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# Soil aggregate size distribution mediates microbial climate change feedbacks

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ABSTRACT

Soil carbon stabilization is known to depend in part on its distribution in structural aggregates, and upon soil microbial activity within the aggregates. However, the influence of climate change on continued soil C storage within aggregates of different size classes is unknown. In this study, we applied a modified dry-sieving technique to separate bulk soil into three fractions (>1 mm large macroaggregate; 0.25–1 mm small macroaggregate; <0.25 mm microaggregate), and measured the activities of seven microbial enzymes involved in the cycling of C, N, and P, in the context of a long-term elevated $CO_2$ and warming experiment. Significant effects of aggregate size were found for most enzyme activities, enzyme stoichiometry, and specific enzyme activities (per unit of microbial biomass), suggesting that aggregate size distribution mediates microbial feedbacks to climate change. C decomposition enzyme activities, the ratios of total C:N and C:P enzyme activity, and the specific enzyme activity for C decomposition were significantly higher in the microaggregates across climate treatments. However, specific enzyme activity for N decomposition was significantly higher in macroaggregates. Increased specific enzyme activity for C decomposition under both elevated $CO_2$ and warming suggests that these climate changes can enhance microbial ability to decompose soil organic matter (SOM). Moreover, changes in the enzyme C:N:P stoichiometry suggest that soil microorganisms may be able to adjust nutrient acquisition ratios in response to climate change. Our study suggests that identifying and modeling aggregate size as a function of SOM decomposition could improve our mechanistic understanding of soil biogeochemical cycling responses to climate change.



## 1. Introduction

Both elevated $CO_2$ ($eCO_2$) and warming have the potential to alter soil carbon (C) cycling processes, which could affect climate-carbon feedbacks (Bardgett et al., 2008; Nie et al., 2013b; Wallenstein and Hall, 2012). Soil microbial respiration resulting from soil organic matter decomposition activities accounts for approximately two-thirds of soil C losses in terrestrial ecosystems (Luo and Zhou, 2006; Silver et al., 2005). Therefore, slight changes in microbial decomposition rates in response to global change could represent large net changes in ecosystem $CO_2$ release (Cusack et al., 2009; Wallenstein et al., 2008).

Climate change can influence the direction and magnitude of ecosystem C storage through a variety of microbial pathways. For example, $eCO_2$ can promote plant productivity and increase soil substrate availability, stimulating microbial decomposition (Carney et al., 2007; Nie et al., 2013a, 2013b). Warming can accelerate microbial metabolic activities (Allison et al., 2010), but in some situations may lead to soil moisture-limiting conditions for microbial decomposition (Liu et al., 2008). Recent studies showed that microbial activity under climate change depends on soil aggregate size, with potential implications for microbial mediation of carbon-cycle feedbacks to climate change (Dorodnikov et al., 2009a,b; Rillig et al., 2002; Six et al., 2001). However, our mechanistic understanding of indirect effects on microbial decomposition rate in soil aggregates is very limited, especially for combined effects of $eCO_2$ and warming.

Soil carbon stabilization and nutrient cycling within the rhizosphere are dependent on microbial activity, and all of these processes may vary spatially in soils at very fine spatial scales. Climate change has been shown to impact microbial activity differentially within different soil aggregate sizes. For example, Six et al. (2001) showed that $eCO_2$ enhanced the proportion of photosynthetic

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carbon with increasing aggregate size, suggesting that SOM turnover accelerated with increasing aggregate size, and that C within smaller aggregates was more stable. Similarly, Dorodnikov et al. (2009b) observed increased fungal SOM turnover in large macroaggregates (>2 mm) relative to microaggregates (<0.25 mm) under $eCO_2$ following glucose amendments. Likewise, warming was shown to increase soil carbon derived from $C_4$ plant species photosynthate inputs corresponding to elevated C decay rates in macroaggregates >2 mm (Cheng et al., 2011).

Soil microbes produce a wide variety of enzymes to degrade organic compounds, thereby driving biogeochemical cycling of essential elements (e.g. C, N, and P) in SOM (Dick et al., 1992; Van Der Heijden et al., 2007). Because activities of soil enzymes are highly sensitive to environmental changes, they have been interpreted as indicators of substrate availability and microbially driven decomposition (Davidson and Janssens, 2006; Nie et al., 2013b; Wallenstein et al., 2008). Phillips et al. (2011) demonstrated that increased root exudation by $eCO_2$ stimulated microbial release of N-acetyl-β-Glucosaminidase, which is a proxy for fungal biomass (Chung et al., 2007; Miller et al., 1998). This microbial enzyme contributes to organic nitrogen (N) mineralization, which could accelerate SOM turnover and prevent soil C accumulation (Phillips et al., 2011). Warming also accelerates SOM turnover due to increased soil enzyme activities (Wallenstein et al., 2011, 2008), supporting suggestions of positive climate-carbon cycle feedbacks (Heimann and Reichstein, 2008; Jones et al., 2005).

There is still much uncertainty about how microbial enzymes activities will impact atmospheric feedbacks in response to climate change. For example, Austin et al. (2009) found that 10-y $CO_2$ fumigation had no significant effects on microbial enzyme activities (such as α-Glucosidase and β-Xylosidase) in a sweetgum plantation, and warming decreased microbial enzyme activities in a boreal forest (Allison and Treseder, 2008) and a tall-grass prairie ecosystem (Zhou et al., 2011). These conflicting results may be mainly attributed to inherent complexity and diversity of SOM, with different physical and chemical stabilities, which leads to variability in microbial decomposition processes (Bardgett et al., 2008; Dorodnikov et al., 2009b). For example, decreased substrate availability due to low quality and accessibility of SOM hampers microbial decomposition and thus can suppress microbial responses to warming (Davidson and Janssens, 2006). Therefore, application of physical fractionation techniques are expected to lend insights into microbial responses to climate change (Dorodnikov et al., 2009a,b; Niklaus et al., 2003; Rillig et al., 2002).

Here, we determined the responses of microbial activity to climate change in the long-term Prairie Heating and $CO_2$ Enrichment (PHACE) experiment, which concurrently simulates the impact of rising atmospheric $CO_2$ and warming on ecosystem dynamics in a semiarid grassland ecosystem. Due to the characteristically low soil moisture in this semi-arid grassland, we chose to use a modified dry-sieving technique to separate bulk soil into three fractions, in which we measured seven microbial enzyme activities involved in the cycling of C (β-Glucosidase, β-D-Cellubiosidase, β-Xylosidase, and α-Glucosidase), N (N-acetyl-β-Glucosaminidase and Leucine amino peptidase), and P (Phosphatase). Our previous work suggested that soil microbes can positively respond to $eCO_2$ and warming (Nie et al., 2013b), and show a high degree of stoichiometric flexibility in their biomass according to altered soil nutrient and moisture availability under climate change (Dijkstra et al., 2012). Therefore, we predicted that $eCO_2$ and warming would increase soil enzyme activities, alter enzyme stoichiometry, and that these microbial functional responses to climate change would vary among aggregate sizes. Our study is unique because we examine the multiple interacting effects of $eCO_2$ and warming on soil enzyme activities within isolated soil aggregates.

## 2. Materials and methods

### 2.1. Study site

The PHACE experiment is located at the US Department of Agriculture Agricultural Research Service (USDA-ARS) High Plains Grasslands Research Station, Wyoming, USA (41°11′N, 104°54′W). The experiment has imposed a factorial combination of two levels of $CO_2$ (ambient and elevated 600 ppmv) since 2006, and two temperature regimes (ambient and elevated (1.5/3.0 °C warmer day/night)) since 2007, with five replicate plots (3.4 m diameter) of each treatment combination (ct, ambient $CO_2$ and ambient temperature; Ct, elevated $CO_2$ and ambient temperature; cT, ambient $CO_2$ and elevated temperature; CT, elevated $CO_2$ and elevated temperature) (Morgan et al., 2011; Nie et al., 2013b). The ecosystem is a northern mixed grass prairie dominated by the $C_3$ grass *Pascopyrum smithii* (Rydb.) and the $C_4$ grass *Bouteloua gracilis* (H.B.K) Lag. Mean annual precipitation is 352 mm and mean air temperatures are 20.2 °C in July and −2.9 °C in January (Morgan et al., 2011; Nie et al., 2013b).

### 2.2. Soil sampling and aggregate-size fractionation

Three soil cores from each treatment plot were collected from 0 to 15 cm depth using a 3-cm-diameter auger in June 2012 and placed in a portable ice box for transport to the laboratory. Field moisture content of all samples was less than 6.0% by weight, which allows limited mechanical stress to induce maximum brittle failure along natural planes of weakness (Dorodnikov et al., 2009b; Kristiansen et al., 2006). To avoid destroying microbial community structure and functioning, therefore, soil aggregate fractions were obtained by sieving field-moist samples by stacking sieves (1 and 0.25 mm). After gentle, manual crumbling to <8 mm, soils were sieved for 3 min on a mechanical shaker to partition the aggregate sizes. Preliminary tests showed that 3 min sieving intervals were sufficient to separate different aggregate size-classes while minimizing aggregate abrasion. All visible gravel and roots were picked out and the aggregates >1 mm were collected (large macroaggregates). The same procedure was carried out to obtain 0.25–1 mm soil aggregates (small macroaggregates) retained on the 0.25 mm sieve. The aggregates <0.25 mm were identified as microaggregates. All samples were processed within 12 h after being collected. As shown in Fig. S1, the modified dry-sieving procedure accomplished aggregate-size fractionation of the PHACE soils.

### 2.3. Soil analysis

Soil samples were oven-dried at 60 °C to constant weight, and weighed to determine soil water content. Soil total C/N concentrations were determined by a Costech 4010 Element Analyzer (Costech Analytical Technologies, CA, USA). OC (organic carbon) concentrations were calculated as the difference between total C concentrations and inorganic C concentrations, which were measured by the modified pressure-calcimeter method (Sherrod et al., 2002).

### 2.4. Enzyme assays

β-Glucosidase (BG), β-D-Cellubiosidase (CB), β-Xylosidase (XYL), α-Glucosidase (AG), N-acetyl-β-Glucosaminidase (NAG), Leucine amino peptidase (LAP), and Phosphatase (PHOS) activities were measured using 4-methylumbelliferyl (MUB) or 4-methylcoumarin hydrochloride-linked (MUC) substrates yielding the highly fluorescent cleavage products MUB or MUC upon hydrolysis