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, excreted by soil microbes, have not been represented in global biogeochemical models, it is thus imperative to elucidate the possible relationships between EEA and SOC, soil N (TN) or soil microbial biomass carbon (MBC) under N fertilization. Based on 59 published studies, we synthesized α-1,4-Gulcosidase (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). The *C-acq*, *N-acq* and *OX* were calculated as the sum of AG, BG, CBH and BX, NAG and LAP, and PHO and PEO, respectively. The relationships were explored between response ratios (RR) of EEA and SOC, TN or MBC when they were reported simultaneously. Results showed N fertilization significantly increased CBH,*C-acq*, BG, BX, AP and UREA activities by 5.9, 9.0, 11.0, 12.0, 12.0 and 19.0%, but decreased PEO, *OX* and PHO by 6.4, 9.2 and 12.0%, respectively. N fertilization enhanced SOC and TN by 7.7% and 16.0%, respectively, but inhibited MBC by 9.0%. Significant positive correlations were found only between the RR of *C-acq* and MBC suggesting changes in combined hydrolases activities might act as a proxy for MBC under N fertilization. In contrast with other variables, the RR of AP, MBC and TN showed monotonic trends under different edaphic, environmental and physiological conditions. Our results provide the first set of evidence on how hydrolase and oxidase activities respond to N fertilization over various ecosystems. To improve long-term model projections, this study highlights the need for further investigations specifically on how slow cycling soil C pools and oxidases respond to global change scenarios.

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 the global reactive nitrogen inputs, which is estimated to increase from 86 Tg N in 1995 to 135 Tg N in 2050 (Gallo et al. 2004). This enhanced N availability can alter decomposition and formation of soil organic matter (SOM) due to 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 meter 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). As extracellular enzymes are important agents of soil C decomposition (Sinsabaugh 1994, Sinsabaugh et al. 2008), it highlights the significance of studying N fertilization effects on extracellular enzyme activities (EEA). In spite of increasing number of field and laboratory studies on this topic, no syntheses have yet explored the N fertilization effect on EEA in fertilization to their relationships with soil C, N and nutrient changes.

A wide range of EEA have been measured associated with C and N turnover (Burns 1982, Dick 1994, Wallenstein and Burns 2011). In general, soil extracellular enzymes include hydrolases and oxidases which decompose different substrates with varying complexity (Sinsabaugh 2010, Sinsabaugh and Shah 2012). Briefly, cellulase is a group of hydrolytic enzymes that soil microbes produce to decompose polysaccharides and they include α-1,4-Gulcosidase (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 acquisitions include β-1,4-N-Acetyl-glucosaminidase (NAG), Leucine amino peptidase (LAP) and urease (UREA), which target chitin, protein and urea, respectively (Tabatabai and

67 Bremner 1972). The enzymes associated with P acquisition cleave PO_4^{3-} from P-containing
68 organic compounds and they include acidic phosphatase (AP) and alkaline phosphatase
69 (Tabatabai and Bremner 1972, Eivazi and Tabatabai 1977, Hui et al. 2013). Oxidative
70 enzymes productions incur high energy costs and they are produced by microbes to
71 specifically decompose slow cycling substrates (i.e. lignin). Phenol oxidase (PHO) and
72 peroxidase (PEO) are two most frequently assayed oxidases (Sinsabaugh 2010, Wang et al.
73 2012).

74 The responses of EEA under N fertilization have been studied for decades and
75 generally showed high variations in both direction and magnitude across studies (Fog 1988,
76 Sinsabaugh et al. 1993, Sinsabaugh and Findlay 1995, Ajwa et al. 1999, Carreiro et al. 2000,
77 Lucas et al. 2011, Ramirez et al. 2012).The effects of N fertilization on BG activities were
78 found to be positive in temperate forest and grassland soils (Saiya-Cork et al. 2002, Waldrop
79 et al. 2004a, Sinsabaugh et al. 2005), while the positive effect could be diminished with the
80 increasing soil age in grassland ecosystems, or remain constant in the oldest grassland soil
81 (Zeglin et al. 2007). However, BG activities decreased by 24% with N fertilization averaged
82 from 28 soils across North America (Ramirez et al. 2012). NAG activities can be stimulated
83 or suppressed with N fertilization among different sites. For example, activities of NAG with
84 N fertilization was 76% of unfertilized soils collected from Duke FACE experiment after an
85 18 hour incubation (Billings and Ziegler 2008), whereas NAG activity increased by 14% in a
86 sugar-maple dominated forest in Michigan (Saiya-Cork et al. 2002). Stimulation of AP
87 activities under N fertilization has been widely observed across studies (Ajwa et al. 1999,
88 Olander and Vitousek 2000, Henry et al. 2005, Stursova et al. 2006). Carreiro et al. (2000)
89 first reported ligninolytic enzyme activities were suppressed under N fertilization and
90 resulted in a declined decay rate of high-lignin oak litter. Responses of oxidative enzymes

activities were also found to vary with litter C:N ratios under N fertilization (Carreiro et al. 2000). Waldrop et al. (2004b) reported that under N fertilization, PHO increased in forest where litter contains lower C:N ratio by 16% to 36%, whereas decreased by 20% in forest had higher C:N ratio. Li et al. (2013) reported that PHO increased by 70~170% with adding litter of high N concentration under warming. Allison et al. (2008) however found no significant N fertilization effect on PHO at a boreal forest soil in Alaska.

N fertilization also affected microbial growth and activities which directly altered SOC turnover and subsequently lead to changes in C and N pool sizes. N fertilization caused reductions by 8%~11% in microbial respiration (Treseder 2008, Liu and Greaver 2010) and by 15%~35% in microbial biomass C (MBC) (Treseder 2008, Liu and Greaver 2010, Ramirez et al. 2010). This suppressing effect is a net effect of either positive or negative changes in relative abundance of different fungal and bacterial communities (Ramirez et al. 2012). For example, N fertilization increased the relative abundance of *Actinobacteria* and *Firmicutes* by 11.8% and 2.0%, respectively, and decreased the relative abundance of *Acidobacteria* and *Verrucomicrobia* by 13.5% and 5%, respectively (Ramirez et al. 2012). A number of studies revealed that N fertilization can enhance soil C storage. Hyvonen et al. (2008) showed that N fertilization increased SOC sequestration in the fertilized plots but not in control plots. Pregitzer et al. (2008) found long-term N fertilization significantly increased surface soil (0-10 cm) C content by 26% due to retarding the decomposition rate, and did not enhance the C input in a boreal forest. However, it was also reported that N fertilization had no effect or a negative effect on soil C concentration in tundra ecosystems (Neff et al. 2002, Mack et al. 2004). A recent meta-analysis showed that N fertilization had no effect on C storage in forests and grasslands, but significantly increased C storage in agricultural ecosystems by 3.5% (Lu et al. 2011b).

N fertilization generally increased N stock in soil and plants (Liu and Greaver 2010, Lu et al. 2011b, Lu et al. 2013, García-Palacios et al. 2015). Various experimental studies showed varying magnitudes of soil N pool response to N fertilization. For example, Koyama et al. (2013) found increase in total N content in the organic horizons in Arctic tundra soils, while Lagomarsino et al. (2009) found no effect of N fertilizer on total N in the bulk soil of a *Triticum durum* and *Zeamais* farmland in central Italy. Lu et al. (2011a) reported that N fertilization increased N content by about 6.0% in both organic and mineral soil horizons.

Given the increasing availability of soil EEA measurements in the last decade, it becomes feasible to use meta-analysis 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 59 independent studies to elucidate the impact of N fertilization on EEA associated with soil C, N and P acquisition, SOC, soil N (TN), and microbial biomass C (MBC) pool sizes. This study also explored the relationships between EEA and C and N pool sizes. We hypothesize that 1) N fertilization would significantly increase EEA associated with C and P acquisitions but depress EEA associated with N and lignin or oxidative C acquisitions. The 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); 2) N fertilization will increase SOC and TN but decrease MBC; and 3) positive correlations exist between SOC and *OX* or MBC and *C-acq*. We further explored these patterns across different edaphic, environmental and physiological conditions. This study is expected to summarize the important microbial extracellular enzyme changes and the subsequent impact on soil C and N dynamics given the increasing N inputs in terrestrial ecosystems.

2. Materials and methods

2.1 Data collection

The search engine of Web of Science was used to locate published journal articles. We used different combinations of terms to including “soil”, “extracellular enzyme” and either “nitrogen fertilization”, “nitrogen deposition” or “chronic nitrogen fertilization”. A total of 400 papers were found and 59 of them were identified to contain at least one of our targeted variables (i.e. EEA in Table 1). Data were extracted according to the following criteria: (1) if data were only reported in graphs and figures, the means and standard deviations (SD) were extracted using GetData Graph Digitizer 2.26 (http://www.getdata-graph-digitizer.com/index.php). If replicate numbers (n) and standard errors (SE) were reported, they were converted to standard deviations (SD) using SD = SE $\times\sqrt{n}$; (2) If one article reports multiple independent manipulative experiments, e.g. two experiments at two 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 change 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 contains results from multiple sampling date and soil depth, we used the measurement of latest sampling time and the upmost soil layer. The complete dataset and 59 publications were attached in Supplementary Material.

In total, data of ten different extracellular enzymes were collected (Table 1). We further integrated individual EEA into combined EEA targeting specific nutrient 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 acquired enzymes (*C-acq*) denoted the average enzyme activity of AG, BG, BX and CBH; N acquired enzyme (*N-acq*) denoted the average enzyme activity of NAG and LAP; and oxidative enzyme (*OX*) denoted 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*. Soil organic carbon content (SOC), soil nitrogen (TN) and microbial biomass carbon (MBC) were also collected based on studies reporting EEA simultaneously, which will allow us to explore relationship between EEA and these C or N pool sizes.

For each site, we also collected its 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 experimental type, duration of experiment, quantity and form of N fertilization. Soil type and soil texture records were extracted from the original publications, or they were determined based upon soil characteristics and literature searching based upon USDA soil taxonomy (Soil Survey Staff, 1999). Given the fact that N fertilization could be carried out in the field or during lab incubation, the experiment type was categorized into “field” and “lab”, respectively. We further divided continuous variables (i.e. MAT, MAP, experimental duration and N fertilization quantity) 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 fertilization quantity as fertilizers was grouped into “≤ 50 kgN/ha/yr”, “50-150

kgN/ha/yr" and "> 150 kgN/ha/yr", while N fertilizer form was grouped into "NO_3^-", "NH_4^+", "NH_4^+&NO_3^-", "urea", "organic N" and "organic N & 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 ($\bar{x}_c$):

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

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 represented standard deviation of treatment group and control groups, replicate numbers of treatment and control groups, respectively. In order to derive the overall response effect, we used the weighted (or average) response ratio RR_{++}, defined as (Hedges et al. 1999, Luo et al. 2006, Lu et al. 2013):

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

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 equation (3) and (4) was defined as weighting factor, the inverse of the pooled variance ($w_{ij} = 1/v$), *m* was the number of compared groups, and *k* was the number of comparisons in the corresponding groups.

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

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

When a 95% CI value of the response valuable did not overlap 0, we considered the effect of nitrogen fertilization on the variable was significantly different between control and treatment group. 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 overall effect of N fertilization on EEA and C and N dynamic. We also applied meta-analysis to explore 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 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 study against RR of EEA by removing one publication from dataset each time and calculating the average RR and 95% CI (Deng et al. 2015). If the 95% CI without a specific publication is significantly different from the entire dataset's 95% CI, the observations in that publication were removed and the rest dataset were reanalyzed.

In fertilization, we conducted regression analyses and plotted RR versus number of observations by randomly selecting a certain number of observations (starting from 20 and adding 5 each time until all observations) and calculating the RR (Philibert et al. 2012, Loladze 2014, Deng et al. 2015). Meta-analysis was conducted by MetaWin 2.1 (SinauerAsSOMiates Inc., Sunderland, MA, USA) using random-effect models. 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,BG, BX, AP and UREA activities by 5.9%, 11.2%, 11.7%, 12.0% and 18.6% ($P < 0.05$), but significantly decreased the activities of PEO and PHO by 6.4% and 11.8%, respectively (Figure. 1). As for the combined enzymes, N fertilization significantly increased *C-acq* by 9.0% but decreased *OX* by 9.2%. AG, NAG, LAP, and *N-acq* were not significantly altered by N fertilization. The ratio of C:N acquisition enzyme activity increased by 1.2% in response to N fertilization, though statistically insignificant. Based on studies reporting SOC, TN, MBC with EEA simultaneously, N fertilization significantly increased SOC and TN contents by 7.7% and 16%, respectively, while significantly decreased MBC content by 9.0% (Figure. 1). The publication bias and independence tests of our dataset satisfied the requirements for our meta-analysis (Supplementary Figs. S1-S2).

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

Significant positive correlations were found between any two of AG, BG, BX and CBH, and between each individual C acquired enzymes with NAG or AP (Table 2). Significant positive correlations were also observed between PHO and NAG, or between PEO and BG (Table 2). Significant correlations were also present between any two of the combined enzyme groups (*C-acq*, *N-acq* and *OX*). Significant positive correlations were found for the *C:N-acq* with *C-acq,* BG or CBH, but significant negative correlations were present for the *C:N-acq* with *N-acq,* NAG or LAP.

Among EEA, SOC, TN and MBC, significant correlations were found between MBC and *C-acq* or MBC and BG (Table 2). Further regression analysis revealed a linear

relationship between the response ratio (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 (Figure 2). Significant difference of the relationships (i.e. slopes in Figure. 2) was found only between forest and farmland (P<0.05).

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

The most pronounced results are presented regarding N fertilization on EEA and C and N pool sizes given specific conditions.

Percentage changes of *C-acq* were significantly negative for Histosols and Aridsols, which contrasted with the significantly positive percentage changes for other soil types (Figure 3). There are either insignificant or significantly positive percentage changes in all other edaphic, climatic and physiological conditions (Figure 3).

Percentage changes of *N-acq* were significantly negative for Histosols, Gelisols and Andisols, but significantly positive for Alfisol and Aridsols (Figure 4). Percentage changes were significantly negative when N load is higher than 150 kg/ha/yr or when inorganic and organic N fertilizers were simultaneously applied, or when MAT is between 10^o C and 20^o C (Figure 4). Percentage change is significantly positive for loamy soils. Percentage changes of *OX* were either significantly negative or insignificant for any specific conditions (Figure 5).

Percentage changes of *C:N_acq* were significantly negative for Aridsoils, for grassland or farmland, for NH_4^+ or urea treated fertilization experiment, but significantly positive for Gelisols, for organic N fertilizer input, for experiments longer than 10 years, for forest ecosystems, for sites with MAP less than 250mm or for N fertilizer input less than 50 or more than 150 kg/ha/yr (Figure 6).

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

Percentage changes of SOC were significantly negative only for Oxisols. There are either insignificant or significantly positive percentage changes in all other edaphic, climatic and physiological conditions (Figure 7). Percentage changes of MBC were either significantly negative or insignificant for specific conditions (Figure 8). Percentage changes of TN were either significantly positive or insignificant for specific conditions (Figure 9).

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 especially for improving mechanistic models and prediction (Tang and Riley 2015). Our study represented a comprehensive synthesis and strived to reveal the effect of N fertilization on soil EEA, and possible linkages between EEA and soil C and N dynamics.

In our first hypothesis, we speculate N fertilization decreased EEA associated with microbial N and oxidative C acquisitions, and increased EEA associated with hydrolytic C and P acquisitions. Results concluded from this meta-analysis partially supported the first hypothesis. Three out of four EEA associated with hydrolytic C acquisition increased significantly, including BG, BX and CBH (Figure 1). Meanwhile, AP increased significantly by 12%, although lower than 46% revealed in a former study (Marklein and Houlton 2012), possibly due to a larger number of observations in this study including all former ones (i.e. 155 vs. 80). As a result of N fertilization, sufficient N supply sustains soil microbes to produce fewer extracellular enzymes associated with hydrolytic C-acquisition to satisfy their metabolism demand and stoichiometry, resulting in overall lower energy acquisition costs. These stimulated EEA responses suggest that soil microbial communities are likely

303 constrained by C or P under N fertilization.

304 We did not observe significant changes in LAP, NAG or their sum, thus our first
305 hypothesis was not supported in this regard. Zeglin et al. (2007) found responses of LAP and
306 NAG to N fertilization were related with LAP to NAG ratio in grassland ecosystems. That is,
307 when the LAP to NAG ratio was high, N fertilization reduced LAP activity and increased
308 NAG; where the ratio was low, N fertilization increased LAP and depressed NAG. This could
309 lead to no significant change in the sum of LAP and NAG, which is supportive to our
310 synthesis result on *N-acq*. Sinsabaugh and Follstad Shah (2012) showed that at ecosystem
311 scale, microbial N-acquiring EEAs didn't have a simple relationship with N availability. As
312 LAP and NAG attack different classes of substrates, this was not unexpected.

313 It is worthy to note, *N-acq* showed significantly negative percentage changes when N
314 load was high (>150 kg ha^{-1} yr^{-1}) or both inorganic and organic N fertilizers were
315 simultaneously applied. This demonstrates a large quantity of soil available N could have
316 substantially relieved N limitations for microbes thus causing more conservative production
317 of N-associated enzymes (Sinsabaugh et al. 2008). Another interesting finding is that NAG
318 activity was significantly depressed in Histosols, Gelisols and Andisols, but significantly
319 enhanced in Alfisols and Aridsols. This demonstrates a modulating effect of edaphic
320 properties in regulating N retention and supply. In particular, Alfisols and Aridsoils represent
321 low fertility soil where the tight N demand may stimulate N acquisition in their respective
322 ecosystems (Sinsabaugh et al. 2008).

323 It should be noted that the ratio of C acquisition to N acquisition enzyme activities
324 (C:N_acq) was not significantly affected by N fertilization, which is consistent with the well
325 constrained stoichiometry of EEA across large scale studies (Sinsabaugh et al. 2008). It
326 reflected the intrinsic microbial acquisitions for C and nutrients even if the environment is

327 below the N saturation conditions.

328 However, PHO, PEO and combined *OX* all significantly decreased under N
329 fertilization (Figure 1). Other studies have also observed inhibited PHO and PEO activities,
330 especially in ecosystems with high lignin litter content (Waldrop et al. 2004b, Sinsabaugh et
331 al. 2009, Hobbie et al. 2012). The possible explanation is that added N drives microbial
332 community toward hydrolytic substrate decomposition resulted from increased aboveground
333 and belowground litter input. There are other possibilities as well, such as added N
334 suppressing fungi that produce these enzymes (Matocha et al. 2004, Allison et al. 2008) or
335 less lignin decomposition due to plentiful N content in lignin (Taylor et al. 1989, Hobbie et al.
336 2012, Talbot and Treseder 2012).

337 **4.2 N fertilization enhanced SOC but depressed MBC pool sizes**

338 Consistent with our second hypothesis, SOC content significantly increased by 7.7%
339 due to N fertilization, which is a little larger than 3.2% in Lu et al. (2011b). In fact, increasing
340 evidence supported that N fertilization enhanced SOC sequestration in terrestrial ecosystems
341 (DeForest et al. 2004, Hyvonen et al. 2008, Pregitzer et al. 2008). One mechanism for
342 explaining the phenomena is that the lignin-rich substrates, as the major portion of total
343 inputs, are preserved from decomposition due to depressed oxidative activities with N
344 fertilizations (Waldrop and Firestone 2004). Our result indeed showed 11.8% and 6.4%
345 decreases in phenol oxidase and peroxidase activities due to N fertilization, respectively.
346 Waldrop and Firestone (2004) also explored relationships between response ratios of SOC
347 pool and phenol oxidase or peroxidase, however, no significant correlations were detected.
348 This creates a need to synthesize the slow cycling SOC pool specifically in order to explore
349 its relationship with oxidative enzyme activities. Although this preserving mechanism is
350 currently derived from a single site, it is likely via data syntheses to establish a sound

relationship between the oxidative C pool sizes and oxidase activities across multiple sites.

In support of our 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* were decreased by 13.5% and 5%, respectively under N fertilization. Because *Acidobacteria* is abundant in the soil (Janssen 2006, Jones et al. 2009), changes of its size and relative abundance may contribute substantially to the overall microbial biomass change under N fertilization. However, it remains unclear why microbial biomass and soil respiration decreased under N fertilization (Treseder 2008, Ramirez et al. 2010), while soil microbially-mediated activities were still 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 group responses to N fertilization and elucidate their contributions to the biomass change.

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

If a time-integrated variable can somewhat be linked with SOC pool size, it will make soil modeling and long-term prediction much easier. Some current soil microbial models all included EEA as an independent C pool and catalyst for SOC decompositions (Allison et al. 2010, Wang et al. 2013, Li et al. 2014). A type of linear correlation between EEA and SOC may be needed because it can substantially simplify the current soil models. A single or a combined EEA is such a candidate variable. MBC and EEA were simultaneously collected at each site, we thus have a unique opportunity to explore their relationship. Furthermore, our analysis can reveal the differences in this relationship under different

conditions, such as by ecosystem or soil type. Our synthetic results showed a significantly positive linear relationship between response ratios of *C-acq* and MBC, but not between *C-acq* and SOC. This supported the modeling efforts 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).

The positive relationships between response ratios of *C-acq* and MBC varied under different ecosystems, experimental duration, MAT and N forms (Figure 2). In particular, the pronounced difference between forests and farmland provided likely useful information to improve the parameterization of the enzyme C pool when models were applied to different ecosystems. This difference could be attributed to varied microbial community compositions and structures. For example, fungi were relatively more abundant in the forest soil and Gram-positive bacteria were more abundant in cropland soil, thus the fungal:bacterial PLFA ratio was higher in the forest soil than most of the agricultural soils (Jangid et al. 2008). The difference in community structure between cropland and pasture soils was not as dramastic as the difference between agricultural and forest soils (Jangid et al. 2008). Bacterial communities in forest soils were less diverse than in agricultural soils (Upchurch et al. 2008). On the other hand, this difference is attributed to the sensitivities of distinct microbial groups in response to N fertilization in these ecosystems. N fertilizer amendments had a larger effect on bacterial communities specifically including *Acidobacteria*, *Baacterioidetes* and *Proteobacteria* than in forests and pastures (Jangid et al. 2008). Because bacteria communities are a primary group of decomposer on cellulose via production of hydrolases (Bayer et al. 2006), this substantial inhibitive effect of N fertilization on bacterial groups can directly lead to depressed production of *C-acq* in agricultural soils.

5. Conclusion

Extracellular enzyme activities (EEA) have not been explicitly represented in global biogeochemical models due to unclear relationship between EEA and soil C and N dynamics. This study summarized hydrolases and oxidases activities, SOC, TN and MBC pool sizes under N fertilizations based on 59 published studies. In general, N fertilization stimulated hydrolases associate with C and P but depressed oxidases, while has no significant effect on hydrolases associated with N. In particular, a significantly positive relationship was found between response ratios (RRs) of the combined hydrolases with C acquisition (i.e. *C-acq*) and MBC suggesting changes in combined hydrolases activities might act as a proxy for MBC under N fertilization. This study provides the first set of evidence on how hydrolase and oxidase activities respond to N fertilization and how they correlate to soil C and N pools over various ecosystems. Due to importance of slow cycling soil C pools for quantifying soil feedback to climate while the scarcity of such measurements, future studies should investigate the relationship between slow cycling soil C and oxidases.

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