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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), leucine amino 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.

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 m of soil (Jobbágy and Jackson, 2000)], elevated N bioavailability could alter soil C turnover and exert strong feedbacks on global

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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; Chen et al., 2016). 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, 2011b, 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, 2013). The C acquiring enzymes (*C-acq*) denote the average enzyme activity of AG, BG, BX