li et al., 2014). A recent inventory of a large EEA dataset found significant linear correlations between log transformed BG, AP, LAP, or NAG with log transformed organic carbon pool size across terrestrial and aquatic ecosystems (Sinsabaugh et al., 2014). This type of linear correlation between EEA and SOC may be needed to substantially simplify the current soil models. However, the correlation conveyed little information about how EEA responses vary under different environmental conditions or management regimes, including N fertilization. Our synthetic results showed that a single (i.e., BG) or a combined EEA (i.e., *C-acq*) correlated significantly with MBC and not with SOC. This supported the

modeling efforts in that hydrolytic enzyme C is linearly proportional to the microbial biomass C pool (Schimel and Weintraub, 2003; Allison et al., 2010; Wang et al., 2013; Li et al., 2014).

4.4 N fertilization-induced changes vary with different soil conditions

The significantly negative responses of *C-acq* to N fertilization were found for Histosols and Aridsols only (Fig. 3), a finding that is consistent with a relatively low N demand in the low temperature ecosystems (i.e., permafrost, peatland, and bog) and in the moisture constrained ecosystems (i.e., desert, and dryland) (Schlesinger et al., 1990; Davidson and Janssens, 2006). For Aridsols in particular, the significantly negative response of *C:N_acq* under N fertilization (Fig. 6) could reflect the strong moisture constraint on nutrient diffusion to reactive sites given a large quantity of bioavailable N forms in soil media (Davidson et al., 2006).

It is worthwhile to note that *N-acq* showed significantly negative changes when the N load was high (>150 kg ha^{-1} yr^{-1}) or when both inorganic and organic N fertilizers were simultaneously applied. This demonstrates that a large quantity of available N in soil could have substantially relieved N limitations for microbes and caused more conservative production of N-associated enzymes (Sinsabaugh et al., 2008). Another interesting finding is that *N-acq* activity was significantly depressed in Histosols, Gelisols, and Andisols, but significantly enhanced in Alfisols and Aridsols (Fig. 4). This demonstrates a modulating effect of edaphic properties in regulating N retention and supply. In particular, Alfisols and Aridsoils represent low fertility soils

where the tight N demand may stimulate N acquisition in their respective ecosystems (Sinsabaugh et al., 2008).

The changes of *OX* were significantly negative for deeper soils, high precipitation regimes, and inorganic N forms (NH_4^+, NO_3^-, and both) (Fig. 5). In deeper soils, the observed response may reflect the relatively greater fraction of slow turnover substrates and the relatively lesser influence of other factors (i.e., climate) compared to the surface horizon. In the regions with high precipitation, oxygen availability at the scale of micro-sites is more likely to constrain oxidase activities due to diffusive limitations (Freeman et al., 2001). Given the readily bioavailable N form, inorganic N fertilizer input will lower soil pH substantially due to nutrient uptake and proton release to the soil solution (Richter et al., 1994). On the other hand, the large quantity of available N input to soil media can potentially moderate the microbial demand and prevent microbes from investing more energy and resources to produce oxidase (Sinsabaugh et al., 2008).

The positive relationships between response ratios of *C-acq* and MBC varied under different ecosystems, experimental duration, MAT, and N forms (Fig. 2). In particular, acknowledging the difference between forest and farmland soils could improve parameterization of the enzyme C pool when models are applied to different ecosystems. This difference could be attributed to varied microbial community compositions and structures. For example, past studies demonstrated that fungi were relatively more abundant in the forest soil, and Gram-positive bacteria were more abundant in cropland soil; thus the fungal: bacterial ratio was higher in the forest soil

than in most of the agricultural soils (Jangid et al., 2008; Upchurch et al., 2008). On the other hand, this difference could be attributed to the sensitivities of distinct microbial functional groups in response to N fertilization. N fertilizer amendments had a larger effect on bacterial communities, specifically including *Acidobacteria*, *Bacterioidetes,* and *Proteobacteria*, in cropland than in forests and pastures (Jangid et al., 2008). Because bacterial communities are the primary group of decomposers of cellulose via production of hydrolases (Bayer et al., 2006), a substantial inhibitive effect of N fertilization on bacterial groups could have reduced production of *C-acq* enzymes in agricultural soils compared to forest soils.

5. Conclusion

Extracellular enzyme activities have not been explicitly represented in global biogeochemical models because the relationship between EEA and soil C and N dynamics is unclear. This study summarized hydrolase and oxidase activities and SOC, TN, and MBC pool sizes under N fertilization based on 65 published studies. In general, N fertilization stimulated hydrolases associated with C and P acquisition, depressed oxidase activities, and had no significant effect on hydrolases for the acquisition of N. In particular, a significantly positive relationship was found between RRs of the combined hydrolases associated with C acquisition enzymes (i.e., *C-acq*) and MBC, suggesting changes in combined hydrolases activities might act as a proxy for MBC under N fertilization. That the linear relationship differed significantly between forest and farmland soils suggests that the huge variations among different

ecosystem and soil types will require further data synthesis under a wide range of environmental and climatic conditions. In addition, a limited sample size for soil or ecosystem types was revealed by this synthesis and others, challenging experimental and modeling communities to make this research a priority. Overall, this study provides the first comprehensive evidence of how hydrolase and oxidase activities respond to N fertilization and how they correlate to soil C and N pools over various ecosystems. Future studies could incorporate the relationship between hydrolase and microbial biomass for different ecosystems under global N enrichment scenarios in large-scale ecosystem models.

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Table 1

Table 1. A detailed description of soil extracellular enzymes in this study.

Extracellular enzyme	EC	Enzyme function	Abbreviation
α-1,4-Glucosidase	3.2.1.20	Hydrolysis of soluble saccharides	AG
β-1,4-Glucosidase	3.2.1.21	Hydrolysis of cellulose	BG
β-D-Cellobiosidase	3.2.1.91	Hydrolysis of cellulose	CBH
β-1,4-Xylosidase	3.2.1.37	Hydrolysis of hemicellulose	BX
Acid phosphatase	3.1.3.2	Cleaving of PO_4 from P-containing OM	AP
β-1,4-N-Acetyl-glucosaminidase	3.1.6.1	Hydrolysis of chitooligosaccharides	NAG
Leucine amino peptidase	3.4.11.1	Cleaving of peptide bonds in proteins	LAP
Phenol oxidase	1.10.3.2	Oxidation of lignin	PHO
Peroxidase	1.11.1.7	Oxidation of lignin	PEO
Urease	3.5.1.5	Hydrolysis of urea	UREA

EC denotes enzyme's commission number.

Table 2

Table 2. Pearson-moment correlation coefficients between response ratios (RR) of extracellular enzyme activities (EEA), SOC, MBC, TN and *C-acq* to *N-acq* ratio. Numbers in bold font represent significant correlation coefficients at P<0.05. The number of pairs is listed in bracket.

	AG	BG	BX	CBH	NAG	LAP	UREA	PHO	PEO	AP	*C-acq*	*N-acq*	*OX*	SOC	MBC	TN	C:N-acq
AG		**0.75** (24)	**0.76** (24)	**0.73** (24)	0.43 (18)	**0.64** (14)	NA	-0.42 （11)	-0.18 (12)	**0.59** (18)	**0.8** (24)	0.4 (19)	-0.33 (12)	0.15 (9)	0.5 (7)	0.15 (11)	0.15 (19)
BG			**0.81** (38)	**0.79** (62)	**0.47** (101)	**0.49** (28)	0.17 (5)	0.18 (106)	**0.28** (77)	**0.43** (107)	**1** (168)	**0.45** (108)	**0.31** (113)	0.19 (58)	**0.56** (44)	0.26 (33)	**0.51** (107)
BX				**0.8** (32)	**0.45** (24)	0.4 (19)	NA	0.12 (17)	0.35 (18)	**0.7** (28)	**0.87** (38)	0.34 (25)	0.3 (18)	0.07 (14)	0.66 (7)	0.27 (16)	0.28 (25)
CBH					**0.62** (63)	0.37 (28)	NA	0.1 (48)	0.29 (45)	**0.31** (57)	**0.9** (77)	**0.52** (70)	0.21 (51)	0.08 (29)	0.19 (21)	0.36 (22)	**0.3** (70)
NAG						**0.47** (27)	NA	**0.23** (88)	0.1 (66)	**0.46** (80)	**0.48** (115)	**0.96** (122)	**0.28** (89)	0.26 (27)	-0.29 (26)	0.16 (22)	**-0.47** (115)
LAP							NA	-0.21 (27)	0.1 (27)	**0.5** (29)	**0.49** (31)	**0.79** (34)	-0.04 (30)	0.44 (8)	-0.87 (3)	-0.01 (13)	**-0.39** (31)
UREA								NA	NA	0.34 (23)	**0.53** (15)	NA	0.84 (3)	0.51 (8)	**0.13** (4)	0.33 (8)	NA
PHO									0.17 (85)	0.22 (69)	0.18 (108)	0.12 (93)	**0.59** (129)	0.15 (33)	0.24 (21)	-0.03 (22)	0 (89)
PEO										0.11 (56)	**0.27** (79)	0.04 (73)	**0.8** (92)	0.4 (23)	0.42 (15)	-0.33 (21)	0 (69)
AP											**0.37** (129)	0.45 (87)	**0.33** (72)	0.18 (47)	-0.47 (18)	-0.05 (33)	-0.08 (81)
C-acq												**0.32** (144)	**0.3** (115)	0.1 (84)	**0.45** (45)	**0.28** (54)	**0.6** (144)
N-acq													0.21 (96)	0.25 (48)	-0.1 (27)	-0.02 (43)	**-0.56** (144)
OX														0.32 (33)	0.24 (25)	-0.22 (23)	-0.02 (92)
SOC															0.29 (27)	0.19 (56)	-0.14 (47)
MBC																0.08 (17)	0.2 (27)
TN																	0.28 (40)
C:N-acq																	

Figure legend

Figure 1. Mean and 95% CI of weighted response ratio of N fertilization effects on (a) individual, (b) combined soil extracellular enzymes activity, and (c) soil C and N pools. The abbreviations were presented in Table 1. The sample size of each variable was displayed beside each bar.

Figure 2. Relationship of response ratio of MBC (RR-MBC) and response ratio of *C-acq* (*RR-C-acq*) under three ecosystems including forest, grassland and farmland.

Figure 3. Nitrogen fertilization effects on soil C-acquisition enzyme activity (*C-acq*) under different edaphic, climatic and experimental conditions. MAT: mean annual temperature; MAP: mean annual precipitation. The sample size of each variable was displayed beside each bar.

Figure 4. Nitrogen fertilization effects on soil N-acquisition enzyme activity (*N-acq*) under different edaphic, climatic and experimental conditions. MAT: mean annual temperature; MAP: mean annual precipitation. The sample size of each variable was displayed beside each bar.

Figure 5. Nitrogen fertilization effects on soil oxidative enzyme activity (*OX*) under different edaphic, climatic and experimental conditions. The sample size was displayed beside each bar. MAT: mean annual temperature; MAP: mean annual precipitation. The sample size of each variable was displayed beside each bar.

Figure 6. Nitrogen fertilization effects on the ratio of EEA associated with C-acquisition over that with N-acquisition (*C:N-acq*) under different edaphic, climatic and experimental conditions. The sample size was displayed beside each bar. MAT: mean annual temperature; MAP: mean annual precipitation. The sample size of each variable was displayed beside each bar.

Figure 1

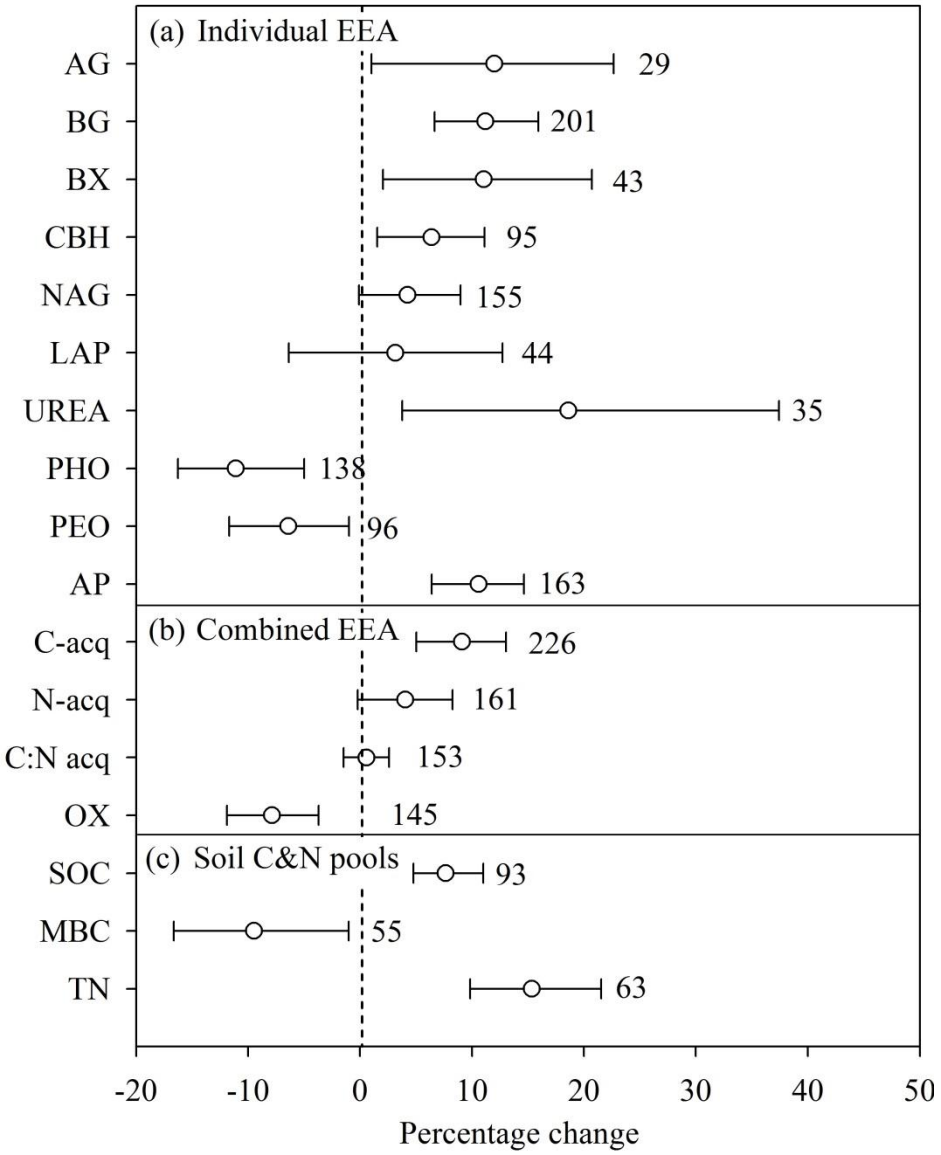

(a) Individual EEA
AG
BG
BX
CBH
NAG
LAP
UREA
PHO
PEO
AP
29
201
43
95
155
44
35
138
96
163
(b) Combined EEA
C-acq
N-acq
C:N acq
OX
226
161
153
145
(c) Soil C&N pools
SOC
MBC
TN
93
55
63
-20
-10
0
10
20
30
40
50
Percentage change

Figure 2

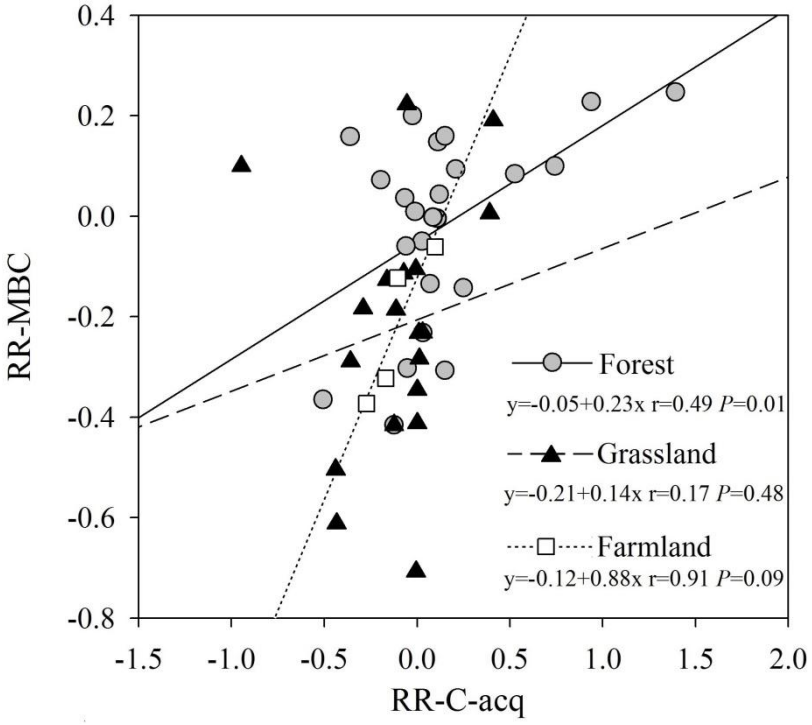

0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
RR-MBC
-1.5
-1.0
-0.5
0.0
0.5
1.0
1.5
2.0
RR-C-acq
Forest
y=-0.05+0.23x r=0.49 P=0.01
Grassland
y=-0.21+0.14x r=0.17 P=0.48
Farmland
y=-0.12+0.88x r=0.91 P=0.09

Figure 3

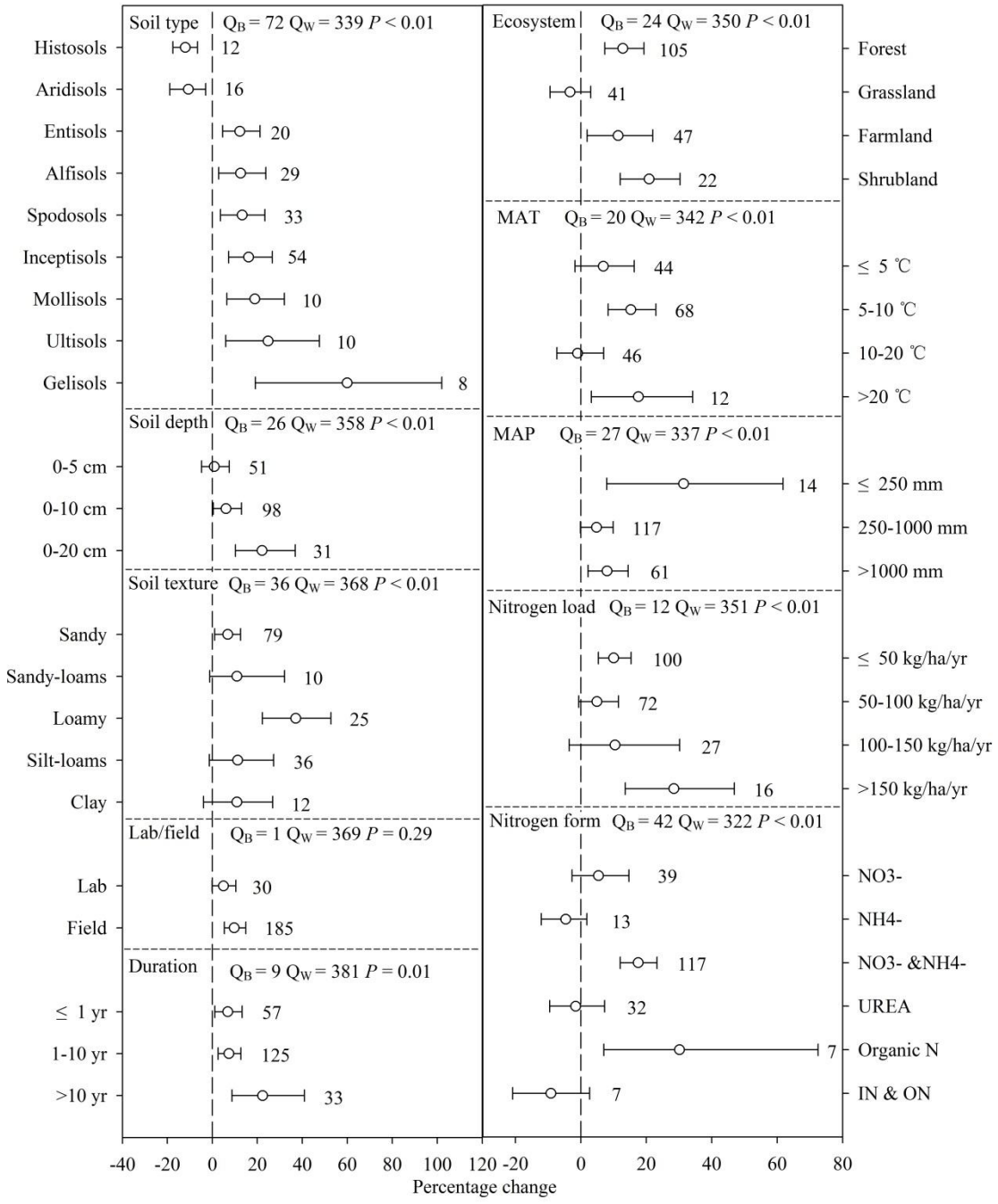

Soil type $Q_B = 72$ $Q_W = 339$ $P < 0.01$
Histosols 12
Aridisols 16
Entisols 20
Alfisols 29
Spodosols 33
Inceptisols 54
Mollisols 10
Ultisols 10
Gelisols 8
Soil depth $Q_B = 26$ $Q_W = 358$ $P < 0.01$
0-5 cm 51
0-10 cm 98
0-20 cm 31
Soil texture $Q_B = 36$ $Q_W = 368$ $P < 0.01$
Sandy 79
Sandy-loams 10
Loamy 25
Silt-loams 36
Clay 12
Lab/field $Q_B = 1$ $Q_W = 369$ $P = 0.29$
Lab 30
Field 185
Duration $Q_B = 9$ $Q_W = 381$ $P = 0.01$
≤ 1 yr 57
1-10 yr 125
>10 yr 33
Ecosystem $Q_B = 24$ $Q_W = 350$ $P < 0.01$
Forest 105
Grassland 41
Farmland 47
Shrubland 22
MAT $Q_B = 20$ $Q_W = 342$ $P < 0.01$
≤ 5 ℃ 44
5-10 ℃ 68
10-20 ℃ 46
>20 ℃ 12
MAP $Q_B = 27$ $Q_W = 337$ $P < 0.01$
≤ 250 mm 14
250-1000 mm 117
>1000 mm 61
Nitrogen load $Q_B = 12$ $Q_W = 351$ $P < 0.01$
≤ 50 kg/ha/yr 100
50-100 kg/ha/yr 72
100-150 kg/ha/yr 27
>150 kg/ha/yr 16
Nitrogen form $Q_B = 42$ $Q_W = 322$ $P < 0.01$
NO3- 39
NH4- 13
NO3- &NH4- 117
UREA 32
Organic N 7
IN & ON 7
-40 -20 0 20 40 60 80 100 120
-20 0 20 40 60 80
Percentage change

Figure 4

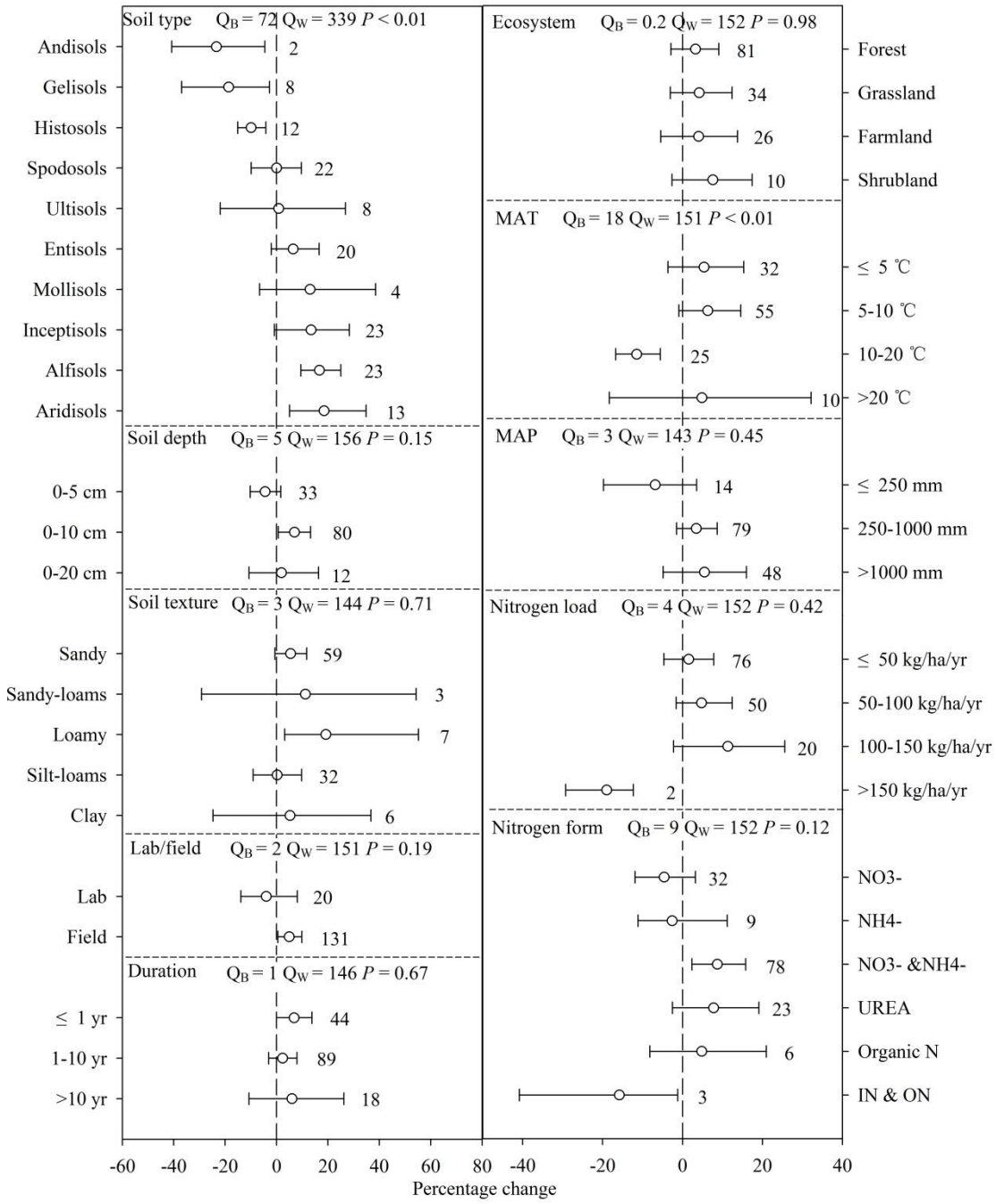

Soil type $Q_B = 72$ $Q_W = 339$ $P < 0.01$
Andisols 2
Gelisols 8
Histosols 12
Spodosols 22
Ultisols 8
Entisols 20
Mollisols 4
Inceptisols 23
Alfisols 23
Aridisols 13
Soil depth $Q_B = 5$ $Q_W = 156$ $P = 0.15$
0-5 cm 33
0-10 cm 80
0-20 cm 12
Soil texture $Q_B = 3$ $Q_W = 144$ $P = 0.71$
Sandy 59
Sandy-loams 3
Loamy 7
Silt-loams 32
Clay 6
Lab/field $Q_B = 2$ $Q_W = 151$ $P = 0.19$
Lab 20
Field 131
Duration $Q_B = 1$ $Q_W = 146$ $P = 0.67$
≤ 1 yr 44
1-10 yr 89
>10 yr 18
Ecosystem $Q_B = 0.2$ $Q_W = 152$ $P = 0.98$
81 Forest
34 Grassland
26 Farmland
10 Shrubland
MAT $Q_B = 18$ $Q_W = 151$ $P < 0.01$
32 ≤ 5 ℃
55 5-10 ℃
25 10-20 ℃
10 >20 ℃
MAP $Q_B = 3$ $Q_W = 143$ $P = 0.45$
14 ≤ 250 mm
79 250-1000 mm
48 >1000 mm
Nitrogen load $Q_B = 4$ $Q_W = 152$ $P = 0.42$
76 ≤ 50 kg/ha/yr
50 50-100 kg/ha/yr
20 100-150 kg/ha/yr
2 >150 kg/ha/yr
Nitrogen form $Q_B = 9$ $Q_W = 152$ $P = 0.12$
32 NO3-
9 NH4-
78 NO3- &NH4-
23 UREA
6 Organic N
3 IN & ON
-60 -40 -20 0 20 40 60 80
-40 -20 0 20 40
Percentage change

Figure 5

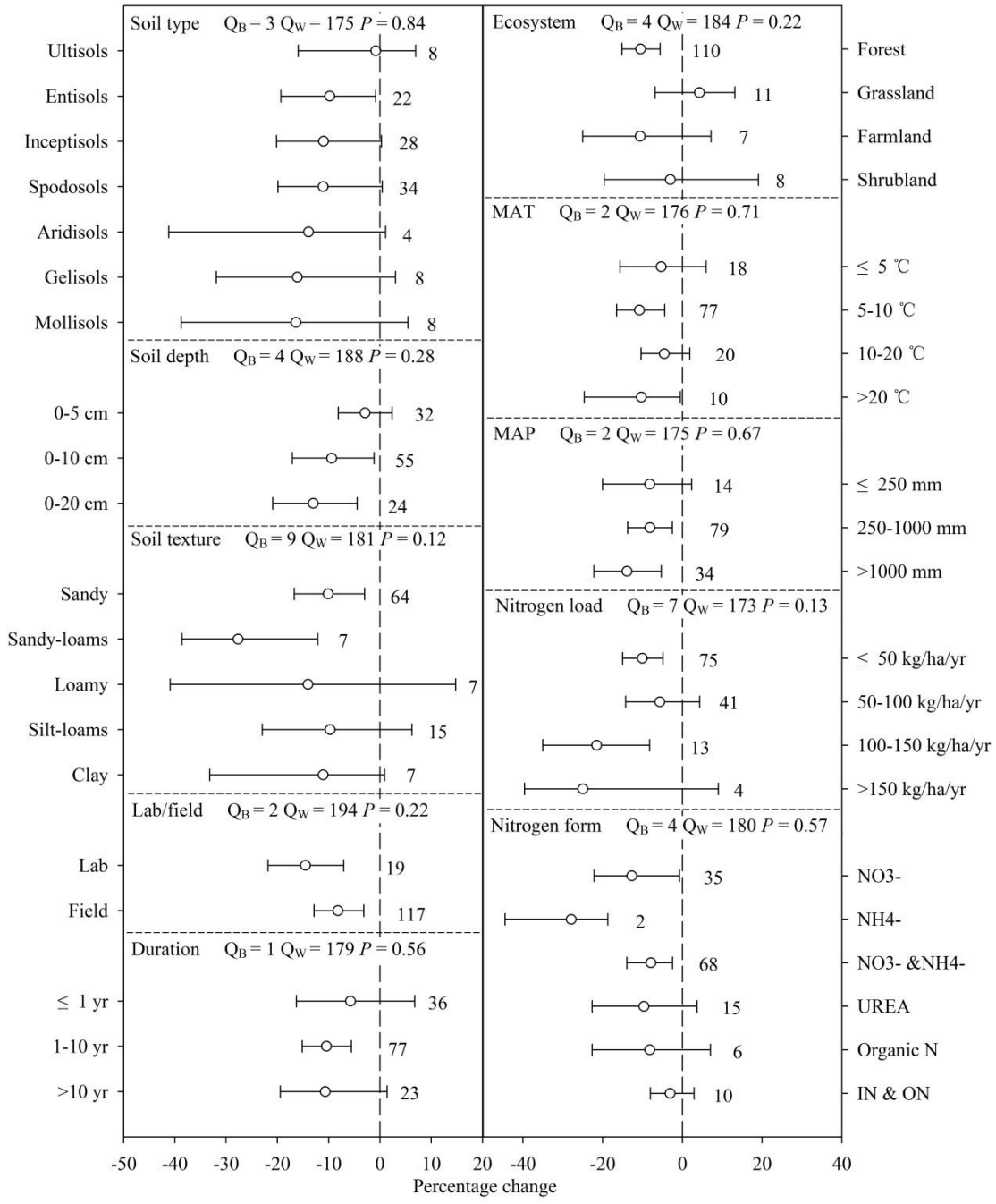
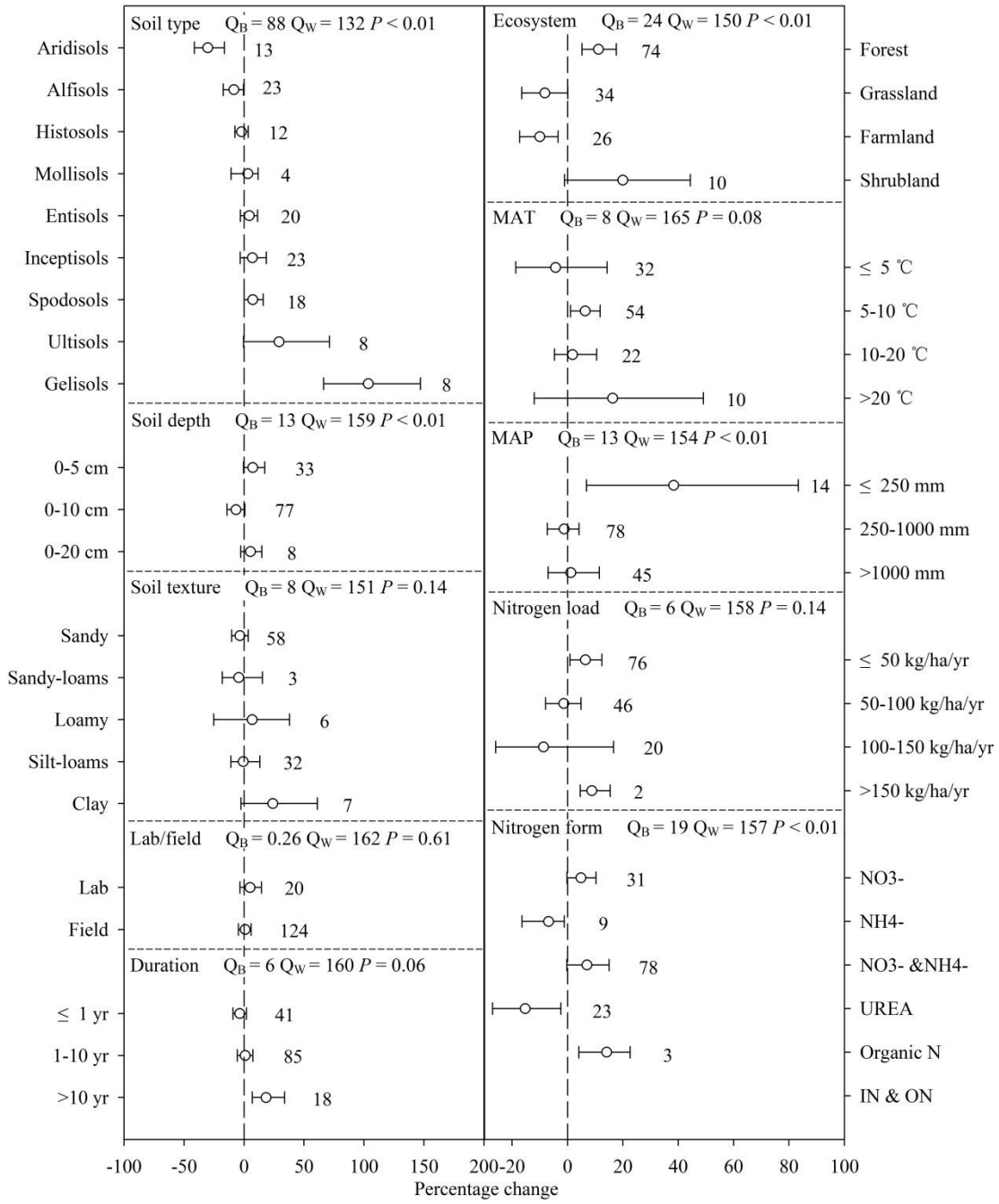

Soil type $Q_B = 3$ $Q_W = 175$ $P = 0.84$
Ultisols 8
Entisols 22
Inceptisols 28
Spodosols 34
Aridisols 4
Gelisols 8
Mollisols 8
Soil depth $Q_B = 4$ $Q_W = 188$ $P = 0.28$
0-5 cm 32
0-10 cm 55
0-20 cm 24
Soil texture $Q_B = 9$ $Q_W = 181$ $P = 0.12$
Sandy 64
Sandy-loams 7
Loamy 7
Silt-loams 15
Clay 7
Lab/field $Q_B = 2$ $Q_W = 194$ $P = 0.22$
Lab 19
Field 117
Duration $Q_B = 1$ $Q_W = 179$ $P = 0.56$
≤ 1 yr 36
1-10 yr 77
>10 yr 23
Ecosystem $Q_B = 4$ $Q_W = 184$ $P = 0.22$
110 Forest
11 Grassland
7 Farmland
8 Shrubland
MAT $Q_B = 2$ $Q_W = 176$ $P = 0.71$
18 ≤ 5 ℃
77 5-10 ℃
20 10-20 ℃
10 >20 ℃
MAP $Q_B = 2$ $Q_W = 175$ $P = 0.67$
14 ≤ 250 mm
79 250-1000 mm
34 >1000 mm
Nitrogen load $Q_B = 7$ $Q_W = 173$ $P = 0.13$
75 ≤ 50 kg/ha/yr
41 50-100 kg/ha/yr
13 100-150 kg/ha/yr
4 >150 kg/ha/yr
Nitrogen form $Q_B = 4$ $Q_W = 180$ $P = 0.57$
35 NO3-
2 NH4-
68 NO3- &NH4-
15 UREA
6 Organic N
10 IN & ON
-50 -40 -30 -20 -10 0 10 20 -40 -20 0 20 40
Percentage change

Figure 6


Soil type $Q_B = 88$ $Q_W = 132$ $P < 0.01$
Aridisols 13
Alfisols 23
Histosols 12
Mollisols 4
Entisols 20
Inceptisols 23
Spodosols 18
Ultisols 8
Gelisols 8
Soil depth $Q_B = 13$ $Q_W = 159$ $P < 0.01$
0-5 cm 33
0-10 cm 77
0-20 cm 8
Soil texture $Q_B = 8$ $Q_W = 151$ $P = 0.14$
Sandy 58
Sandy-loams 3
Loamy 6
Silt-loams 32
Clay 7
Lab/field $Q_B = 0.26$ $Q_W = 162$ $P = 0.61$
Lab 20
Field 124
Duration $Q_B = 6$ $Q_W = 160$ $P = 0.06$
≤ 1 yr 41
1-10 yr 85
>10 yr 18
Ecosystem $Q_B = 24$ $Q_W = 150$ $P < 0.01$
74 Forest
34 Grassland
26 Farmland
10 Shrubland
MAT $Q_B = 8$ $Q_W = 165$ $P = 0.08$
32 ≤ 5 ℃
54 5-10 ℃
22 10-20 ℃
10 >20 ℃
MAP $Q_B = 13$ $Q_W = 154$ $P < 0.01$
14 ≤ 250 mm
78 250-1000 mm
45 >1000 mm
Nitrogen load $Q_B = 6$ $Q_W = 158$ $P = 0.14$
76 ≤ 50 kg/ha/yr
46 50-100 kg/ha/yr
20 100-150 kg/ha/yr
2 >150 kg/ha/yr
Nitrogen form $Q_B = 19$ $Q_W = 157$ $P < 0.01$
31 NO3-
9 NH4-
78 NO3- &NH4-
23 UREA
3 Organic N
IN & ON
-100 -50 0 50 100 150 200-20 0 20 40 60 80 100
Percentage change