Soil Biology & Biochemistry 68 (2014) 357–365

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Soil aggregate size distribution mediates microbial climate change feedbacks

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ARTICLE INFO

Article history:
Received 31 May 2013
Received in revised form
30 September 2013
Accepted 6 October 2013
Available online 18 October 2013

Keywords:
Climate change
Elevated CO_2
Warming
Soil aggregate
Extracellular enzyme activity
Specific enzyme activity
Enzyme stoichiometry

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.

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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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http://dx.doi.org/10.1016/j.soilbio.2013.10.012

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

(Wallenstein et al., 2008). All of the enzyme assays were set up in 96-well microplates. Briefly, 2.75 g of fresh soil was homogenized with 91 ml of 50 mM acetate buffer in a blender. Then 800 μl of sample slurry was added into wells which contained 200 μl of 200 μM substrate for enzyme activity measurement. Standard curves were created with 200 μl of MUB or MUC solution and 800 μl of sample slurry. Twelve replicate wells were set up for each sample and each standard concentration. The assay plates were incubated in the dark at 25 °C for 3 h to mimic the average soil temperature during the plant growing season. Enzyme activities were corrected using a quench control (Wallenstein et al., 2008; Nie et al., 2013b). Fluorescence was measured using a Tecan infinite M200 microplate fluorometer (Grödig, Austria) with 365 nm excitation and 460 nm emission filters. The activities were expressed in units of nmol h^{-1} g^{-1} dry soil. For recovery of each enzyme activity, the sum of enzyme activity as the weight distributions of aggregate classes was calculated as percentage of this enzyme activity in the bulk soils.

2.5. Specific enzyme activity and phospholipid fatty acid assays

To normalize activity to the size of the microbial community, specific enzyme activity was calculated as the rate of enzyme activity per gram of phospholipid fatty acids (PLFAs) biomass of total community. We quantified microbial biomass by PLFAs because they are specific biomarkers (Hassett and Zak, 2005; Nie et al., 2013b; Zak et al., 2000). Lipids were extracted from 5 g of lyophilized soil in a chloroform-methanol-phosphate buffer mixture (1:2:0.8), and the phospholipids were separated from other lipids on a solid phase silica column (Agilent Technologies, Palo Alto, CA). The phospholipids were subjected to mild-alkaline methanolysis, dissolved in chloroform, and purified with a solid phase amino column (Agilent Technologies). The resulting fatty acid methyl esters were dissolved in 0.2 ml 1:1 hexane:methyl *t*-butyl ether containing 0.25 mg 20:0 ethyl ester ml^{-1}, separated in an Agilent 6890 gas chromatograph with an Agilent Ultra 2 column (Agilent Technologies, and identified according to the MIDI eukaryotic method with Sherlock software (MIDI, Inc., Newark, DE). The PLFAs were classified as Gram-positive bacteria (i14:0, i15:0, a15:0, i16:0, i17:0, and a17:0), Gram-negative bacteria (16:1ω9c, cy17:0, 18:1ω9c, and cy19:0), saprotrophic fungi (18:2ω6c) and arbuscular mycorrhizal fungi (AMF) (16:1ω5c) (Zak et al., 2000). Each PLFA and the sum of all PLFAs are expressed as μg PLFA g^{-1} dry soil.

2.6. Statistical analyses

To determine the effects of eCO_2, warming, and aggregate size on soil chemical properties and enzyme variables across soil aggregates, we used a three-way ANOVA with eCO_2, warming, and aggregate size as fixed effects by SPSS 13.0. *Post hoc* means were determined using least squares means separation by SPSS. The significance level (*P* value) of post-hoc LSD (least significant difference) test was set to 5%. Data not meeting assumptions of normality and homogeneity of variance were log-transformed before statistical testing. Pearson correlation analyses were performed to evaluate relationships between soil chemical properties and microbial activity by SPSS.

3. Results

3.1. Weight distribution, water content and C/N ratios

Under ambient CO_2 and temperature (control), weight distribution in the large macroaggregates was significantly higher than two smaller aggregates (Table 1). Compared to the control, Ct and cT treatments did not affect the weight distribution of aggregates (Table 1). However, CT decreased weight distribution in the large macroaggregates (Table 1).

Table 1
Weight distribution (%) in soil aggregates.

Treatment	Aggregate-size classes (mm)		
	>1	1–0.25	<0.25
ct	37.2 ± 2.9^{Aa}	31.9 ± 1.1^{Ba}	30.9 ± 2.0^{Bb}
Ct	37.0 ± 1.4^{Aa}	33.1 ± 0.8^{ABa}	29.9 ± 1.5^{Bb}
cT	33.1 ± 3.2^{Aa}	33.7 ± 1.2^{Aa}	33.2 ± 2.1^{Ab}
CT	27.0 ± 1.0^{Bb}	34.5 ± 1.1^{Aa}	38.5 ± 0.9^{Aa}

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. Values are given as mean ± standard error ($n = 5$). Treatments followed by the same letters are not significantly different between aggregate size classes in the same climatic treatment (uppercase letters) and climatic treatments in the same class of aggregate size (lowercase letters) at $P < 0.05$.

Compared to the control, CT significantly decreased water content except in microaggregates (Table 2; Fig. 1a). Under Ct treatment, the highest water content was found in the large macroaggregates (Fig. 1a).

Only aggregate size significantly affected organic C and N concentrations (Table 2). The microaggregates had higher C and N concentrations than both macroaggregates at the same climate treatment (Fig. 1b and c). Neither aggregate size nor climate factors affected C:N ratios (Table 2; Fig. 1d).

3.2. Enzyme activity

Although climate treatments did not change the activities of C degrading enzymes BG, CB, XYL, or AG in bulk soils (Fig. 2a–d), aggregate size had significant effects on distribution of these enzyme activities (Table 2), which increased as aggregate size decreased (Fig. 2a–d). In the large macroaggregates, cT increased BG activity (Fig. 2a), all climate treatments increased CB activity (Fig. 2b), Ct and cT increased XYL activity (Fig. 2c), and Ct and CT increased AG activity (Fig. 2d). cT and CT significantly decreased NAG activity across all aggregates (Fig. 2e), with significant warming effects (Table 2). Climate treatments did not significantly change LAP activity in bulk soils, whereas CT significantly increased LAP activity in the large and small macroaggregates, compared to the control (Fig. 2f). Ct significantly increased PHOS activity in the small macroaggregates, and cT increased PHOS activity in microaggregates, leading to increases in PHOS activity of the bulk soils (Fig. 2g). However, when eCO_2 and warming were combined, no effect on PHOS was observed.

3.3. Enzyme stoichiometry

Aggregate size had significant effects on the ratio of total C:N and C:P enzyme activity (Table 2), which increased as aggregate size decreased across climate treatments (Fig. 3a and b). Compared with the large macroaggregates, a higher ratio of total C:P enzyme activity in the microaggregates was observed under the control and Ct but not in cT and CT (Fig. 3b). cT decreased the ratio of total N:P enzyme activity across all aggregates (Fig. 3c). Ct and CT had no effects on the ratio of total N:P enzyme activity across all aggregates (Fig. 3c). Ct had no effects on the ratio of total C:N enzyme activity (Table 2). However, cT significantly increased the ratio of total C:N enzyme activity across all aggregates (Fig. 3a). Under CT treatment, a significantly higher ratio of total C:N enzyme activity was observed in the large macroaggregates compared with the control

Table 2
Effects of elevated CO_2, warming (T), and soil aggregate-size (Size) on microbial enzyme activity, enzyme stoichiometry, and specific enzyme activity (SEA) across soil aggregates (*P*-values from three-factor ANOVA, $n = 5$).

	CO_2	*T*	Size	$CO_2 \times T$	$CO_2 \times$ size	$T \times$ size	$CO_2 \times T \times$ size
Water content	0.593	$P < 0.001$	$P < 0.001$	$P < 0.05$	0.645	0.260	0.475
Organic C concentration	0.103	0.936	$P < 0.001$	0.688	0.734	0.988	0.643
N concentration	0.103	0.480	$P < 0.001$	0.552	0.664	0.961	0.639
Organic C:N ratio	0.056	3.709	9.634	0.630	0.969	0.845	1.185
β-Glucosidase	0.455	0.157	$P < 0.001$	$P < 0.05$	0.975	0.848	0.881
β-D-Cellubiosidase	0.115	0.265	$P < 0.001$	$P < 0.05$	0.870	0.653	0.890
β-Xylosidase	0.210	0.887	$P < 0.001$	0.059	0.854	0.609	0.951
α-Glucosidase	0.330	0.466	$P < 0.001$	0.192	0.835	0.499	0.857
N-acetyl-β-Glucosaminidase	0.331	$P < 0.001$	0.661	0.634	0.983	0.935	0.874
Leucine amino peptidase	$P < 0.001$	0.199	0.710	0.940	0.713	0.977	0.911
Phosphatase	0.933	0.870	0.869	$P < 0.001$	0.811	0.630	0.587
Total C:N enzyme ratio	0.331	$P < 0.001$	$P < 0.001$	$P < 0.01$	0.998	0.924	0.880
Total C:P enzyme ratio	0.211	0.378	$P < 0.01$	0.453	0.940	0.585	0.843
Total N:P enzyme ratio	$P < 0.05$	0.101	0.987	$P < 0.05$	0.800	0.681	0.630
SEA for C decomposition	0.102	$P < 0.05$	$P < 0.01$	$P < 0.001$	0.915	0.268	0.783
SEA for N decomposition	$P < 0.001$	0.077	$P < 0.01$	$P < 0.05$	0.589	0.526	0.930
SEA for P decomposition	0.592	0.340	0.078	$P < 0.001$	0.620	0.724	0.642

(Fig. 3a). Ct and cT had no effects on the ratio of total C:P enzyme activity (Table 2 and Fig. 3b) across all aggregates.

3.4. Specific enzyme activity

Ct, cT, and CT all significantly increased the specific enzyme activity (SEA) for C decomposition in bulk soils (Fig. 4a). Ct increased the SEA for C decomposition across soil aggregates, whereas cT and CT increased the SEA for C decomposition in the large and small macroaggregates (Fig. 4a). Aggregate size had significant effects on the SEA for C decomposition (Table 2), which increased as aggregate size decreased across all climate treatments (Fig. 4a). All climate treatments had no effects on the SEA for N decomposition in bulk soils (Fig. 4b). However, Ct increased the SEA for N decomposition in the large and small macroaggregates. Compared with the control, Ct significantly increased the SEA for P decomposition in the bulk soils and small macroaggregates (Fig. 4c), and cT significantly increased the SEA for P decomposition in the bulk soils and all aggregates with the highest in the large macroaggregates (Fig. 4c). However, CT had no effects on the SEA for P decomposition across all aggregates (Fig. 4c).

4. Discussion

Responses of soil microbial communities to rising atmospheric CO_2 concentration and climate warming play a key role in mitigating or accelerating carbon-climate feedbacks (Bardgett et al., 2008; Nie et al., 2013b). This study revealed that long-term simulated climate change strongly affected soil microbial function by both changing the distribution of aggregate sizes within soils, and also through altering function within each aggregate class. Significant effects of aggregate-size were found for most enzyme activities, enzyme stoichiometry, and specific enzyme activities, suggesting that soil aggregate size to a large extent determines soil microbial feedbacks to climate change. Increased enzyme activity under climate change could enhance microbial ability to

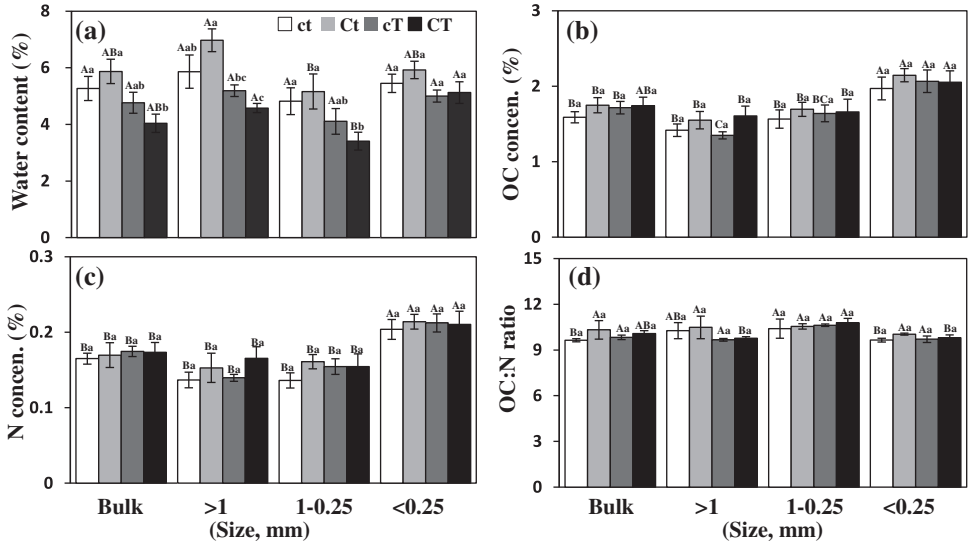

Fig. 1. Soil water content, organic carbon (OC) concentration, nitrogen (N) concentration, and OC:N ratio in bulk soil and three aggregates across climate treatments. ct: ambient CO_2 and ambient temperature; Ct: elevated CO_2 and ambient temperature; cT: ambient CO_2 and elevated temperature; CT: elevated CO_2 and elevated temperature. Values are given as mean ± standard error ($n = 5$). Treatments followed by the same letters are not significantly different between aggregate size classes in the same climatic treatment (uppercase letters) and climatic treatments in the same class of aggregate size (lowercase letters) at $P < 0.05$.

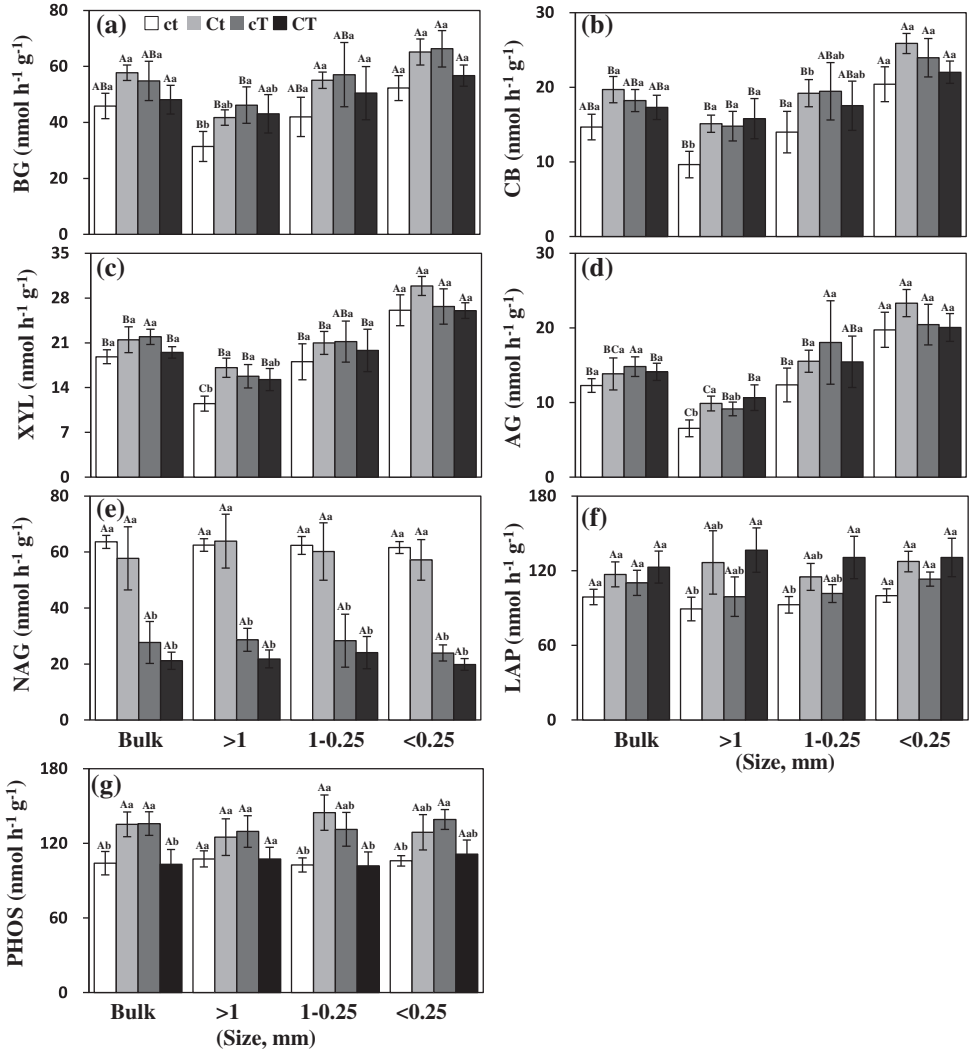

Fig. 2. β-Glucosidase (BG), β-D-Cellubiosidase (CB), β-Xylosidase (XYL), α-Glucosidase (AG), N-acetyl-β-Glucosaminidase (NAG), Leucine amino peptidase (LAP), and Phosphatase (PHOS) activities in bulk soil and three aggregates across climate treatments. ct: ambient CO_2 and ambient temperature; Ct: elevated CO_2 and ambient temperature; cT: ambient CO_2 and elevated temperature; CT: elevated CO_2 and elevated temperature. Values are given as mean ± standard error ($n = 5$). Treatments followed by the same letters are not significantly different between aggregate size classes in the same climatic treatment (uppercase letters) and climatic treatments in the same class of aggregate size (lowercase letters) at $P < 0.05$.

decompose SOM, which may have affected the distribution of SOM within aggregates. Moreover, because of the changes in enzyme stoichiometry, our findings suggest that microbial communities are able to change nutrient acquisition ratios in response to climate change, which could affect nutrient availability and the chemical composition of SOM.

4.1. Soil fractionation and aggregate-size effects

Microbial functional shifts can occur among different aggregate sizes due to differences in the chemical composition of SOM and the accessibility of substrates within soil particles (von Lutzow et al., 2007). Wet and dry fractionation schemes have been developed to separate bulk soil into different fractions to quantify SOM dynamics in soil aggregates (Kristiansen et al., 2006; Lagomarsino et al., 2012; Rillig et al., 2002). However, the wet sieving process could potentially alter *in-situ* microbial activity by leaching microbial enzymes (most of which are water-soluble) (Davidson and Janssens, 2006; Rietz and Haynes, 2003), change microbial biomass (Denef et al., 2001), and create anoxic conditions within aggregates (Zhang and Zak, 1998). Alternatively, an 'optimal moist' sieving technique has been suggested to study microbial community structure and functioning among aggregates without adding water (Dorodnikov et al., 2009a, 2009b; Schutter and Dick, 2002). However, this technique often requires soil drying under room temperature or 4 °C for several hours to days. Therefore, the wet or 'optimal moist' sieving techniques, at some level, may influence sensitive microbial activities. Due to the characteristically low soil moisture in our semi-arid grassland field study, the dry sieving technique has allowed us a unique opportunity to study the heterogeneous distribution of microbial activities among isolated soil aggregates without introducing the additional moisture complications associated with the other sieving techniques.

Our results are consistent with previous studies which found that soil aggregate size exerted strong impacts on soil C dynamics and microbial activity (Cheng et al., 2011; Dorodnikov et al., 2009b; Schutter and Dick, 2002). All enzyme activities related to C decomposition (BG, CB, XYL, and AG) increased as aggregate size decreased (Fig. 2a–d). Aggregates with higher OC showed higher activity of C decomposition enzymes (e.g. BG and XYL)

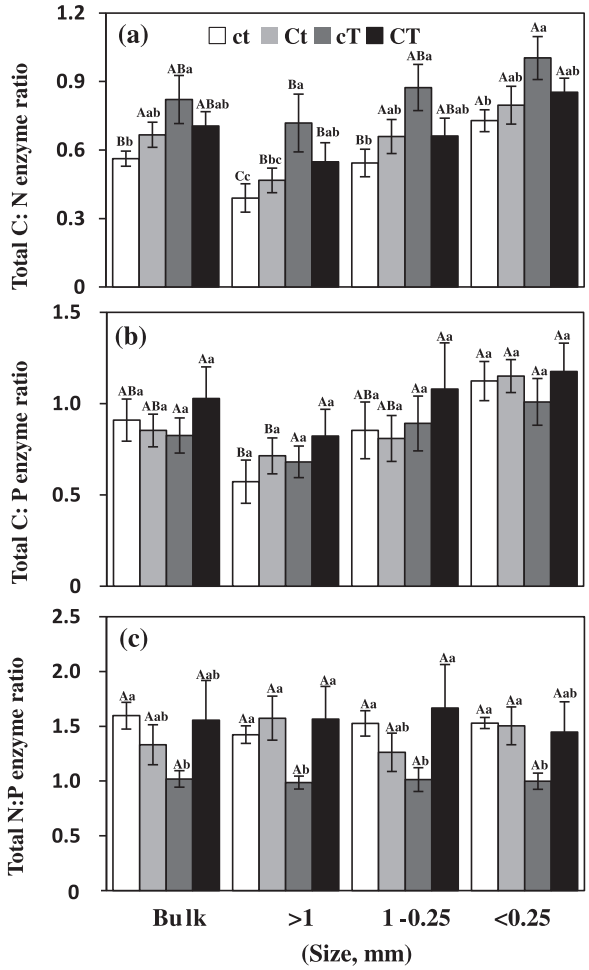

Fig. 3. Enzyme stoichiometry of C:N, C:P, and N:P. ct: ambient CO_2 and ambient temperature; Ct: elevated CO_2 and ambient temperature; cT: ambient CO_2 and elevated temperature; CT: elevated CO_2 and elevated temperature). Values are given as mean ± standard error ($n = 5$). Treatments followed by the same letters are not significantly different between aggregate size classes in the same climatic treatment (uppercase letters) and climatic treatments in the same class of aggregate size (lowercase letters) at $P < 0.05$.

(Lagomarsino et al., 2012). Similarly, enzyme activities for C decomposition in different aggregates were highly correlated to the OC concentration in an agro-ecosystem (Qin et al., 2010). In our study, this positive relationship was also found across all aggregates by Pearson correlation analysis ($R = 0.72$; $P < 0.0001$; Fig. S2), suggesting that increased enzyme activities related to C decomposition with decreasing aggregate size may be due to a higher OC concentration in microaggregates compared with macroaggregates. Likewise, N concentration across all aggregates was positively related to enzyme activities for N decomposition ($R = 0.46$; $P < 0.001$; Fig. S2). In the PHACE soil, therefore, SOM with high OC and N concentrations in microaggregates may have higher turnover than in macroaggregates, possibly offsetting the additional C input by roots in soils (Dorodnikov et al., 2009a).

Microbial decomposition efficiency in terms of SEA for C and N degrading enzyme activities appears to differ among soil aggregate size, and was affected by experimental climate change. For example, the SEA for C decomposition was significantly higher in the small macro- and microaggregates than large macroaggregates under Ct treatment (Fig. 4a), possibly because eCO_2-induced increases in rhizosphere effects by plant fine roots have a stronger influence on the small macro- and microaggregates than large macroaggregates (Dorodnikov et al., 2009b; Morgan et al., 2011; Nie et al., 2013a,b). We therefore provide additional evidence that the microbial ability to decompose SOM increases from macro- to microaggregates under Ct treatment (Cheng et al., 2011; Dorodnikov et al., 2009a). However, cT had no effects on the SEA for C decomposition among aggregates (Fig. 4a), possibly because temperature effects are relatively homogeneous among aggregates. Moreover, our findings demonstrate that the effects of CT on the SEA for C decomposition will produce positive feedbacks to climate change with non-additive effects, which did not differ among size classes of soil aggregates (Fig. 4a). In contrast, the SEA for N decomposition decreased with decreasing soil aggregate size under all climate treatments (Fig. 4b), such that enzyme C:N stoichiometry was lower in large macroaggregates than microaggregates (Table 2 and Fig. 3a), suggesting a greater demand for N by microbes in macroaggregates which may reflect the high (no statistical significance) OC/N ratio of these aggregates (Lagomarsino et al., 2012). Our results showed that enzyme C:N stoichiometry differs among soil aggregate size classes adding to a growing body of research suggesting that stoichiometry of soil enzyme activity can respond

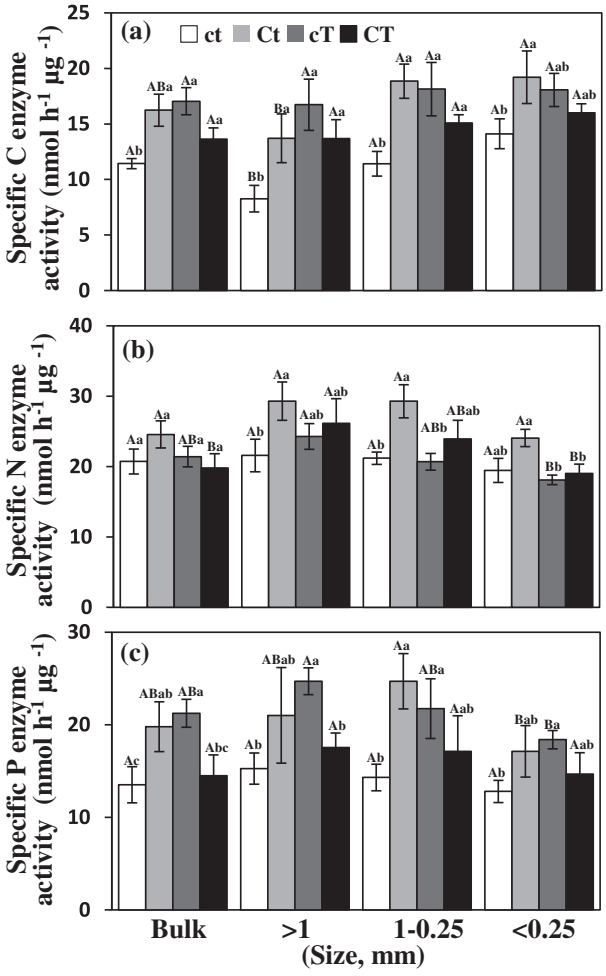

Fig. 4. Specific enzyme activities for C, N, and P decompositions (b). ct: ambient CO_2 and ambient temperature; Ct: elevated CO_2 and ambient temperature; cT: ambient CO_2 and elevated temperature; CT: elevated CO_2 and elevated temperature. Error bars show standard error of the mean ($n = 5$). Treatments followed by the same letters are not significantly different between aggregate size classes in the same climatic treatment (uppercase letters) and climatic treatments in the same class of aggregate size (lowercase letters) at $P < 0.05$.

to changes in the availability of C and N (Lagomarsino et al., 2012; Sinsabaugh et al., 2009, 2008).

Aggregate size did not interact with climate factors to affect microbial activity (Table 2), suggesting that soil physical structure largely determines the basic framework of microbial enzyme activity, enzyme stoichiometry, and specific enzyme activity. These microbial activities differ among soil aggregates, but total activities in bulk soil comprised of different aggregates may not change in response to climate change as a whole.

4.2. Elevated CO_2 effects

Ct had no statistically significant effects on microbial enzyme activities or their stoichiometry in the bulk soils (Figs. 2 and 3). While some studies found that rising atmospheric CO_2 concentration significantly increased microbial C and N decomposition enzyme activities (Carney et al., 2007; Phillips et al., 2011), other studies documented no significant difference of these enzyme activities (Austin et al., 2009; Dorodnikov et al., 2009b). Therefore, aggregate fractionation is a tool to reveal the effects of climate change on soil processes. We observed that Ct increased C decomposition enzyme activities in the large macroaggregates rather than smaller aggregates, possibly because enhanced photosynthetic C input to soil by Ct is mainly allocated to the large macroaggregates (Six et al., 2001). This observation may suggest decreasing microbial community C utilization efficiency (thus increasing soil losses via CO_2 respiration) under Ct conditions in this prairie ecosystem as has been suggested for other ecosystems (Dorodnikov et al., 2009a; Lipson et al., 2005). This result is also consistent with a previous study at this research site which demonstrated microbial respiration per unit of PLFA increased under Ct treatment (Nie et al., 2013b). In a sandstone grassland, Hu et al. (2001) found that Ct reduced microbial respiration per unit biomass because of eCO_2-induced progressive nitrogen limitation. However, our previous study suggests that Ct increased plant N uptake from soils, while concurrently stimulating microbial biomass N immobilization (Dijkstra et al., 2010). Furthermore, Ct consistently caused an increase in modeled and measured soil heterotrophic respiration in the PHACE experiment (Parton et al., 2007; Pendall et al., 2013). Consistent with this prediction, we observed that Ct had no effects on the ratio of total C:N enzyme activity (Fig. 3a), suggesting that Ct increased the N decomposition (especially within the larger and small macroaggregates) together with that of C. After 7-y eCO_2 fumigation at PHACE, therefore, progressive nitrogen limitation appears not to constrain soil microbial C decomposition activity under Ct treatment, as suggested in the Duke Forest (Phillips et al., 2011).

P availability is limited in this PHACE grassland, where nearly 60% of soil P is in organic form (Dijkstra et al., 2012). The eCO_2-induced increase in PHOS that we observed could ameliorate soil P availability to plants and microorganisms, possibly because eCO_2-induced increases in plant root growth and root exudation can mobilize P (Dijkstra et al., 2010; Morgan et al., 2011; Phillips et al., 2006). In addition, the ratio of total N:P enzyme activity was decreased by Ct treatment (Table 2) supporting our previous results that Ct increased P availability to plants and microbes relative to that of N (Dijkstra et al., 2012).

4.3. Warming effects

cT enhanced the potential for soil C decomposition by microbes, as suggested by the SEA of C-degrading enzymes (Fig. 4). This supports the modeling prediction by Parton et al. (2007) that soil heterotrophic respiration rate would increase under cT treatment during most years of a 10-yr simulation period for the PHACE experiment. However, it contradicts with field measurements of slightly reduced heterotrophic respiration (Pendall et al., 2013). This difference could be explained by frequent moisture limitation *in situ*, which may decrease enzyme activities in the field soils. However, we measured potential enzyme activities under optimal moisture and temperature conditions in this study.

Increased N availability can result in increased C utilization efficiency of some microbial groups in a forest ecosystem (Ziegler and Billings, 2011), and promote enzyme activity for C decomposition relative to N in some grasslands (Stursova et al., 2006; Tiemann and Billings, 2011). In the PHACE experiment, cT was shown to significantly increase both soil inorganic and organic N availabilities (Carrillo et al., 2012; Dijkstra et al., 2010). Thus, warming-induced increase in soil N availability may lead to increased C decomposition relative to that of N under cT treatment (Fig. 3a). In addition, increased soil N availability could shift microbial enzyme activity away from N acquisition and toward P acquisition (Sinsabaugh et al., 2002), possibly resulting in narrower N:P enzyme activity ratios (across all aggregates) under cT treatment (Fig. 3c). It should be noted that changes in N availability due to cT treatment occurred without N fertilization in the PHACE experiment. cT exerts negative impacts on plant biomass, especially fine root biomass (Carrillo et al., 2011; Morgan et al., 2011), which may result in less plant N uptake. Moreover, soil water availability is a strongly limiting factor for biological activity, and cT decreased soil water content in the semiarid grassland (Dijkstra et al., 2010; Morgan et al., 2011). Previous findings showed that soil nitrogen transformation could either decrease or increase under cT treatment depending on yearly differences in water stress (Dijkstra et al., 2010; Parton et al., 2007). Many studies have shown that microbial N acquisition activity is more sensitive to water deficit than that of C (Geisseler et al., 2011; Pascual et al., 2007; Zelikova et al., 2012), which could result in wider C:N enzyme activity ratios under cT treatment (Fig. 3a).

4.4. Elevated CO_2 × warming effects

As expected, eCO_2 × warming (CT) interacted to increase the SEA for C decomposition (Table 2 and Fig. 4a), which could potentially create a positive climate feedback from soil C losses (Pendall et al., 2013). CT also had no significant effects on the ratio of total C:P enzyme activity (Fig. 3b), and SEA for N decomposition (Fig. 4b). However, CT elicited non-additive effects on PHOS (Fig. 2g), SEA for P decomposition, and total N and P enzyme activities (Fig. 4c). Similarly plants do not exhibit differences in N:P ratios under CT treatment at the PHACE site (Dijkstra et al., 2012) or in other studies (Menge and Field, 2007). Regardless, we suggest that over time, plant and microbial P demands will likely shift under persistently warmer and drier conditions (Dijkstra et al., 2012).

5. Conclusions

Our results from a long-term field manipulation provide evidence that soil aggregate size independently mediates soil microbial feedbacks to multiple climate change factors. Altered microbial enzyme activities, enzyme stoichiometry, and specific enzyme activities under climate change were mainly consistent across soil aggregate size classes. An exception was that C degrading enzyme activities were greatest where C concentrations were highest, namely in the microaggregates. Moreover, climate change increased SEA for C decomposition, suggesting positive feedbacks between microbial activities related to SOM decomposition and climate change. The distribution of aggregates within soils is affected by both physical and biological processes, and therefore not only affects microbial function, but is affected by it. It is

challenging to disentangle the interactions between microbes and aggregates. Given the important role of soil aggregate distribution in determining microbial feedbacks to climate change, there is a clear need to enhance our understanding of the processes controlling soil aggregate dynamics.

Acknowledgments

We thank Prof. Karl Ritz and anonymous referees for their constructive comments on the early version of this manuscript, which greatly improved the quality of our article. We also thank Dan LeCain for overall project management and Jack Morgan for leading the PHACE experiment. This material is based upon work supported by the US Department of Agriculture, US Department of Energy's Office of Science (BER), through the Terrestrial Ecosystem Science program, and by the National Science Foundation (DEB# 1021559). Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2013.10.012.

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