



Nonlinear responses of the V_{max} and K_m of hydrolytic and polyphenol oxidative enzymes to nitrogen enrichment

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ABSTRACT

The kinetics of extracellular enzymes produced by soil microorganisms regulate soil organic matter decomposition and other ecosystem functions. Atmospheric nitrogen (N) deposition creates a soil environment with elevated reactive N, which is expected to affect the kinetic parameters (V_{max} and K_m) of hydrolytic and polyphenol oxidative enzymes, as well as soil microbial respiration. We measured the V_{max} and K_m of seven soil hydrolytic enzymes and polyphenol oxidase (PPO), as well as soil microbial respiration and physicochemical properties, in a temperate steppe after 15 years of multi-level N addition treatments. As the N addition increased, the V_{max} of most carbon (C)-degrading and N-degrading hydrolytic enzymes decreased, and this was associated with a decline in soil pH. In contrast, the V_{max} of acid phosphatase (AP) increased with increasing N addition. Higher N addition was associated with lower K_m of most hydrolytic enzymes, except for AP and β -xylosidase (BX). There was a quadratic relationship between N addition and the V_{max} and K_m of PPO, which reached a maximum with the addition of $8 \text{ g ;N m}^{-2} \text{ y}^{-1}$, and decreased at higher N addition levels. Structural equation modeling indicated that the decline in soil microbial respiration with increasing N deposition was directly mediated by the BG kinetics, due to the fact N-induced acidification negatively impacted the V_{max} and K_m of BG. Our empirical data on soil enzyme (i.e., V_{max} , K_m) and its relationship with microbial respiration should be useful for modelling how microbes and substrates interact to regulate soil carbon cycling under N enrichment.

1. Introduction

The increased availability of reactive nitrogen (N) stimulates plant production and has widespread effects on carbon (C) cycling in terrestrial ecosystems (Cusack et al., 2011). A growing body of literature found that high N deposition inhibited microbial respiration, resulting in slower decomposition of soil organic matter (SOM) (Janssens et al., 2010; Frey et al., 2014; Riggs et al., 2015). Microbial communities secrete extracellular enzymes to degrade complex polymers into soluble substrates for assimilation and respire CO_2 during the decomposition of SOM (Sinsabaugh et al., 2008; Burns et al., 2013; Bödeker et al., 2014). It is generally accepted that the catalysis by extracellular enzymes is the rate-limiting step for the degradation of organic matter in terrestrial ecosystems (Sinsabaugh et al., 2008; Cenini et al., 2016). The reduction in decomposition under anthropogenic N deposition should be achieved

by altering enzyme activities in some ways. Therefore, exploring the microbial enzyme catalytic process will facilitate the understanding of biological mechanisms by which soil organic matter decomposition responds to anthropogenic N deposition.

Extracellular enzymes in the soil catalyze the degradation of organic matter primarily through hydrolysis (for the breakdown of celluloses, hemicelluloses, chitins, and proteins) and oxidation (for the breakdown of more recalcitrant lignin or humified organic matter) (Sinsabaugh, 2010). Based on cost-efficiency, soil microbes adjust their allocation of resources in the synthesis of various extracellular C-, N-, and phosphorus (P)-acquiring enzymes (Sinsabaugh and Moorhead, 1994; Sinsabaugh and Shah, 2012; Burns et al., 2013). Thus, the synthesis of soil enzymes, as a form of foraging strategy, should vary under different soil nutrient conditions (Allison, 2005; Sinsabaugh et al., 2014). Under high N deposition, microbes allocate more resources to synthesize C-acquiring

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