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Warming rate drives microbial nutrient demand and enzyme expression during peat decomposition

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ABSTRACT

Recent developments of enzyme-based decomposition models highlight the importance of enzyme kinetics with warming, but most modeling exercises are based on studies with a step-wise warming. This approach may mask the effect of temperature in controlling in-situ activities as in most ecosystems the rate of warming is more gradual than these step warming studies. We conducted an experiment to test the effects of contrasting warming rates on the kinetics of carbon (C), nitrogen (N), and phosphorus (P) degradation enzymes in subtropical peat soils. We also wanted to evaluate if the stoichiometry of enzyme kinetics shifts under contrasting warming rates and if so, how does it relate to the stoichiometry in microbial biomass. Contrasting warming rates altered microbial biomass stoichiometry leading to differing patterns of microbial demand for C vs. nutrient (N and P) and enzyme expression following the optimum foraging strategy. Activity (higher Vmax) and efficiency (lower Km) of C acquisition enzymes were greater in the step treatment; however, expressions of nutrient (N and P) acquiring enzymes were enhanced in the ramp treatment at the end of the experiment. In the step treatment, there was a typical pattern of an initial peak in the Vmax and drop in the Km for all enzyme groups followed by later adjustments. On the other hand, a consistent increase in Vmax and decline in Km of all enzyme groups were observed in the ramp treatment. These changes were sufficient to alter microbial identity (as indicated by enzyme Km and biomass stoichiometry) with two apparently different endpoints under contrasting warming rates. This observation resembles the concept of alternate stable states and highlights a need for improved representation of warming effects on enzymes in decomposition models. Using peat soils of Florida Everglades, here we have demonstrated that contrasting warming rates can influence the dynamics of microbial and enzymatic kinetics. Hence, we suggest that future laboratory and field warming studies could consider our approach to accurately represent microbial and enzymatic kinetics in biogeochemical models.

1. Introduction

Extracellular enzymes synthesized by soil microbes are the proximate agents of soil organic matter (SOM) degradation and can provide key information on microbial nutrient demand (Turner and Romero, 2010). As they catalyze the rate-limiting step in soil organic matter (SOM) decay, heterotrophic respiration depends on the activity of extracellular enzymes since much of the dead plant material entering soils are in polymeric form. Thus, patterns of extracellular enzyme activity have potential to provide insights into the biochemical controls on SOM storage (Sinsabaugh et al., 2008).

Temperature has been shown to stimulate the growth and metabolic functions of microbes and microbial-mediated enzymatic processes (Kadlec and Reddy, 2001; Weedon et al., 2013). Increased enzymatic activity with greater conformational stability at higher temperature accelerates soil respiration rates as microbial activity increases (reviewed by Conant et al., 2011; Wallenstein et al., 2011). According to the biochemical theory, maximum catalytic rates of enzymes increase under warming until temperature optima is achieved, beyond which the tertiary structure of the enzyme protein denatures (Davidson and Janssens, 2006). Studies of aquatic carbon (C) processing showed isoenzymes of different temperature optima are also maintained by microorganisms (Hochachka and Somero, 2002; Grzymski et al., 2008).

Allison et al. (2010b) further proposed that as enzyme activity of the extant pool increases with temperature, microbes may downregulate enzyme synthesis in response to limiting substrates or other resources. If that is true, depolymerization of soil C will decrease at some point under sustained warming in response to lower concentrations of

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catalysts. Thermal adaptation in enzyme functions has also been suggested to affect heterotrophic respiration in soil (Bradford et al., 2008, 2010). Thus, a better understanding of the temperature sensitivity of soil enzymatic reactions would improve predictions of the soil C storage in a warmer world.

Until recently, most studies dealing with the temperature sensitivity of soil enzymes have not been focused on kinetic parameters (Trasar-Cepeda et al., 2008; Wang et al., 2012; but see Stone et al., 2012, German et al., 2012, and Drake et al., 2013b). Maximum catalytic activity (Vmax) and substrate binding-affinity (Km) are the two parameters that determine the kinetic behavior of an enzyme as a function of the substrate concentration. When the enzyme-mediated reactions in the soil are operating under non-saturating levels of substrates, Km becomes an important parameter that demands a detailed investigation (Marx et al., 2005; Davidson et al., 2006; German et al., 2011).

In addition to Vmax, Km of an enzyme is also believed to increase with warming resulting from increased destabilization of the enzyme–substrate complex associated with the higher kinetic energy of both enzymes and substrates (Davidson and Janssens, 2006; Davidson et al., 2006), which could potentially offset the positive feedback between the Vmax of hydrolytic enzymes and warming, and thus counteract loss of SOM under warming. Therefore, warming responses of soil enzyme kinetics demand more attention so that the fate of soil carbon (C) stocks can be determined under future climate change (Conant et al., 2011).

Soil warming studies further demonstrated that microbial carbon use efficiency (CUE) decreases with warming (Sihi et al., 2017; Steinweg et al., 2008; Frey et al., 2013), resulting in greater production of carbon di-oxide (CO_2) per unit of substrate C assimilated into microbial biomass. Thus, it is likely that if C allocation is altered, enzyme production can also be expected to change with warming (Wallenstein et al., 2009). Steinweg et al. (2013) also suggested that the warming-induced increase in maintenance costs have the potential to increase microbial nutrient demand and thereby increase mass-specific enzyme activities. A shift in the abundance of microbial groups and associated expressions of enzymes under warming has also been reported to alter SOM dynamics (Zhang et al., 2005; Frey et al., 2008).

Recent developments of microbial enzyme-based decomposition models (Allison et al., 2010a; Davidson et al., 2012; German et al., 2012; Sihi et al., 2016a, 2018) highlighted the importance of enzyme kinetics with warming. Most of these modeling exercises are based on recent studies investigating temperature effects on soil enzyme catalysis by step-wise increases in temperature (e.g. 5 °C, 10 °C), either in the laboratory- or field-manipulation studies. This approach may mask the effect of temperature in controlling in-situ activities (Wallenstein and Weintraub, 2008) as in most ecosystems soil temperature changes more gradually and raise the question of the significance of these step-warming experiments (Rustad, 2006; Wetterstedt and Ågren, 2011).

A slow warming rate is likely to ease the degree of adaptation of soil microbial community and can allow overlapping of different microbial groups competing for limiting resources that may also be linked to altered expressions of enzyme kinetics. Therefore, the rate of environmental perturbation (i.e. warming) is likely to play an instrumental role in the enzymatic reactions that may stem from a differential capacity of microbes producing enzyme to adapt to the rate of the changing environment (Ayo et al., 2017). Given climate change will involve gradual warming over time, evaluation of microbial biomass and enzymatic response to gradual warming is warranted in addition to the frequently observed response to a large-step warming.

Further, measures of enzyme kinetics with the warming of wetland soils, especially tropical/subtropical wetlands, have not been well investigated yet despite their critical importance to global C cycle, which may have contributed to observed uncertainties in the current estimates of global climate models (Sjögersten et al., 2014). Based on this research gap, we conducted an experiment to test the effects of contrasting warming rate on the kinetics of C (Enz_C)-, N (Enz_N)-, and P (Enz_P)-degradation enzymes in subtropical peat soils. We chose subtropical peat soils because of their importance for emissions of greenhouse gas methane (Megonigal et al., 2004; Sihi et al., 2016b), a significant component of wetland C fluxes.

Here we presented results of a 100-day laboratory incubation study where we exposed peat soils from Florida Everglades to contrasting warming rates. By doing so, we intend to demonstrate how other laboratory and field warming studies across different terrestrial ecosystems could also adopt this approach to accurately capture temperature response of microbial and enzymatic kinetics. We hypothesized that temperature response of enzyme kinetic parameters (Vmax and Km) would be lower in a gradual temperature change @ 0.1 °C day^{-1} (the ramp treatment) to that of a single large-step change in temperature @ 10 °C within a day (the step treatment). Furthermore, we also hypothesized that the stoichiometry (i.e. ratios of C- to nutrient- (N, P) and N- to P- degrading enzymes) of enzyme kinetics would shift under contrasting warming rates and that should correlate with the stoichiometry in microbial biomass and dissolved pool.

2. Materials and methods

2.1. Site selection and soil sample collection

Peat samples were collected from the wet prairies of Florida Everglades, inside the water Conservation area 2-A (WCA-2A), which was primarily characterized to be a P-limited system (Craft and Richardson, 1993). However, historical inputs of P from the Everglades Agricultural Area in the north resulted in a shift toward increasing N limitation near the inflow site of WCA-2A (Verhoeven et al., 1996; White and Reddy, 2000; Inglett et al., 2004; Inglett and Reddy, 2006).

For this study, we chose an N-limited site dominated by *Typha domingensis* (F1 site, Latitude: 26.35°N, Longitude: 80.36°W), and another, *Cladium jamaicense*-dominated peat site which is designated as P-limited (U3 site, Latitude: 26.29°N, Longitude: 80.41°W) (Craft and Richardson, 1993; Inglett et al., 2004). Total soil P (TP) content in the P-limited interior site i.e. U3 site (TP: 226 ± 3 mg kg^{-1}) was almost 6-folds lower than that at the P-rich inflow site i.e. F1 site (TP: 1420 ± 6 mg kg^{-1}) (See Table 1 in Sihi et al., 2017). Because total nitrogen (TN) content was similar between these two sites, P input in the F1 site lowered the N:P ratio by 6-folds (23 ± 2 in F1 vs 155 ± 16 in U3 site) which invoked N-limitation in F1-site (stoichiometry values are calculated from Table 1 in Sihi et al., 2017).

Three field replicates were collected at each site on 17 October, 2013, covering an area of approximately 15 m × 15 m. Each field replicate was a composite of five intact soil cores (0–40 cm deep, 10 cm id), covering an area of approximately 2.5 m × 2.5 m. We used the top soils (0–10 cm) for this study. After collection, the peat samples were transported to the Wetland Biogeochemistry Laboratory, University of Florida and stored at 20 °C (which is close to the in-situ temperature) in dark for up to 24 h. Field moist samples were homogenized manually by

Table 1
Soil extracellular enzymes assayed for kinetic parameters.

Enzyme group	Enzyme name	Abbreviations	Substrates
C acquisition enzyme (Enz_C)	ß-D-Glucosidase	BG	4-MUF ß-D-glycopyranoside
	ß-D-Xylosidase	XYL	4-MUF ß-D-xylopyranoside
N acquisition enzyme (Enz_N)	Leucine aminopeptidase	LAP	L-leucine hydrochloride
	N-Acetyl-ß-D glucosaminidase	NAG	N-MUF ß-D glucosaminide
P acquisition enzyme (Enz_P)	Phosphomonoesterase	PHO	MUF phosphate
	Phosphodiesterase	BPHO	Bis-(MUF) phosphate

MUF: methylumbelliferyl.