LETTER

Positive climate feedbacks of soil microbial communities in a semi-arid grassland

Ming Nie,[1]* Elise Pendall,[1] Colin Bell,[2] Caley K. Gasch,[3] Swastika Raut,[1] Shanker Tamang[1] and Matthew D. Wallenstein[2]

Abstract

Soil microbial communities may be able to rapidly respond to changing environments in ways that change community structure and functioning, which could affect climate–carbon feedbacks. However, detecting microbial feedbacks to elevated CO_2 (eCO_2) or warming is hampered by concurrent changes in substrate availability and plant responses. Whether microbial communities can persistently feed back to climate change is still unknown. We overcame this problem by collecting microbial inocula at subfreezing conditions under eCO_2 and warming treatments in a semi-arid grassland field experiment. The inoculant was incubated in a sterilised soil medium at constant conditions for 30 days. Microbes from eCO_2 exhibited an increased ability to decompose soil organic matter (SOM) compared with those from ambient CO_2 plots, and microbes from warmed plots exhibited increased thermal sensitivity for respiration. Microbes from the combined eCO_2 and warming plots had consistently enhanced microbial decomposition activity and thermal sensitivity. These persistent positive feedbacks of soil microbial communities to eCO_2 and warming may therefore stimulate soil C loss.

Keywords

Climate change, climate–carbon feedback, common garden experiment, elevated CO_2, enzyme stoichiometry, microbial community, soil decomposition, temperature sensitivity.

INTRODUCTION

Feedbacks between terrestrial C cycling and climate change determine the capacity for terrestrial ecosystem C storage (Pendall *et al.* 2004; Luo 2007). For example, warming can stimulate microbial decomposition of litter and SOM, which accounts for two-thirds of soil C losses in terrestrial ecosystems (Luo & Zhou 2006). However, microorganisms may be able to rapidly respond to changing environments in ways that alter community structure and functioning, which could affect climate–carbon feedbacks (Luo 2007; Bardgett *et al.* 2008; Wallenstein & Hall 2012). Numerous studies have reported that climate change significantly affects microbial composition and biomass (Carney *et al.* 2007; He *et al.* 2010), enzyme activities (Carney *et al.* 2007; Zhou *et al.* 2012) and physiological profiles (Zhou *et al.* 2012). Nevertheless, our understanding of microbial feedbacks to climate change is still limited, especially for combined effects of eCO_2 and warming, despite their importance for soil C dynamics (Bardgett *et al.* 2008; Dieleman *et al.* 2012).

Microbial mediation of soil C cycling can be affected by climate change primarily through two mechanisms. First, heterotrophic microbial metabolism is regulated by the quantity and quality of substrates, which can be affected by climate change (Hu *et al.* 2001; He *et al.* 2010). eCO_2 often results in increased labile C inputs to soils through plant root exudation and litter production (Phillips *et al.* 2011). Because of diverse plant species and soil properties, microbial metabolism responses to increased substrate availability at eCO_2 have been observed to exhibit highly variable patterns, ranging from positive (Carney *et al.* 2007), neutral (Zak *et al.* 2000; Austin *et al.* 2009) to negative feedbacks (Hu *et al.* 2001). Increased labile C inputs resulting from eCO_2 can also stimulate SOM decomposition in a process known as priming (Kuzyakov *et al.* 2000). For example, eCO_2 increased the relative abundances of fungi and soil enzyme activity in a scrub–oak ecosystem, and the stimulation of SOM decomposition offset more than 50% of the stimulation in plant belowground productivity, leading to a net decrease in soil C (Carney *et al.* 2007). However, in other ecosystems belowground C storage was enhanced by eCO_2 (Jastrow *et al.* 2000).

Second, warming may induce changes in the temperature sensitivity of microbially mediated processes (Wallenstein & Hall 2012). Warming typically accelerates soil microbial respiration rates due to increased soil enzyme activities, which drive decomposition (Wallenstein *et al.* 2009, 2011). Primarily as a result of this response, a 2 °C global average temperature increase is predicted to stimulate soil C loss by 10 Pg year^{-1} (Pendall *et al.* 2004). However, this stimulation of soil C loss could be entirely mitigated by a decline in the temperature sensitivity of microbial activity (Allison *et al.* 2010). For example, one field experiment showed that temperature sensitivity of soil respiration decreased at high temperatures, possibly reducing the potential for C losses in tallgrass prairie (Luo *et al.* 2001). Similarly, microbial temperature sensitivity for respiration decreased in response to long-term soil warming in a hardwood forest, even when incubated under non-

[1]*Department of Botany and Program in Ecology, University of Wyoming, Laramie, WY, USA*

[2]*Natural Resource Ecology Laboratory, Colorado State University, Fort Collins, CO, USA*

[3]*Department of Ecosystem Science and Management and Program in Ecology, University of Wyoming, Laramie, WY, USA*

Correspondence: E-mail: mnie@uwyo.edu

limiting substrate conditions (Bradford *et al.* 2008). By removing plant-mediated labile C availability in Arctic soil, Hartley *et al.* (2008) used experimental cooling to avoid the confounding factor of warming-induced substrate depletion and reported no declines in microbial community temperature sensitivity. Microbial communities have also been observed to respond to variable precipitation regimes (Evans & Wallenstein 2012) and redox fluctuations (DeAngelis *et al.* 2010), suggesting other environmental drivers which can initiate microbial feedbacks besides those associated with atmospheric CO_2.

Another challenge in assessing microbial feedbacks to climate change is that responses may vary seasonally or interannually. For example, SOM decomposition driven by microbial activity can shift in response to temporal precipitation patterns (Parton *et al.* 2007; Carrillo *et al.* 2011). The inherent complexity and diversity of microbial communities and the many ways that they can be affected by climate change and other environmental factors hamper our ability to understand microbial mediation of soil C cycling (Bardgett *et al.* 2008). Determining microbial feedbacks to climate change requires the separation of such changes from substrate availability and other environmental factors (Bradford *et al.* 2008; Wallenstein & Hall 2012). Soil microorganisms can shift seasonally from dormant states in frozen soils to a resuscitated state with high activity during the plant growing season (Panikov 2009; Lennon & Jones 2011). For microbial feedbacks to climate change to be important for ecosystem response, they should be persistent. For example, dormant microorganisms which can recover from winter and perform ecosystem processes during the plant growing season may represent a persistent change in soil microbial activity in response to the previous years' climate conditions (Panikov 2009; Lennon & Jones 2011).

Here, we collected soils in cold winter conditions from the long-term Prairie Heating and CO_2 Enrichment (PHACE) experiment to use as dormant microbial inocula in a sterile-soil incubation experiment. This study site is unique because it is one of only a few field studies in the world where both atmospheric CO_2 and temperature are manipulated. We designed this experiment to address whether any persistent soil microbial feedbacks exist in response to long-term continuous climate change treatments. Our goal was to focus on changes in microbial community structure and functioning by excluding differences in plant activity, SOM properties and other environmental factors by collecting off-plot soil as the substrate for an incubation experiment. We measured differences in SOM decomposition associated with C turnover and microbial temperature sensitivity (Q_{10}) by assessing thermal responses for SOM decomposition. Our previous work suggests that eCO_2 and combined eCO_2 and warming gradually decrease soil organic C pool relative to expectations from high plant belowground biomass and C inputs (Parton *et al.* 2007; Morgan *et al.* 2011; Carrillo *et al.* 2012), and warming could increase soil respiration and decrease SOM concentrations over years (Parton *et al.* 2007; Carrillo *et al.* 2011). Therefore, we hypothesised that (1) soil microbial communities exposed to eCO_2 exhibit increased soil microbial respiration rates as a result of increased microbial enzyme activity and subsequent SOM decomposition, (2) soil microbial communities exposed to warming increase their metabolic temperature sensitivity and (3) the combined effects of eCO_2 and warming on microbial activities will produce positive feedbacks with possible additive or synergistic effects.

MATERIALS AND METHODS

Experimental design

Soil microbial inocula were prepared from the PHACE experiment, which 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 PHACE ecosystem is a northern mixed-grass prairie dominated by C_4 grass *Bouteloua gracilis* (H.B.K) Lag and C_3 grass *Pascopyrum smithii* (Rydb.). Mean annual precipitation (2006–2011) was 352.5 ± 32.6 mm (mean ± standard error) and mean air temperature (2006–2011) was 20.2 ± 0.6 °C in July and −2.9 ± 1.0 °C in January. The soil is a fine-loamy, mixed, mesic Aridic Argiustoll with pH of 7.9 (Dijkstra *et al.* 2010; Morgan *et al.* 2011). 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). Warming is induced using infrared heaters placed above the plant canopy (Dijkstra *et al.* 2010; Morgan *et al.* 2011).

Three 5-cm-depth soil cores were collected from each treatment plot on Dec 13 2011 using a 2.5-cm-diameter auger and then mixed. In the field, soil temperatures were monitored to assess when they reached ≤ 0 °C before sampling (which occurred on Dec 3 2011; Figure S1). PHACE soils were handpicked to remove rocks and plant residual roots. Twenty soil–water slurry subsamples for each experimental plot were prepared using 20 g soil and 20 mL sterile DI water (van de Voorde *et al.* 2012). Off-plot soil was collected from 0- to 5-cm depth near the PHACE plots to use as a common growth substrate for the incubation. Off-plot soil was passed through a 2-mm sieve to remove stones and plant residues, and then homogenised to best produce an unbiased composite sample. After sterilisation by autoclaving (121 °C, 45 min) twice in succession and again 24 h later, the off-plot soil was again homogenised and then pre-incubated at room temperature for a week in sterilised jars with frequent ventilation by 0.45-μm filtered CO_2-free air. We measured respiration rates in the soil to evaluate sterilisation efficiency prior to inoculating the microbial inocula. A very low amount of activity (*c.* 0.2 μg C g^{-1} day^{-1}) was detected among all jars, which could be due to abiotic CO_2 production, extracellular enzyme activities or remnant microbial populations. However, this remnant CO_2 flux contributed only *c.* 1.2% of the respiration in inoculated samples. Each soil slurry (i.e. soil community) was inoculated by adding 1.5 mL of soil slurry solution to each specimen cup containing 30 g sterile off-plot soil (*c.* 60% of WHC) in a 500-mL incubation jar. The 1.5 mL of inoculum contained < 5.7 μg C g^{-1}, compared with 23.6 mg C g^{-1} in soil originally present.

Each of the 20 PHACE soil communities were inoculated into four replicate specimen cups for two different incubation experiments: (1) Microbial community C dynamics: three jars were incubated at 25 °C for 30 days. Headspace gas samples (30 mL) of one jar were collected at 1, 2, 3, 5, 7, 10, 14, 20 and 30 days of incubation for measuring microbial respiration, and the other two jars were harvested and combined after 10 days of incubation for measuring microbial community structure and functioning. This was jus-

tified because microbial respiration rates on day 10 fell between the stages of slow and rapid respiration decay (Figure S2); thus incubation samples on day 10 captured the major responses to the treatments while avoiding longer term feedbacks that may have occurred and (2) Temperature sensitivity: sample jars were incubated at 35 °C and 25 °C for 30 days and measured on the same days as in Experiment 1 for microbial respiration to evaluate Q_{10} of microbial SOM decomposition. We chose the 25 °C incubation temperature based on the average soil ambient temperature during the main period of vegetative growth from June to August (22.9 ± 0.6 °C); and 35 °C based on the highest daytime temperature during this stage. All jars were thoroughly flushed with ambient air immediately after gas sampling. Both incubation experiments had five laboratory replicates, following the PHACE field replication. Uninoculated blank jars (i.e. additions of 1.5 mL sterile DI water to sterile soil) were incubated under the same conditions for each experiment.

Respiration

On each sampling date, a headspace sample of 30 mL was collected by a syringe. Gas samples were directly analysed for CO_2 concentration using an infrared gas analyser (Li-Cor 820; LICOR Inc., Lincoln, NE, USA) calibrated with standard gases (Carrillo *et al.* 2011). Temperature sensitivity (Q_{10}) of SOM decomposition was determined using eqn 1:

$$Q_{10} = (R_2/R_1)^{(10/\Delta T)} \qquad (1)$$

where R_2 and R_1 are the mean respiration rates at 35 °C and 25 °C within 30 days of incubation (Balser & Wixon 2009) respectively. Thus, ΔT (the difference in incubation temperatures) is 10 °C in this study.

Physiological profiles

Physiological profiles of microbial communities were assessed on day 10 using the MicroResp system (Macaulay Institute, Aberdeen, UK), which allows substrate-induced CO_2 measurements on whole soil rather than dilutions in microbial growth media. A total of 15 C sources were chosen to cover a range of rhizodeposits, including sugars, amino acids and carboxylic acids (Figure S3). Each well of a 96-well deep-well microplate was inoculated with 300 mg of the soil and a solution of pre-dispensed C sources (Campbell *et al.* 2003). The deep-well plate was then immediately sealed with the gasket and a detection plate by a metal clamp. Colour development on the detection plate was measured immediately before and after 6 h of incubation at 25 °C using a Biotek microplate spectrophotometer (BioTek Instruments Inc., Winooski, VT, USA) at 590 nm, and converted to CO_2 evolution based on a standard calibration curve prepared with standard gas mixtures (Campbell *et al.* 2003).

Enzyme assays

On day 10, activities of seven extracellular enzymes [β-Glucosidase (BG; EC: 3.2.1.21), β-D-Cellubiosidase (CB; EC: 3.2.1.91), N-acetyl-β-Glucosaminidase (NAG; EC: 3.2.1.14), Phosphatase (PHOS; EC: 3.1.3.1), β-Xylosidase (XYL; EC: 3.2.1.37), α-Glucosidase (AG; EC: 3.2.1.20) and leucine amino peptidase (LAP; EC: 3.4.11.1)] 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.* 2009). All the enzyme assays were set up in 96-well microplates. Briefly, 2.75 g of fresh soil was added into blender, and homogenised with 91 mL of 50 mM acetate buffer. 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 mL 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. 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.

Microbial community structure

Microbial community structure was assessed by analysing the composition of extractable ester-linked phospholipid fatty acids (PLFAs) on day 10 of the incubation. Lipids were extracted from 5 g of lyophilised 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, USA). The phospholipids were subjected to mild-alkaline methanolysis, dissolved in chloroform and purified using 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 using 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, USA). The dominant 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.

Statistical analyses

To determine the effects of eCO$_2$ and warming on cumulative microbial respiration, physiological profiles, enzyme activities and community structure, we used a two-way ANOVA with eCO$_2$ and warming as fixed effects using SPSS 13.0 (SPSS Inc., Chicago, IL, USA). Data not meeting assumptions of normality and homogeneity of variance were log-transformed before statistical testing. Power regression and Pearson correlation analysis were performed to evaluate relationships underlying microbial processes using SPSS. Power regression ($y = ax^b$) was used to fit cumulative respiration (y) against incubation day (x). In the power-regression equation, the coefficient value (a) and exponent value (b) stand for initial respiration efflux and cumulative respiration efficiency respectively. Canonical correspondence analysis (CCA) was performed to determine how microbial community structure was related to microbial function variables using Canoco v4.5 (Microcomputer Power, NY, USA). Overall CCA plot and their axes were significantly constructed (all $P < 0.05$) by a Monte Carlo permutation test in Canoco. Permutational multivariate analysis of variance (PERMANOVA) was used to test whether eCO$_2$ and warming affected overall community-level
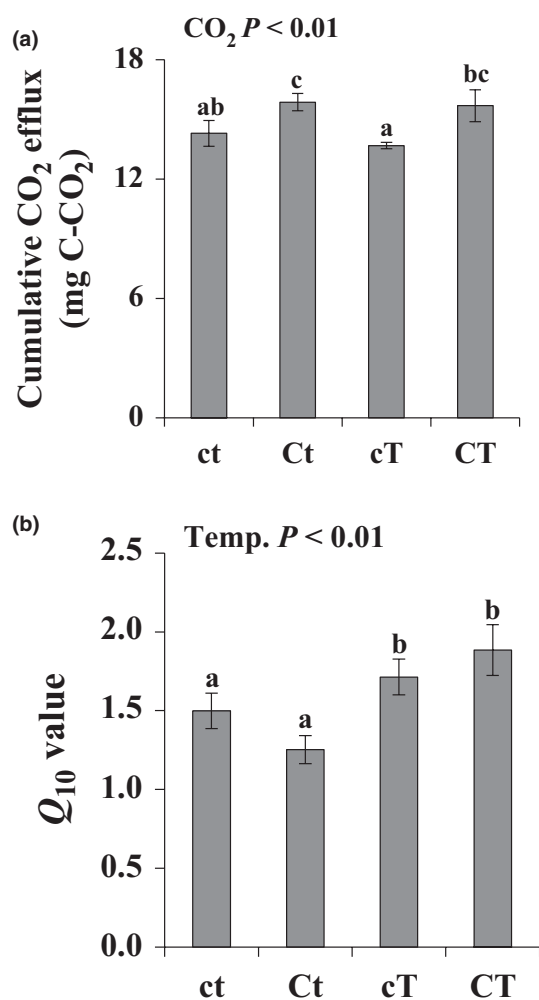
Figure 1 Cumulative respiration by microbial communities for the entire jars after 30-days incubation at 25 °C (a), and temperature sensitivity (Q_{10}) of decomposition (b). Cumulative CO_2 effluxes were adjusted by the organic C in the 1.5 mL inoculants ($C_{cumulative\ efflux} - C_{inoculant}$). ct: 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 ($n = 5$). The same letters denote non-significant differences between treatments ($P > 0.05$).

physiological profiles using PRIMER v6.1 (PRIMER-E Ltd., Plymouth, UK). The Shannon–Weaver index (H') and evenness index (J) were used to determine whether eCO_2 and warming affected

physiological specificity for different C sources based on MicroResp: $H' = -\Sigma\ Pi\ (\ln\ Pi)$, where Pi is the ratio of the activity of a particular substrate and the sum of activities of all substrates; $J = H'/H'_{max}$, where H'_{max} is the maximum level of physiological diversity (Zak *et al.* 1994).

RESULTS

Respiration

After 30 days of incubation at 25 °C, microbes inoculated from eCO_2 plots had significantly greater cumulative CO_2 efflux ($F_{1,\ 19} = 9.92$; $P < 0.01$; Fig. 1a) than communities from ambient plots when averaged across warming treatments, whereas warming treatment had no effect on cumulative CO_2 efflux when averaged across CO_2 treatment ($F_{1,\ 19} = 0.52$; $P > 0.05$; Fig. 1a). The cumulative CO_2 efflux from CT plots marginally increased compared with those from ct plots ($P = 0.10$; Fig. 1a). The exponent values (b) from power regressions were significantly higher in soils inoculated from Ct and CT than those from ct plots (all $P < 0.05$; Table 1), suggesting that microbial communities from Ct and CT plots were able to decompose the standardised SOM more rapidly than communities from ct plots. Soils inoculated with microbes from warmed
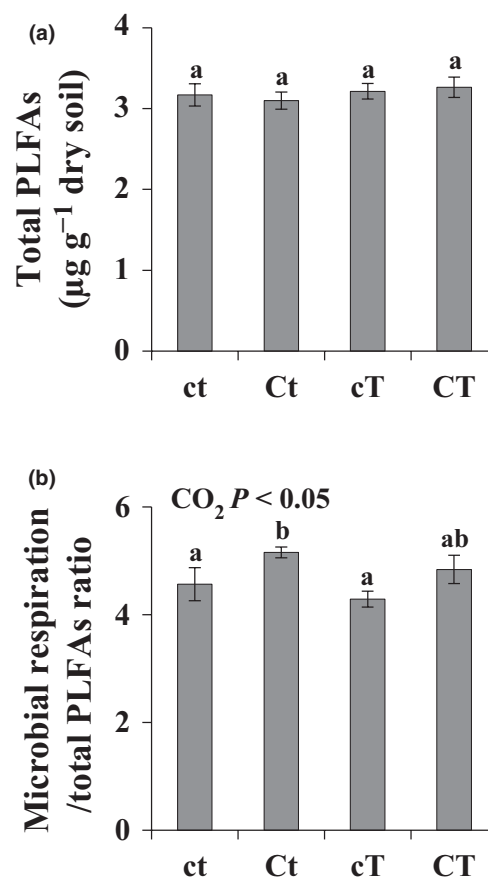

Figure 2 Total PLFAs for soil microbial biomass (a), and microbial respiration rate on a per-unit-PLFA basis (b). ct: ambient CO_2 and ambient temperature; Ct: elevated CO_2 and ambient temperature; cT: ambient CO_2 and elevated temperature; CT: elevated CO_2 and elevated temperature. Error bars show standard error of the mean ($n = 5$). The same letters denote non-significant differences between treatments ($P > 0.05$).
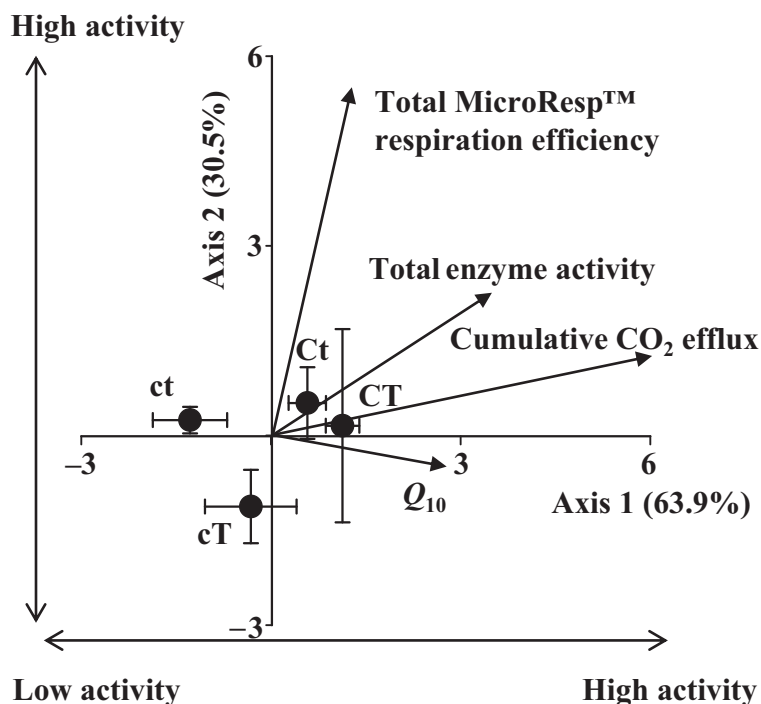
Table 1 Power-regression coefficients ($Y = aX^b$) of cumulative respiration after 30 days incubation at 25 °C

Treatment	a	b	Curve-Fitting P
ct	2.22 ± 0.17 a	0.57 ± 0.02 a	all <0.0001
Ct	1.94 ± 0.16 a	0.64 ± 0.02 b	all <0.0001
cT	1.86 ± 0.12 a	0.60 ± 0.02 ab	all <0.0001
CT	1.91 ± 0.15 a	0.63 ± 0.01 b	all <0.0001

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 ($n = 5$). Values in each column followed by the same letter are not significantly different at $P < 0.05$.

Figure 3 Canonical correspondence analysis (CCA) ordination of microbial communities based on PLFAs. Arrow length indicates the importance of each microbial function variables and their relationships with microbial communities. The community-function correlations were 0.73 and 0.53 for axis 1 and axis 2 respectively. 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. The data are means ± standard errors ($n = 5$).

plots had a greater Q_{10} of respiration measured in laboratory incubations than those inoculated from unwarmed plots ($F_{1, 19} = 10.32$; $P < 0.01$; Fig. 1b).

Physiological profiles

Treatments did not change the overall substrate use by PERMA-NOVA (all $P > 0.05$). Microbial physiological diversity (H') and evenness (J) were also not changed by any treatment (all $P > 0.05$; Table S1). However, microbes inoculated from eCO_2 plots were significantly increased by 43.8% in total substrate respiration rate from Ct than those from ct plots ($P < 0.05$; Figure S3).

Enzyme assays

eCO_2 alone significantly increased total enzyme activity, CB, NAG and PHOS; warming alone significantly increased PHOS; and combined eCO_2 and warming significantly increased total enzyme activity and NAG (Table S2; Figure S4). Cumulative CO_2 efflux after 30 days of incubation at 25 °C significantly increased with increasing NAG ($r = 0.45$; $P < 0.05$), PHOS ($r = 0.39$; $P < 0.05$) and total of seven enzyme activities ($r = 0.40$; $P < 0.05$) across the treatments.

Microbial community structure

Although total PLFA biomass of inoculated soils was not changed by any treatments (all $P > 0.05$; Fig. 2a), eCO_2 significantly increased microbial respiration per unit of PLFAs ($F_{1, 19} = 6.69$; $P < 0.05$; Fig. 2b), which was calculated from total PLFA biomass on day 10 and cumulative CO_2 efflux after 30 days of incubation.

Microbial communities represented by PLFA's were separated by treatment in canonical correlation analysis (Fig. 3). Axis 1 and axis 2 can be interpreted as the microbial activity gradient because of their positive correlations with measured microbial functions (Fig. 3; Table S3). Along axis 1 and axis 2, microbial communities from Ct and CT plots appeared to have higher activity than those from ct and cT plots (Fig. 3). Moreover, eCO_2 alone significantly decreased 16:1ω9c (Gram-negative bacteria), warming alone significantly increased a17:0 (Gram-positive bacteria), 18:1ω9c (Gram-negative bacteria), cy19:0 (Gram-negative bacteria) and 18:2ω6c (saprotrophic fungi) and combined eCO_2 and warming significantly increased 18:2ω6c (Figure S5; Table S4).

DISCUSSION

Our findings add to a growing body of evidence that microbial communities respond to climate change through functional changes that affect the rates of ecosystem processes (Bardgett *et al.* 2008; Wallenstein & Hall 2012). Most previous studies of microbial responses to climate change were performed using individual climate factor studies; for example eCO_2 (e.g. Hu *et al.* 2001; Carney *et al.* 2007; He *et al.* 2010; Phillips *et al.* 2011) or warming (e.g. Frey *et al.* 2008; Hartley *et al.* 2008; Bradford *et al.* 2010; Zhou *et al.* 2012). The multiple interacting effects of eCO_2 and warming on soil microbial communities and their feedbacks to climate change are not well known (Bardgett *et al.* 2008; Dieleman *et al.* 2012). In part, our current lack of understanding is hampered by concurrent climate interactions with changes in substrate availability and/or plant responses (Bardgett *et al.* 2008). In this study, we were able to test for potential microbial feedbacks under eCO_2 and warming while controlling for confounding effects of

soil substrate heterogeneity and root activity (van de Voorde *et al.* 2012). Our results demonstrated positive feedbacks of soil microbial communities to eCO_2 and warming, which may accelerate soil C losses under future climate conditions. These results can explain, at least partially, the lower rates of soil C accumulation relative to expectations from high plant C inputs to soils or even a decline in the soil C pool under climate treatments in our field observations and model predictions (Parton *et al.* 2007; Carrillo *et al.* 2011, 2012; Morgan *et al.* 2011).

Increased microbial activities at eCO_2

Results from this experiment are consistent with model predictions of increased soil respiration under eCO_2 at the PHACE experiment (Parton *et al.* 2007), but suggest that these effects can be attributed to microbial responses that are independent of changes in substrate availability. eCO_2 has a significant influence on plant biomass accumulation, and promotes increased C allocation to fine roots at the PHACE experimental site (Morgan *et al.* 2011). Greater plant belowground C allocation to soil through root exudation and root litter would increase labile C availability and thus increase microbial biomass and activity in the PHACE soils (Dijkstra *et al.* 2010; Carrillo *et al.* 2011, 2012). Previous studies suggest that eCO_2 could increase the diversity of microbial genes related to decomposition of C-rich substrates, such as cellulose (He *et al.* 2010; Weber *et al.* 2011). Increased cumulative CO_2 efflux in laboratory incubations from Ct and CT plots (Fig. 1a; Table 1), despite similar microbial biomass, suggests that these eCO_2 observations represent greater microbial community metabolism, indicating community-level feedbacks to eCO_2.

In our experiment, autoclaving may have increased the pool of labile C through the production of solubilised substrates (Trevors 1996), and thus overestimated potential SOM decomposition rates. However, our observations followed a similar temporal pattern to direct laboratory incubations of PHACE field soils under the same incubation conditions, suggesting that the contribution of these solubilised substrates to respiration was transient (Figure S2) (Carrillo *et al.* 2010). The average range of cumulative CO_2 efflux from the inoculated soils was about half of that from PHACE soils (Carrillo *et al.* 2010). This difference is probably explained by lower microbial biomass in inoculated soils than field soils.

We suggest that the mechanism accounting for increased microbial respiration by communities exposed to eCO_2 was increased respiration per unit biomass (Hu *et al.* 2001); because we observed enhanced eCO_2 respiration on a per-unit-PLFA basis with no change in total PLFA biomass (Fig. 2). Thus, these findings suggest that eCO_2 may decrease microbial C utilisation efficiency and increase respiration loss in this prairie ecosystem. Our study demonstrates respiration per unit biomass in microcosms. Further assessment is needed to extend these findings to a field experiment.

Microbial taxa are known to specialise in the degradation and metabolism of specific substrates, and substrate availability may drive community composition through species sorting (Hanson *et al.* 2008). Thus, microbial communities have the potential to alter respiration rates for specific C sources in response to altered soil C availability under climate change. We observed greater CO_2 emission rates by Ct communities compared with the other treatment communities for 15 of the substrates assessed (Figure S3). However, soil microbial communities within the cT plots did not show a higher ability to decompose C than those from Ct plots based on

CO_2 respiration rates. This suggests that microbial communities in the eCO_2 plots have a higher capacity to decompose SOM than microbial communities from warming plots, possibly because eCO_2 induced microbial communities to prefer the environment of high quantity of plant-derived C inputs with low substrate quality (high C : N) (van Veen *et al.* 1991). We also observed that experimental climate change did not alter microbial community-level C substrate specificity, as no differences in microbial physiological diversity (H') or evenness (J) were observed among treatment plots (Table S1). This suggests that the differences in physiological profiles between microbial communities were mainly due to community-level changes in respiration efficiencies rather than their specificities for different C sources. Previous studies suggested that microbial functional genes involved in labile C decomposition and enzyme activities were increased by eCO_2 (Carney *et al.* 2007; He *et al.* 2010). Therefore, increased community-level decomposition rates may be due to elevated microbial metabolic activity occurring within the eCO_2 plots.

The extracellular enzyme data showed that the microbial communities from Ct and CT plots had higher enzyme activities pertinent to SOM decomposition, including CB, NAG, PHOS and AG, than ct and cT communities (Figure S4). Our results are consistent with previous studies which found that eCO_2 stimulated soil enzyme activities and thus accelerated SOM decomposition (Carney *et al.* 2007; Phillips *et al.* 2011). Out of the seven enzyme activities measured, PHOS had the highest activity across all treatments (Figure S4), probably due to P limitation in this prairie ecosystem (Dijkstra *et al.* 2012). As an indicator of potential N : P acquisition activity (Sinsabaugh *et al.* 2008), the ratios of (NAG + LAP)/(PHOS) were lower in microbial communities from Ct ($P < 0.05$) and CT ($P = 0.06$) plots compared with ct communities, suggesting microbial N/P acquisition activities could be mediated by eCO_2. Our previous study found that soil P availability increased relative to N in eCO_2 (Dijkstra *et al.* 2012), which could be due to deceased ratios of (NAG + LAP)/(PHOS).

Increased microbial temperature sensitivity under warming

We compared the abilities of microbial communities to decompose SOM at two different incubation temperatures. The highest Q_{10} was observed in soil inoculums from the increased temperature plots (cT and CT plots) (Fig. 1b). This implies that warming can amplify the microbial community's temperature sensitivity, which could result in a further increase in microbial respiration with rising temperature. Warming could increase C-decomposition genes, such as cellulose- and chitin-degradation genes (Yergeau *et al.* 2012; Zhou *et al.* 2012), which may account for a high temperature sensitivity of microbial respiration. The current study measured the temperature sensitivity of microbial respiration rates based on the data taken at 25 °C and 35 °C. The temperature sensitivity of microbial respiration rates at lower temperature range may have to be further studied.

Previous studies showed that microbial temperature sensitivity had a negative response to warming (Luo *et al.* 2001; Bradford *et al.* 2008). However, this phenomenon is difficult to parse from changes in substrate availability in field studies or laboratory incubations. For example, Belay-Tedla *et al.* (2009) demonstrated that warming significantly increased labile C/N fractions through plant biomass input, which increased soil microbial biomass. In our study, we were able to avoid influences from soil initial heterogeneity (e.g. initial substrate availability) and root activity. In laboratory incubations, sub-

strates become depleted faster at higher temperatures, which may lead to an apparent decline in temperature sensitivity (Bradford *et al.* 2010; Tucker *et al.* 2012). Therefore, we compared microbial respiration during the initial 1 or 2 days to calculate Q_{10}, to test whether microbial temperature sensitivity changed with minimum variation in substrate availability during incubation (Steinweg *et al.* 2008). Likewise, we found that microbes inoculated from warmed plots exhibited a significantly greater Q_{10} of respiration measured within initial 1–2 days than those from unwarmed plots (all $P < 0.05$; Figure S6). We determined that microbial temperature sensitivity was not affected by incubation duration (which may have affected substrate availability) (three-factor ANOVA, CO_2 and warming as fixed effects and incubation day as random effect; all $P > 0.05$; Figure S6). In fact, warming ($F_{1, 159} = 106.49$; $P < 0.0001$; Figure S6) and combined CO_2 and warming effect ($F_{1, 159} = 6.54$; $P < 0.05$; Figure S6) significantly increased microbial temperature sensitivity during all 30 days of incubation. These results suggest that warming increases microbial temperature sensitivity regardless of changes in substrate availability during the incubation.

Increased temperature sensitivity in response to warming suggests that there are little to no trade-offs associated with community-physiological constraints under changing temperature (Allison *et al.* 2010). If trade-offs occur, microbial respiration rates will negatively respond to a warmer environment (Allison *et al.* 2010). Although warming may decrease microbial respiration due to substrate depletion, microbial baseline respiration can be stimulated by a high temperature under substrate saturation (Tucker *et al.* 2012). Similar to our observations, Hartley *et al.* (2007) demonstrated that microbial respiration from warmed plots was higher than those from control plots, when measurements were made at a common temperature; and any positive microbial response to warming can be maintained through cold winter season. Balser & Wixon (2009) found that the temperature sensitivity of microbial respiration increased with site mean annual temperature in a latitudinal gradient study. Our study demonstrates temperature sensitivity between 25 and 35 °C. Further assessment is needed to extend these findings to a wider range of temperatures.

Combined effects of eCO$_2$ and warming

A positive effect of combined eCO_2 and warming on cumulative respiration efficiency was observed in this study (Table 1). The combined mechanisms of increased respiration efficiency with eCO_2 and increased Q_{10} with warming may potentially interact to amplify individual effects on soil communities and their feedback to climate change. In addition, the CT microbial communities had the highest Q_{10} among all treatments (Fig. 1b). It seems that combined effects of eCO_2 and warming on microbial activities will produce positive feedbacks with non-additive effects, which could create a positive feedback on C loss from soils.

Shifts in turnover time of the microbial biomass or possible changes of composition and functioning of the microbial community may cause higher rates of SOM decomposition estimated from the respiration rate on a per-unit-PLFA basis of Ct and CT communities (Fig. 2b) (Kandeler *et al.* 2008). The CCA biplot supports that eCO_2 and warming led to microbial community structural and functional shifts of inoculated soils (Fig. 3). Microbial communities from Ct and CT plots were more similar to one another than to other communities, and had higher activity than those from ct and cT plots

(Fig. 3). Therefore, our results provide support for a recent meta-analysis (Dieleman *et al.* 2012), which suggests that soil process responses to combined eCO_2 and warming are more similar to those in individual eCO_2 treatment than individual warming treatment.

CONCLUSIONS

This research demonstrated that microbial community-level responses to climate change affected the ecosystem function of SOM decomposition. Our results highlighted two major mechanisms by which the microbial community can mediate terrestrial C cycling feedback to climate change. First, microbial ability to decompose SOM increased with elevated CO_2 at ambient and warmed temperatures due to increased substrate respiration rates or/and enzyme activities. Second, microbial temperature sensitivity increased in response to warming at ambient and elevated CO_2, suggesting a positive feedback between microbial activities related to SOM decomposition and climate warming. Overall, our study indicates that positive microbial community feedbacks in response to eCO_2 and warming can accelerate microbial decomposition and potentially lead to soil C losses.

AUTHORSHIP

MN, EP and MW designed the study, MN, EP, CB, CG, SR and ST performed the experiment, MN, EP, CB and MW analysed the data and MN, EP, CB and MW wrote the paper.

REFERENCES

Allison, S.D., Wallenstein, M.D. & Bradford, M.A. (2010). Soil-carbon response to warming dependent on microbial physiology. *Nat. Geosci.*, 3, 336–340.

Austin, E.E., Castro, H.F., Sides, K.E., Schadt, C.W. & Classen, A.T. (2009). Assessment of 10 years of CO_2 fumigation on soil microbial communities and function in a sweetgum plantation. *Soil Biol. Biochem.*, 41, 514–520.

Balser, T.C. & Wixon, D.L. (2009). Investigating biological control over soil carbon temperature sensitivity. *Glob. Change Biol.*, 15, 2935–2949.

Bardgett, R.D., Freeman, C. & Ostle, N.J. (2008). Microbial contributions to climate change through carbon cycle feedbacks. *ISME J.*, 2, 805–814.

Belay-Tedla, A., Zhou, X.H., Su, B., Wan, S.Q. & Luo, Y.Q. (2009). Labile, recalcitrant, and microbial carbon and nitrogen pools of a tallgrass prairie soil in the US Great Plains subjected to experimental warming and clipping. *Soil Biol. Biochem.*, 41, 110–116.

Bradford, M.A., Davies, C.A., Frey, S.D., Maddox, T.R., Melillo, J.M., Mohan, J. E. *et al.* (2008). Thermal adaptation of soil microbial respiration to elevated temperature. *Ecol. Lett.*, 11, 1316–1327.

Bradford, M.A., Watts, B.W. & Davies, C.A. (2010). Thermal adaptation of heterotrophic soil respiration in laboratory microcosms. *Glob. Change Biol.*, 16, 1576–1588.

Campbell, C.D., Chapman, S.J., Cameron, C.M., Davidson, M.S. & Potts, J.M. (2003). A rapid microtiter plate method to measure carbon dioxide evolved from carbon substrate amendments so as to determine the physiological profiles of soil microbial communities by using whole soil. *Appl. Environ. Microbiol.*, 69, 3593–3599.

Carney, K.M., Hungate, B.A., Drake, B.G. & Megonigal, J.P. (2007). Altered soil microbial community at elevated CO_2 leads to loss of soil carbon. *Proc. Natl Acad. Sci. USA.*, 104, 4990–4995.

Carrillo, Y., Pendall, E., Dijkstra, F.A., Morgan, J.A. & Newcomb, J.M. (2010). Carbon input control over soil organic matter dynamics in a temperate grassland exposed to elevated CO_2 and warming. *Biogeosciences Discuss.*, 7, 1575–1602.

Carrillo, Y., Pendall, E., Dijkstra, F.A., Morgan, J.A. & Newcomb, J.M. (2011). Response of soil organic matter pools to elevated CO_2 and warming in a semi-arid grassland. *Plant Soil*, 347, 339–350.

Carrillo, Y., Dijkstra, F., Pendall, E., Morgan, J. & Blumenthal, D. (2012). Controls over soil nitrogen pools in a semiarid grassland under elevated CO_2 and warming. *Ecosystems*, 15, 761–774.

DeAngelis, K.M., Silver, W.L., Thompson, A.W. & Firestone, M.K. (2010). Microbial communities acclimate to recurring changes in soil redox potential status. *Environ. Microbiol.*, 12, 3137–3149.

Dieleman, W.I., Vicca, S., Dijkstra, F.A., Hagedorn, F., Hovenden, M.J., Larsen, K.S. *et al.* (2012). Simple additive effects are rare: a quantitative review of plant biomass and soil process responses to combined manipulations of CO_2 and temperature. *Glob. Change Biol.*, 18, 2681–2693.

Dijkstra, F.A., Blumenthal, D., Morgan, J.A., Pendall, E., Carrillo, Y. & Follett, R.F. (2010). Contrasting effects of elevated CO_2 and warming on nitrogen cycling in a semiarid grassland. *New Phytol.*, 187, 426–437.

Dijkstra, F.A., Pendall, E., Morgan, J.A., Blumenthal, D., Carrillo, Y., LeCain, D. *et al.* (2012). Climate change alters stoichiometry of phosphorus and nitrogen in a semiarid grassland. *New Phytol.*, 196, 807–815.

Evans, S.E. & Wallenstein, M.D. (2012). Soil microbial community response to drying and rewetting stress: does historical precipitation regime matter? *Biogeochemistry*, 109, 101–116.

Frey, S.D., Drijber, R., Smith, H. & Melillo, J. (2008). Microbial biomass, functional capacity, and community structure after 12 years of soil warming. *Soil Biol. Biochem.*, 40, 2904–2907.

Hanson, C.A., Allison, S.D., Bradford, M.A., Wallenstein, M.D. & Treseder, K. K. (2008). Fungal taxa target different carbon sources in forest soil. *Ecosystems*, 11, 1157–1167.

Hartley, I.P., Heinemeyer, A., Evans, S.P. & Ineson, P. (2007). The effect of soil warming on bulk soil vs. rhizosphere respiration. *Glob. Change Biol.*, 13, 2654–2667.

Hartley, I.P., Hopkins, D.W., Garnett, M.H., Sommerkorn, M. & Wookey, P.A. (2008). Soil microbial respiration in arctic soil does not acclimate to temperature. *Ecol. Lett.*, 11, 1092–1100.

He, Z.L., Xu, M.Y., Deng, Y., Kang, S.H., Kellogg, L., Wu, L.Y. *et al.* (2010). Metagenomic analysis reveals a marked divergence in the structure of belowground microbial communities at elevated CO_2. *Ecol. Lett.*, 13, 564–575.

Hu, S., Chapin, F.S., Firestone, M.K., Field, C.B. & Chiariello, N.R. (2001). Nitrogen limitation of microbial decomposition in a grassland under elevated CO_2. *Nature*, 409, 188–191.

Jastrow, J.D., Miller, R.M. & Owensby, C.E. (2000). Long-term effects of elevated atmospheric CO_2 on below-ground biomass and transformations to soil organic matter in grassland. *Plant Soil*, 224, 85–97.

Kandeler, E., Mosier, A.R., Morgan, J.A., Milchunas, D.G., King, J.Y., Rudolph, S. *et al.* (2008). Transient elevation of carbon dioxide modifies the microbial community composition in a semi-arid grassland. *Soil Biol. Biochem.*, 40, 162–171.

Kuzyakov, Y., Friedel, J.K. & Stahr, K. (2000). Review of mechanisms and quantification of priming effects. *Soil Biol. Biochem.*, 32, 1485–1498.

Lennon, J.T. & Jones, S.E. (2011). Microbial seed banks: the ecological and evolutionary implications of dormancy. *Nat. Rev. Microbiol.*, 9, 119–130.

Luo, Y.Q. (2007). Terrestrial carbon-cycle feedback to climate warming. *Annu. Rev. Ecol. Evol. Syst.*, 38, 683–712.

Luo, Y. & Zhou, X. (2006). *Soil Respiration and the Environment*. Elsevier, San Diego.

Luo, Y.Q., Wan, S.Q., Hui, D.F. & Wallace, L.L. (2001). Acclimatization of soil respiration to warming in a tall grass prairie. *Nature*, 413, 622–625.

Morgan, J.A., LeCain, D.R., Pendall, E., Blumenthal, D.M., Kimball, B.A., Carrillo, Y. *et al.* (2011). C_4 grasses prosper as carbon dioxide eliminates desiccation in warmed semi-arid grassland. *Nature*, 476, 202–205.

Panikov, N.S. (2009). Microbial activity in frozen soils. In: *Permafrost Soils* (ed Margesin, R.). Springer, Berlin, pp. 119–147.

Parton, W.J., Morgan, J.A., Wang, G.M. & Del Grosso, S. (2007). Projected ecosystem impact of the prairie heating and CO_2 enrichment experiment. *New Phytol.*, 174, 823–834.

Pendall, E., Bridgham, S., Hanson, P.J., Hungate, B., Kicklighter, D.W., Johnson, D.W. *et al.* (2004). Below-ground process responses to elevated CO_2 and temperature: a discussion of observations, measurement methods, and models. *New Phytol.*, 162, 311–322.

Phillips, R.P., Finzi, A.C. & Bernhardt, E.S. (2011). Enhanced root exudation induces microbial feedbacks to N cycling in a pine forest under long-term CO_2 fumigation. *Ecol. Lett.*, 14, 187–194.

Sinsabaugh, R.L., Lauber, C.L., Weintraub, M.N., Ahmed, B., Allison, S.D., Crenshaw, C. *et al.* (2008). Stoichiometry of soil enzyme activity at global scale. *Ecol. Lett.*, 11, 1252–1264.

Steinweg, J.M., Plante, A.F., Conant, R.T., Paul, E.A. & Tanaka, D.L. (2008). Patterns of substrate utilization during long-term incubations at different temperatures. *Soil Biol. Biochem.*, 40, 2722–2728.

Trevors, J.T. (1996). Sterilization and inhibition of microbial activity in soil. *J. Microbiol. Methods.*, 26, 53–59.

Tucker, C.L., Bell, J., Pendall, E. & Ogle, K. (2012). Does declining carbon-use efficiency explain thermal acclimation of soil respiration with warming? *Glob. Change Biol.*, DOI: 10.1111/gcb.12036.

van Veen, J.A., Liljeroth, E., Lekkerkerk, L.J.A. & Vandegeijn, S.C. (1991). Carbon fluxes in plant-soil systems at elevated atmospheric CO_2 levels. *Ecol. Appl.*, 1, 175–181.

van de Voorde, T.F.J, van der Putten, W.H. & Bezemer, T.M. (2012). Soil inoculation method determines the strength of plant–soil interactions. *Soil Biol. Biochem.*, 55, 1–6.

Wallenstein, M.D. & Hall, E.K. (2012). A trait-based framework for predicting when and where microbial adaptation to climate change will affect ecosystem functioning. *Biogeochemistry*, 109, 35–47.

Wallenstein, M.D., McMahon, S.K. & Schimel, J.P. (2009). Seasonal variation in enzyme activities and temperature sensitivities in Arctic tundra soils. *Glob. Change Biol.*, 15, 1631–1639.

Wallenstein, M., Allison, S.D., Ernakovich, J., Steinweg, J.M. & Sinsabaugh, R. (2011). Controls on the temperature sensitivity of soil enzymes: a key driver of *in situ* enzyme activity rates. In: *Soil Enzymology* (eds Shukla, G.C. & Varma, A.). Springer, Berlin, pp. 245–257.

Weber, C.F., Zak, D.R., Hungate, B.A., Jackson, R.B., Vilgalys, R., Evans, R.D. *et al.* (2011). Responses of soil cellulolytic fungal communities to elevated atmospheric CO_2 are complex and variable across five ecosystems. *Environ. Microbiol.*, 13, 2778–2793.

Yergeau, E., Bokhorst, S., Kang, S., Zhou, J.Z., Greer, C.W., Aerts, R. *et al.* (2012). Shifts in soil microorganisms in response to warming are consistent across a range of Antarctic environments. *ISME J.*, 6, 692–702.

Zak, J.C., Willig, M.R., Moorhead, D.L. & Wildman, H.G. (1994). Functional diversity of microbial communities: a quantitative approach. *Soil Biol. Biochem.*, 26, 1101–1108.

Zak, D.R., Pregitzer, K.S., Curtis, P.S. & Holmes, W.E. (2000). Atmospheric CO_2 and the composition and function of soil microbial communities. *Ecol. Appl.*, 10, 47–59.

Zhou, J., Xue, K., Xie, J., Deng, Y., Liyou, W., Xiaoli, C. *et al.* (2012). Microbial mediation of carbon-cycle feedbacks to climate warming. *Nat. Clim. Chang.*, 2, 106–110.

SUPPORTING INFORMATION