Global Change Biology (2011) 17, 505–514, doi: 10.1111/j.1365-2486.2010.02296.x

Soil carbon storage under simulated climate change is mediated by plant functional type

ELISE PENDALL*, YUI OSANAI†, AMITY L. WILLIAMS† and MARK J. HOVENDEN†
*Department of Botany and Program in Ecology, University of Wyoming, Laramie, WY 82071, USA, †School of Plant Science,
University of Tasmania, Hobart, Tas. 7001, Australia

Abstract

The stability of soil organic matter (SOM) pools exposed to elevated CO₂ and warming has not been evaluated adequately in long-term experiments and represents a substantial source of uncertainty in predicting ecosystem feedbacks to climate change. We conducted a 6-year experiment combining free-air CO₂ enrichment (FACE, 550 ppm) and warming (+2°C) to evaluate changes in SOM accumulation in native Australian grassland. In this system, competitive interactions appear to favor C_4 over C_3 species under FACE and warming. We therefore investigated the role of plant functional type (FT) on biomass and SOM responses to the long-term treatments by carefully sampling soil under patches of C₃- and C₄-dominated vegetation. We used physical fractionation to quantify particulate organic matter (POM) and long-term incubation to assess potential decomposition rates. Aboveground production of C₄ grasses increased in response to FACE, but total root biomass declined. Across treatments, C:N ratios were higher in leaves, roots and POM of C₄ vegetation. CO₂ and temperature treatments interacted with FT to influence SOM, and especially POM, such that soil carbon was increased by warming under C₄ vegetation, but not in combination with elevated CO₂. Potential decomposition rates increased in response to FACE and decreased with warming, possibly owing to treatment effects on soil moisture and microbial community composition. Decomposition was also inversely correlated with root N concentration, suggesting increased microbial demand for older, N-rich SOM in treatments with low root N inputs. This research suggests that C₃-C₄ vegetation responses to future climate conditions will strongly influence SOM storage in temperate grasslands.

Keywords: C₃, C₄, carbon cycle, climate change, elevated CO₂, grassland, plant functional type, priming, soil organic matter, warming

Received 10 July 2009; revised version received 26 May 2010 and accepted 3 June 2010

Introduction

Soil carbon (C) storage is a function of inputs from aboveground and belowground biomass and losses due to decomposition of residues, all of which are likely to be affected by climate change. A recent meta-analysis showed that elevated atmospheric CO2 concentrations stimulated plant biomass on average by 21% (n = 189studies), and was associated with increased soil C storage of 5.6% (n = 40 studies; Luo et al., 2006). However, decomposition of soil organic matter (SOM) may be enhanced by elevated CO₂ (Pendall et al., 2003; Langley et al., 2009), contributing to lower rates of SOM accumulation relative to expectations from biomass inputs (e.g., Fontaine et al., 2004), or even a decline in the SOM pool (Carney et al., 2007). Climate warming contributes further uncertainty about the stability of SOM pools, because warming has both positive and negative effects on net primary production (NPP)

Correspondence: Elise Pendall, tel. + 1 307 766 6293, e-mail: pendall@uwyo.edu

(Rustad *et al.*, 2001; Luo *et al.*, 2008), and temperature sensitivity varies among SOM pools with different turnover rates (Davidson & Janssens, 2006). Furthermore, respiratory losses of C from soil exposed to experimental warming can be dependent on soil moisture availability (Wan *et al.*, 2007). Simulation models predict the combination of elevated CO₂ and warming to increase long-term C storage in grasslands, as NPP is stimulated more than decomposition due to enhanced N mineralization and soil moisture availability (Parton *et al.*, 2007; Luo *et al.*, 2008). However, no experimental evidence exists from grassland or any other ecosystem to confirm these model predictions.

Plant species and/or plant functional type (FT) may affect soil C storage because vegetation characteristics can alter SOM from both the input and loss sides of the equation (De Deyn *et al.*, 2008). Temperate grasslands tend to have higher soil C contents than forests because plant traits such as high root: shoot ratios and relative growth rate increase inputs belowground, and low litter quality (e.g., high C:N ratio) reduces decomposability (De Deyn *et al.*, 2008). In forests, high litter quality may

enhance decomposability in early stages of decomposition, but retard it in later stages (Fog, 1988; Berg, 2000). Variations in SOM storage within temperate grasslands are associated with root:shoot allocation and differences in FTs, particularly in photosynthetic (C₃ vs. C₄) pathway (Burke *et al.*, 1998). Litter quality, as measured by lignin content and C:N ratio, is lower in C₄ than C₃ grasses, leading to lower decomposability of C₄ residues (Vivanco & Austin, 2006), thus reducing nutrient availability and feeding back to biomass growth (Burke *et al.*, 1998) and SOM storage (Vinton & Burke, 1997).

Responses of ecosystems to climate change are likely to be mediated by changes in plant species composition, such as different C₃-C₄ responses (e.g., Morgan et al., 2004a; Wan et al., 2005), and via differential effects on plant traits related to allocation (De Deyn et al., 2008). In Australian temperate grassland exposed to elevated CO₂ and warming, seedling germination, growth and mortality appear to limit the ability of several C₃ species to compete successfully against an increasingly dominant C₄ species (Williams et al., 2007; Hovenden et al., 2008b). Elevated CO₂ increases belowground C allocation, C:N ratios (Pendall et al., 2004), root production and turnover (Milchunas et al., 2005), potentially contributing to SOM storage in grasslands (Allard et al., 2005). On the other hand, warming can reduce root biomass and root: shoot ratios (Wan et al., 2004), decrease C:N ratios and increase litter decomposition (Hobbie, 1996), leading to SOM losses (Saleska et al., 2002). However, no experiment that we are aware of has evaluated how plant traits associated with FT may mediate the combined effects of elevated CO2 and warming on soil C storage.

We therefore conducted an experiment in an Australian native temperate grassland exposed to warming and elevated CO₂ to evaluate how C₃ and C₄ vegetation may mediate SOM storage via the responses of biomass inputs and C losses due to decomposition. Evaluating ecosystem responses that are mediated by C3 and C4 vegetation is especially critical, because increasing atmospheric CO₂ and warming are expected to interact to shift competitive interactions and geographic ranges of these key grassland functional groups (Hughes, 2003). C₃ plants generally increase in productivity in the presence of elevated CO₂ due to direct stimulation of photosynthesis (Sage & Kubien, 2003) and indirect enhancement of soil moisture (Owensby et al., 1999; Morgan et al., 2004b), whereas C₄ plant growth responds positively to warming (Sage & Kubien, 2003; Luo, 2007) and has neutral to positive responses to elevated CO₂ due to enhanced water relations (Morgan et al., 2001; Sage & Kubien, 2003).

We tested the following hypotheses: (1) Elevated CO₂ increases plant growth and belowground C allocation

(root:shoot ratio and labile soil C), plant C:N ratios, potential SOM decomposition due to enhanced priming, and leads to increased SOM because inputs increase more than decomposition rate. (2) Warming decreases plant growth, belowground C allocation, C/ N ratios, and decomposition (due to soil drying), with a net effect of decreasing SOM as inputs decrease more than decomposition. (3) Elevated CO₂ and warming together stimulate plant growth and belowground C allocation, have no net effect on C/N ratios, and enhance decomposition rate but to a lesser degree than biomass, leading to increased SOM. (4) Soil C storage is mediated by differential responses of C₃ and C₄ vegetation to treatments via differences in quantity and quality (C:N ratio) of root and leaf litter, leading to interactive effects of FT, elevated CO₂ and warming on SOM.

Materials and methods

Study area and experimental design

The TasFACE climate change impacts experiment was established in a species-rich temperate grassland in southeastern Tasmania (42°42′S, 147°16′E, 40 m a.s.l.) in February 2002. Freeair CO₂ enrichment (FACE, 550 ppm year-round) using pure CO₂ injection and warming (2 °C) by ceramic infrared heat lamps (Salamanda ESE250 240 V/250 W Emerson Solid Ceramic Infrared Emitter, Delta T, Melbourne, Australia) were applied in a full factorial design on 12, 1.5 m diameter plots (three replicates). Atmospheric [CO2] was controlled to $549 \pm 0.1 \,\mu\text{mol mol}^{-1}$ in FACE plots, compared with $372 \pm 0.3 \,\mu\text{mol mol}^{-1}$ in Control plots. Soil temperature at 5 mm depth (measured with Type-T thermocouples with a 1min sample interval) in Warmed plots was 1.8 ± 0.1 °C higher than Unwarmed plots at nighttime, and $0.14 \pm 0.01\,^{\circ}\text{C}$ higher during the day. Soil water potential was logged weekly from each of two gypsum blocks buried in each plot at 10 cm depth. Further details about the technical performance of the experimental system can be found in Hovenden et al. (2006).

Vegetation is dominated by a perennial C₄ grass, Themeda triandra Forssk. (the only widespread and abundant C₄ species in Tasmania and the sole C4 species at the site) and the C3 grasses Austrodanthonia caespitosa (Gaudich.) H. P. Linder, Austrodanthonia carphoides (Benth.) H. P. Linder and Austrostipa mollis (R. Br.) S. W. L. Jacobs & J. Everett. Together these species average $\sim 85\%$ of aboveground production at the site. Introduced annual grasses and both native and introduced perennial herbaceous dicots are present in interspaces between grass tussocks. The soil is a clayey black Vertisol formed in alluvium with mixed mineralogy, and is carbonate-free with neutral pH and low N content. Bulk density (1.8 \pm 0.32 g cm⁻³, n = 42) did not vary among treatments in October 2009 (P > 0.05). The climate of the study area has warm, dry summers and cool, moist winters, with mean annual precipitation of 560 mm and mean annual temperature of 11.6 °C. Further details about the site including a full species list can be found in Hovenden et al.

Biomass harvests and soil sampling

Aboveground biomass was clipped from a 100 cm² area on each plot three times per year, with the timing depending upon rainfall patterns. Clipped samples were separated by species in the laboratory. We calculated average aboveground net primary production (ANPP) by summing the yield of green tissues of C₄ (T. triandra) and C₃ vegetation (all other green vegetation) for the period 2004-2007 and dividing by the number of harvests per year. Belowground biomass was sampled in December 2007, the time of peak aboveground biomass, by taking two, 3 cm diameter soil cores to 20 cm depth at random locations within the plots. The cores were immersed in 0.5% sodium hexametaphosphate solution overnight, and roots were separated by sieving in stacked sieves of 2 mm, 500 and 250 µm mesh sizes. Roots were collected manually and combined for each plot. Soils were sampled in October 2007, by taking 2, 3 cm diameter cores (0-5 and 5-10 cm depths) under C₃ and C₄ patches (approximately 40 cm apart within the plots) to evaluate the influence of plant FT on SOM responses to climate change treatments. The two cores per FT were composited for each plot. Because bulk density was not determined on these cores we report SOM results as concentrations.

Soil processing and analysis

A long-term incubation experiment was started within a few days of soil sampling to determine the labile, microbially active soil C fraction (C1) and turnover dynamics of active and slow C pools under ideal temperature and moisture conditions (Pendall and King, 2007). Soil samples were sieved to 4 mm to remove gravel, roots and aboveground litter. Visible roots that passed through the 4 mm sieve were removed by hand. Samples of 20 g fresh weight were placed into specimen cups, and soils were brought to 60% of field capacity with deionized water. The cups were placed into 500 mL canning jars fitted with blue butyl rubber septa after ventilating with fresh air and incubated in a dark cabinet at 23 °C. A syringe was used to remove 20 mL of headspace from the jars on 18 occasions during the 200-day experiment, frequently during the first 3 weeks, and every 2-3 weeks for the remainder. The CO₂ concentration of the headspace sample was analyzed with a LiCor LI-6262 infrared gas analyzer (John Morris Scientific, Melbourne, Australia). CO2 was allowed to accumulate in the jars between sampling times, and jars were vented immediately after sampling. Concentrations never exceeded 0.5% and were usually around 0.1%. A three-parameter exponential decay model Eqn (1) was fitted to the daily respiration rates from each replicate sample to obtain the parameters C_1 , k and r:

$$R_t = C_1 k e^{-kt} + r, (1)$$

where R_t is the microbial respiration rate at time t, C_1 is the labile C pool and k is the intrinsic decay constant of the labile pool (e.g., Dijkstra et al., 2006b; Dijkstra & Cheng, 2007). The asymptote value, r, is the decomposition rate of resistant pool C, which is considered to be constant (Townsend et al., 1997; Dijkstra et al., 2006b). This two-pool, three-parameter model is a simplified form of a two-pool, four-parameter model proposed by Robertson & Paul (2000), because it does not require assumptions regarding the size of the resistant pool. Data were fitted to Eqn (1) using the nonlinear curve fitting routine in SAS v. 9.1 (PROC NLIN), with the default Gauss-Newton algorithm (SAS Institute Inc., 2003). Curve fits were all highly significant (P<0.0001). Because soil organic C (SOC) concentration can be correlated with the labile pool size and decomposition rate, we normalized the values of C1 and r by dividing them by SOC in each sample to reflect the intrinsic labile C proportion and decomposition rate, respectively.

We separated the particulate organic matter (POM) and mineral organic matter (MOM) fractions from the total SOM by dispersing it in 0.5% sodium hexametaphosphate solution and wet sieving at 53 µm (Cambardella & Elliott, 1992). The MOM, or silt + clay fraction ($<53 \mu m$), was flocculated by adding a few drops of 5% alum (K Al (SO₄)₂12H₂O), excess water was decanted, and both fractions were dried at 45 °C. All soil and biomass samples were analyzed for C and N concentration on a Perkin Elmer 2400 Series II elemental analyzer (Analytical Development Company, Adelaide, Australia; precision of standards \pm 0.2% for both C and N).

Statistics

Factorial analysis of variance was used to evaluate the main and interacting effects of CO2 and warming on biomass and ANPP, and the main and interacting effects of CO₂, warming, FT (C₃ vs. C₄ vegetation) and depth on soil C properties. Post hoc means were determined using least squares means separation with the Tukev-Kramer adjustment. Data not meeting assumptions of normality and homogeneity of variance were log-transformed before statistical testing. While significant effects of depth were found for most soil properties, no significant interactions with other factors were observed, so treatment effects on soil properties were graphed by pooling across depths. Simple linear regression was performed to evaluate relationships underlying decomposition rates. All statistical tests were done with SAS v. 9.1.

Results

Elevated CO₂ and warming interacted to alter soil moisture in the treatment plots (Fig. 1). Soil water potential was highest in FACE Unwarmed plots from 2003 through 2005, and lowest in FACE Warmed plots. In early 2006, a drought reduced soil water potential in all plots and reduced biomass production; the transient increase in water potential in Control Warmed plots was explained by drought-induced mortality (M. J. Hovenden, unpublished results). Over the period of study, precipitation averaged only 70% of the long-term mean, reflecting ongoing drought conditions in southeastern Australia (Fig. 1).

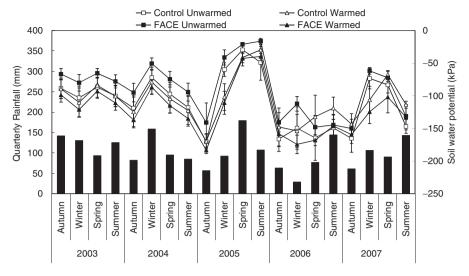


Fig. 1 Quarterly rainfall and soil water potential at the TasFACE experiment from 2003 to 2007. FACE, free-air CO₂ enrichment.

In December 2007, at the peak growing season harvest, aboveground biomass (including standing litter) averaged $297 \pm 56 \,\mathrm{g \, m^{-2}}$ and was not significantly affected by CO₂ or temperature treatments (Fig. 2a). Root biomass (to 20 cm depth) declined under FACE, from 653 ± 97 to $429 \pm 75 \,\mathrm{g}\,\mathrm{m}^{-2}$ (P<0.02; Fig. 2a). Accordingly, root: shoot ratio of standing biomass in 2007 decreased from 3.1 ± 0.6 in Control plots to 1.3 ± 0.4 in FACE plots (P < 0.04). ANPP (excluding standing litter), averaged for the years 2004-2007, was increased by nearly 60% under FACE (Control, $179 \pm 27 \,\mathrm{g}$ $m^{-2}yr^{-1}$; FACE, $285 \pm 40 g m^{-2}yr^{-1}$; P < 0.06), with ANPP of the C₄ species, T. triandra, increasing from $45 \pm 17 \,\mathrm{g}\,\mathrm{m}^{-2}\,\mathrm{yr}^{-1}$ on Control plots to $115 \pm 28 \,\mathrm{g}$ $m^{-2}yr^{-1}$ on FACE plots (P<0.06; Fig. 2b). ANPP of C₃ species was not affected by experimental treatments or interaction effects. The proportion of C4 (standing crop) biomass was 25% on Control plots and 39% on FACE plots (P = 0.2); species composition in terms of biomass has thus been changing toward C₄ dominance, but by December 2007, the difference was not significant. C:N ratios of aboveground biomass were increased by FACE (P = 0.03) and reduced by warming (P = 0.03; Fig. 3a). The interaction between temperature and FT (P < 0.07) was explained by the stronger warming-induced reduction in C:N of A. caespitosa (representative dominant C3 species) than T. triandra (the C4 species; Fig. 3a). The C:N ratios of roots collected in October 2007 were increased by FACE (P < 0.01), reduced by warming (P < 0.04) and higher in C_4 than C_3 vegetation (P < 0.01; Fig. 3b). The warming-induced reduction of root C:N ratios was stronger under C₄ than C_3 vegetation (temperature \times FT interaction, P < 0.07), and stronger in plots at elevated than ambient CO_2 (temperature × CO_2 interaction, P < 0.07; Fig. 3b).

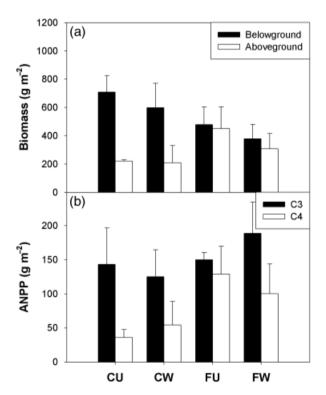


Fig. 2 (a) Total standing crop of aboveground (open bars) and belowground (black bars) biomass in December, 2007; (b) Aboveground net primary production of C_3 and C_4 functional types averaged over the years 2004–2007. Analysis of variance P-values < 0.05 are shown in bold and < 0.1 are shown in italics. Error bars show standard error of the mean. FACE, free-air CO_2 enrichment.

Total SOC concentration was not directly affected by either CO_2 or temperature, but it was subject to a nearly significant $CO_2 \times$ temperature \times FT interaction (P < 0.06;

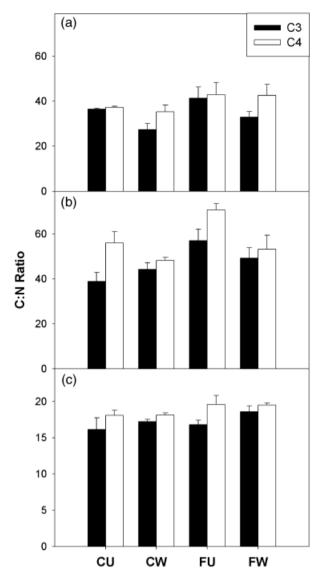


Fig. 3 C: N ratios in biomass and soil organic matter in 2007 (a) aboveground biomass (standing crop in December); (b) root biomass (standing crop in December); (c) particulate organic matter (October). Analysis of variance *P*-values < 0.05 are shown in bold and <0.1 are shown in italics. Error bars show standard error of the mean. FACE, free-air CO₂ enrichment.

supporting information Table S1), which was associated with higher SOC under warmed, C4 vegetation but not under warmed, C3 vegetation at ambient CO2 (Fig. 4a; supporting information Table S1). Total SOC and mineral OM showed similar interactive responses to CO₂ and FT, with higher C concentrations under C₄ vegetation at ambient but not elevated CO_2 (P = 0.01 in both cases; supporting information Table S1). Total SOC was $28.3 \pm 3.0 \,\mathrm{g\,kg^{-1}}$ under ambient C₄ vegetation, $19.6 \pm$ $1.3\,\mathrm{g\,kg^{-1}}$ under ambient $\mathrm{C_3}$ vegetation and 23.6 ± 1.6

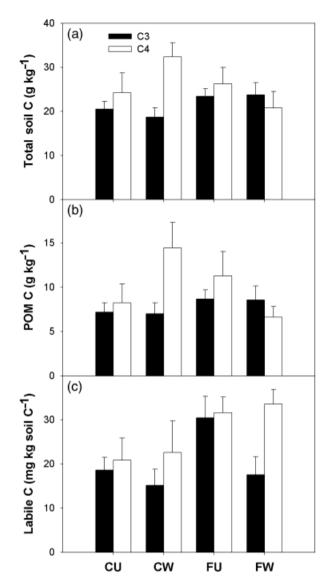


Fig. 4 Mean soil organic matter components in top 10 cm after 6 years of treatments in the TasFACE experiment, (a) total soil organic C; (b) particulate soil organic matter C; and (c) treatment differences in proportion of organic matter that is labile C. Analysis of variance P-values < 0.05 are shown in bold and < 0.1 are shown in italics. Error bars show standard error of the mean. POM, particulate organic matter; FACE, free-air CO₂ enrichment.

and $24.1 \pm 2.7 \,\mathrm{g\,kg}^{-1}$ under FACE C_3 and C_4 vegetation, respectively. Mineral OM C concentrations followed a similar pattern (supporting information Table S1). FT also interacted with CO₂ and temperature treatment to affect POM (three-way interaction, P < 0.03; supporting information Table S1). Under C₃-dominated vegetation, POM was largely unchanged by either temperature or CO₂, whereas under C₄ vegetation, warming increased POM in control plots but not in FACE plots (Fig. 4b).

The significant interaction between CO₂ and temperature (P<0.01) was explained by an increase in POM with FACE or warming separately but not in combination ($7.6 \pm 1.0\,\mathrm{g\,kg^{-1}}$ in Control Unwarmed; $10.0 \pm 1.6\,\mathrm{g\,kg^{-1}}$ in FACE Unwarmed; $10.0 \pm 1.4\,\mathrm{g\,kg^{-1}}$ in Control Warmed; $7.8 \pm 1.07\,\mathrm{g\,kg^{-1}}$ in FACE Warmed). POM constituted about 30–50% of total SOC, and was strongly correlated with SOC (P<0.01; r^2 = 0.88). POM C:N ratios were higher under C₄ (18.9 ± 0.5) than C₃ (17.2 ± 0.5 ; P = 0.01) vegetation and under FACE than control (18.5 ± 0.5 vs. 17.3 ± 0.5 ; P<0.06; Fig. 3c).

Respiration rates and cumulative CO₂ evolved during long-term incubations are plotted in supporting information Figs S1 and S2. Respiration rates fit the two-pool, three-parameter model Eqn (1) significantly (P < 0.0001 in all replicates). The incubations demonstrated that FACE increased the proportion of SOC that was labile, C_1 (P < 0.01); furthermore, warming significantly reduced C₁ in soil under C₃ vegetation but not under C_4 vegetation (temperature \times FT, P < 0.04; Fig. 4c). The mean residence time (MRT) of the C₁ pool was increased by nearly 40% in FACE plots (18 \pm 1.7 days) compared with control plots (13 \pm 1.2 days; P = 0.04) but was not sensitive to any other factor or interaction of factors. The intrinsic decomposition rate of resistant C (normalized to SOC), determined under ideal moisture and temperature conditions in the laboratory from r, the asymptote value of the exponential decay function Eqn (1), was significantly increased in soils from the FACE treatment (Control, $119 \pm 12 \, mg$ kg soil C^{-1} day⁻¹; FACE, 152 ± 10 mg kg soil C^{-1} day⁻¹; P<0.01) and reduced in soils from the warmed treatment (Unwarmed, $155 \pm 12 \,\mathrm{mg \, kg \, soil \, C^{-1} \, day^{-1}}$; Warmed, $115 \pm 12 \,\mathrm{mg \, kg \, soil \, C^{-1} \, day^{-1}}; \ P < 0.01; \ \mathrm{Fig. \ 5}). \ \mathrm{The \ in}$ trinsic decomposition rate of resistant SOC was not affected by FT (P = 0.13) but it was inversely correlated with root N concentration (P < 0.03; Fig. 6).

Discussion

Average annual temperature in Australia is expected to increase by 0.4–2 °C (relative to 1990) by 2030 (Hughes, 2003), and atmospheric CO_2 concentration will reach 550 ppm before the end of the century at the present rate of increase. Ecosystem C-cycle responses to the combination of these two major drivers of global change are likely to play a key role in mitigating or enhancing the rate of warming (Heimann & Reichstein, 2008). Our results demonstrated that soil C storage is dependent on plant FT in an ecosystem exposed to simulated climate change. In particular, warming increased SOC and POM concentrations under C_4 vegetation, but not under C_3 vegetation or in combination with elevated CO_2 . The interaction between FT and elevated CO_2 was

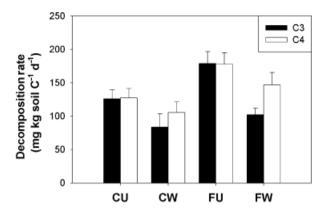


Fig. 5 Treatment effects on resistant pool decomposition rates. Analysis of variance P-values are <0.01 for both CO_2 and warming treatments. Error bars show standard error of the mean. FACE, free-air CO_2 enrichment.

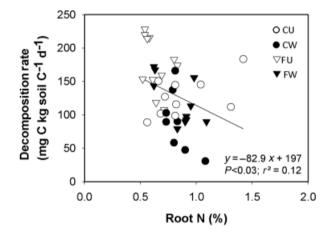


Fig. 6 Relationship between resistant pool decomposition rate and root N concentration. Triangles, free-air CO₂ enrichment; Circles, Control; filled symbols, Warmed; unfilled symbols, Unwarmed.

significant in the mineral (silt + clay) fraction, demonstrating that long-lived C pool responses to climate change can vary at the patch scale in ecosystems. Although our experiment was conducted on one soil type with a limited number of species, the results suggest that shifts in C₃–C₄ dominance in response to climate change in temperate grasslands could strongly alter SOM storage worldwide. Moreover, some of our findings (summarized in Table 1) run counter to general expectations based on biomass and soil C responses in short-term, single-factor experiments, emphasizing the importance of evaluating the role of species or FTs within long-term (>5 years) multifactor experiments (Bardgett *et al.*, 2008).

Biomass responses to elevated CO₂ and warming

The increase in ANPP observed with elevated CO₂ (Fig. 2, Table 1) was consistent with results in other grasslands (e.g., Owensby et al., 1999), but we expected that growth response would be mediated by C₃ rather than C₄ species, as has been observed in other mixed C_3/C_4 systems (Morgan *et al.*, 2004a). The positive response of the C₄ grass in our system (T. triandra) may have resulted from responses of population dynamics to the treatments. Population growth of A. caespitosa, the dominant C₃ grass, declined under elevated CO₂ (Williams et al., 2007) which was probably associated with reduced seed N and seedling vigor and increased seedling mortality (Hovenden et al., 2008b). Soil nutrient limitation is the most common explanation for a lack of biomass response to elevated CO₂ such as in C₃ annual grassland (Shaw et al., 2002), and we cannot rule out its role in the neutral response of C₃ biomass to elevated CO₂ in our experiment (Hovenden et al., 2008a). We did not observe negative effects of warming on plant productivity. T. triandra population growth was highest in warmed, elevated CO2 plots (Williams et al., 2007), suggesting enhanced competitiveness that may have resulted in part from higher N use efficiency in C₄ than C₃ vegetation (An et al., 2005; Vivanco & Austin, 2006) as demonstrated by its higher C:N ratios. Warming favored growth of C₄ over C₃ grasses in tallgrass prairie, possibly because of their higher N use efficiency (An et al., 2005; Luo, 2007). T. triandra may also have benefitted from higher water use efficiency (Wan et al., 2005).

The observed decrease in root:shoot ratio under FACE (Fig. 2a) was contrary to experimental evidence showing enhanced belowground allocation with elevated CO₂ (Pendall et al., 2004). Reduced root:shoot ratio with FACE was due to reduced root biomass and also to increased aboveground C4 biomass. Allocation

to roots was consistently suppressed under elevated CO₂ in a C₃ annual grassland (Shaw et al., 2002), and may be explained by higher soil resource availability, because soil moisture was consistently higher under FACE at ambient temperature (Fig. 1) and soil N availability was higher on warmed, FACE plots (Hovenden et al., 2008a). Warming did not influence root:shoot ratios, but the lowest root biomass was observed on warmed, elevated CO₂ plots.

Higher C: N ratios were observed in leaves and roots of C₄ (T. triandra) than C₃ (A. caespitosa) vegetation, in agreement with other comparisons of grassland species (Vivanco & Austin 2006). FACE increased C: N ratios of leaves, roots and POM, supporting expectations from the literature (e.g., Cotrufo et al., 2005; Dijkstra et al., 2006b). In Colorado grassland, reduced N concentration was associated with lower crude protein concentration and in vitro digestibility in both C3 and C4 plants exposed to elevated CO₂, with negative implications for forage quality (Milchunas et al., 2005) but potentially contributing to SOM storage (Pendall et al., 2004). At TasFACE, warming reduced C:N ratios of leaves and roots, partially offsetting the increases observed under elevated CO₂.

The effect of warming on C:N ratios interacted with FT, with opposite effects on roots and leaves; C:N was reduced in leaves of A. caespitosa but not of T. triandra, whereas C: N ratios were reduced in roots of T. triandra and not A. caespitosa (Table 1). These interactions suggest different N allocation strategies in C₃ and C₄ grasses in response to warming, as found for different Mediterranean shrub species exposed to warming and drought (Sardans et al., 2008). By contrast, warming increased leaf C:N ratios in both C3 and C4 species in tallgrass prairie due to reductions in N mineralization, leading to significant improvement of N use efficiency for the C_4 species (An *et al.*, 2005). The interactive effects of warming and FT on leaf and root C:N ratios suggest

Table 1 Ecosystem C cycling responses observed after 6 years in the TasFACE experiment

Treatment	FT	Growth	C:N ratio	Labile C	Decomposition	Soil C storage
			Leaves, roots			SOC, POM
FACE	C ₃	0	+,+	+	+	0, 0
	C_4	+	+,+	+	+	0, +
Warming	C_3	0	-, 0	_	_	0, 0
	C_4	0	0, –	+	_	+,+
FACE + warming	C_3	0	0, +	0	0	0, 0
	C_4	+	0, –	+	+	0, 0

Plus symbols indicate an increase in the variable, minus symbols indicate a decrease, and zero values indicate no difference relative to ambient CO₂ and temperature conditions.

FACE, free-air CO₂ enrichment; FT, functional type; POM, particulate organic matter; SOC, soil organic carbon.

that species will respond differentially to warming-enhanced soil N availability (Hovenden *et al.*, 2008a), potentially altering competitive interactions (Luo, 2007). Warming did not affect POM C:N, suggesting that N was resorbed from tissues before senescence (An *et al.*, 2005), or that differences in C:N disappeared during decomposition of POM (Robertson & Paul, 2000).

SOM storage, decomposition and priming are dependent on plant FT

We hypothesized that soil C storage would increase with FACE, decrease with warming and increase when the factors were combined. In contrast to our hypotheses, the highest SOC and POM concentrations were observed in warmed plots at ambient CO₂ under C₄ vegetation, despite a lack of biomass response in this treatment (Table 1). Possibly, T. triandra roots had higher turnover rates in the warming treatment, but went undetected by our methods. High lignin concentration of C4 grass roots (Vivanco & Austin, 2006) may help explain SOC accumulation under C4 vegetation in warmed plots, which had higher N availability (Hovenden et al., 2008a), because species with high root lignin concentration were shown to increase soil C stabilization in the presence of abundant soil N in an experimental temperate grassland (Dijkstra et al., 2004). The proportion of SOC that was labile (C₁) decreased with warming under C₃ vegetation. Experimental soil warming in temperate forests reduced soil carbohydrate content after only 14 months (Feng et al., 2008) and led to cumulative loss of labile C pools and reduced soil respiration rates (Melillo et al., 2002), consistent with the diminished labile C contents observed after 6 years of exposure to warming at TasFACE.

Warming in our experiment significantly increased C₄ root N concentration, which was inversely correlated with SOM decomposition rate, albeit weakly (Fig. 6). This observation was also made in an N-fertilized grassland (Dijkstra et al., 2006b), and suggests that abundant root N reduced decomposition of older, N-rich SOM, which could be considered a negative priming effect (Kuzyakov et al., 2000). In conifer forests, a negative relationship between decomposition rate and N concentration has been explained by lignin - N complexation reactions or suppression of lignolytic enzymes (Berg, 2000). By contrast, added N stimulated cellulose-degrading enzymes, but did not affect decomposition rates or lignin-degrading enzymes in grassland and forested ecosystems (Keeler et al., 2009). Clearly, mechanisms underlying the relationships among N availability, lignin content and litter and SOM decomposition rate require further study (Fog, 1988; Keeler et al., 2009).

High soil C concentrations in warmed soils are partly explained by reduced decomposability of resistant SOM, which could also be due to soil drying (Fig. 1). Warminginduced drying was partly responsible for reduced in situ decomposition of shrub litter (Saleska et al., 2002), but we observed reduced decomposability under optimal moisture conditions in the laboratory. Therefore it seems likely that changes in the microbial community or substrate quality explain our results. Reduced decomposition with warming may be attributed to a shift in microbial community structure toward fungi (Feng et al., 2008), which are less temperature and moisture sensitive than bacteria, and which are associated with increased SOM stabilization (Zhang et al., 2005). Thermal adaptation by the microbial community with long-term exposure to warming (e.g., Bradford et al., 2008) may also contribute to lower decomposition rates. If SOM decomposition is reduced by warming in some ecosystems, whether by negative priming effects, enhanced N availability, reduced soil moisture content, or microbial community effects, potential losses of SOM could be diminished over the long term. These findings complicate theoretical predictions of future SOM stability in warmer climates (e.g., Davidson & Janssens, 2006).

POM was somewhat higher with FACE under C₄ vegetation, which may be partly explained by higher C₄ biomass production with FACE. However, the 37% increase of POM under C₄ vegetation in Unwarmed FACE plots was much less than the >3-fold increase of C₄ ANPP in the same plots. We observed that labile C pools and decomposability of resistant SOM both increased under FACE, suggesting that priming partially offset the C inputs from biomass. Although our method did not directly quantify the amount of older SOM decomposed via the labile-C enhanced priming effect (Kuzyakov et al., 2000), normalizing the r-values to SOC mainly reflects the effect of priming. Enhanced litter inputs and soil labile C were associated with losses of older, resistant SOM in the Italian POPFACE experiment (Lagomarsino et al., 2009). In a Florida scrub oak community, a $\sim 20\%$ reduction of resistant SOM after 6 years was explained by priming deduced from long-term incubation (Langley et al., 2009), and by a shift in microbial community composition (Carney et al., 2007). A larger labile C pool and enhanced decomposition of older SOM were also found in Colorado semiarid grassland under doubled CO₂ (Pendall et al., 2003; Pendall & King, 2007). Although priming has been viewed as a short-term (e.g., days to weeks) response to labile C inputs (e.g., Dalenberg & Jager, 1989; Kuzyakov et al., 2000), stimulation of SOM decomposition can be effective in experiments lasting a year or longer (Dijkstra & Cheng, 2007). Taken together, these results suggest that priming may compromise SOC storage as atmospheric CO₂ increases, whereas negative priming with warming may offset SOC losses, and indicate a need to incorporate this mechanism into C cycle models (e.g., Fontaine & Barot, 2005).

In the present study, large labile C pools and high decomposition rates were observed in FACE Warmed plots under C₄ vegetation, indicating a role for plant FT in mediating priming (Cheng et al., 2003). Significant species effects on priming have been related to biomass production (Dijkstra et al., 2006a), which is consistent with the enhanced ANPP under elevated CO2 we observed for the C₄ species, T. triandra. It should be noted that only one C₄ species was present at the site, so further research is needed to evaluate if other C₄ plants have similar priming effects with climate change, and to identify the key functional traits associated with these responses. In some plant communities, priming of SOM may provide a latent source of N that could offset effects of progressive N limitation (Hovenden et al., 2008a; Langley et al., 2009; Dijkstra et al., 2010a), and contribute to altered species composition in future climates (Dijkstra et al., 2010b). Our observations of contrasting functional group responses to climate change and plant-soil interactions support the idea that changes in species composition may be more likely to alter C and N cycling than direct effects of elevated CO₂ or N fertilization (Knops et al., 2007).

Conclusions

Our results from a 6-year-long field manipulation provide the first evidence that plant FT interacts with global change treatments to affect SOM storage, via mechanisms related to changes in biomass production, litter quality and root N concentration that led to differences in labile C production and SOM decomposition rates. In C₄ vegetation, elevated CO₂ increased soil C storage because growth increased and decomposition did not keep up with it; warming increased soil C storage because decomposition rates decreased, and in elevated CO₂ plus warming plots, soil C was the same as in ambient conditions because the C₄ growth increase was offset by primingenhanced decomposition. In C₃ vegetation, soil C storage was not significantly altered by climate change treatments. TasFACE and other long-term climate-manipulation experiments are beginning to provide mechanistic insights into how terrestrial ecosystems will feed back to climate change (Heimann & Reichstein, 2008), and are certain to improve predictive understanding as results are incorporated into models (Bardgett et al., 2008).

Acknowledgements

We thank the Australian Federal Department of Defence for access to the Pontville Small Arms Range Complex. Funding

was provided by USDA – CSREES (grant nos. 2008-35107-04527 and -18655) and the Australian Research Council Discovery Projects Scheme. Comments from F. Dijkstra and Y. Carrillo improved an earlier version of this manuscript.

References

- Allard V, Newton PCD, Lieffering M, Soussana JF, Carran RA, Matthew C (2005) Increased quantity and quality of coarse soil organic matter fraction at elevated CO2 in a grazed grassland are a consequence of enhanced root growth rate and turnover. Plant and Soil, 276, 49-60.
- An YA, Wan SQ, Zhou XH, Subedar AA, Wallace LL, Luo YQ (2005) Plant nitrogen concentration, use efficiency, and contents in a tallgrass prairie ecosystem under experimental warming. Global Change Biology, 11, 1733-1744.
- Bardgett RD, Freeman C, Ostle NI (2008) Microbial contributions to climate change through carbon cycle feedbacks. Isme Journal, 2, 805-814.
- Berg B (2000) Litter decomposition and organic matter turnover in northern forest soils. Forest Ecology and Management, 133, 13-22.
- Bradford MA, Davies CA, Frey SD et al. (2008) Thermal adaptation of soil microbial respiration to elevated temperature. Ecology Letters, 11, 1316-1327.
- Burke IC, Lauenroth WK, Vinton MA et al. (1998) Plant-soil interactions in temperate grasslands. Biogeochemistry, 42, 121-143.
- Cambardella CA, Elliott ET (1992) Particulate soil organic-matter changes across a grassland cultivation sequence. Soil Science Society of America Journal, 56, 777-783.
- Carney KM, Hungate BA, Drake BG, Megonigal JP (2007) Altered soil microbial community at elevated CO2 leads to loss of soil carbon. Proceedings of the National Academy of Sciences of the United States of America, 104, 4990-4995.
- Cheng W, Johnson DW, Fu S (2003) Rhizosphere effects on decomposition: controls of plant species, phenology, and fertilization. Soil Science Society of America Journal, 67,
- Cotrufo MF, De Angelis P, Polle A (2005) Leaf litter production and decomposition in a poplar short-rotation coppice exposed to free air CO2 enrichment (POPFACE). Global Change Biology, 11, 971-982.
- Dalenberg JW, Jager G (1989) Priming effect of some organic additions to C-14-labeled soil. Soil Biology and Biochemistry, 21, 443-448.
- Davidson EA, Janssens IA (2006) Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. Nature, 440, 165-173.
- De Deyn GB, Cornelissen JHC, Bardgett RD (2008) Plant functional traits and soil carbon sequestration in contrasting biomes. Ecology Letters, 11, 516-531.
- Diikstra FA, Blumenthal D, Morgan IA, Pendall E, Carrillo Y, Follett RF (2010a) Contrasting effects of elevated CO2 and warming on nitrogen cycling in a semiarid grassland. New Phytologist, 187, 426-437.
- Dijkstra FA, Cheng WX (2007) Interactions between soil and tree roots accelerate longterm soil carbon decomposition. Ecology Letters, 10, 1046-1053.
- Dijkstra FA, Cheng WX, Johnson DW (2006a) Plant biomass influences rhizosphere priming effects on soil organic matter decomposition in two differently managed soils. Soil Biology and Biochemistry, 38, 2519-2526.
- Dijkstra FA, Hobbie SE, Knops JMH, Reich PB (2004) Nitrogen deposition and plant species interact to influence soil carbon stabilization. Ecology Letters, 7, 1192-1198.
- Dijkstra FA, Hobbie SE, Reich PB (2006b) Soil processes affected by sixteen grassland species grown under different environmental conditions. Soil Science Society of America Journal, 70, 770-777.
- Dijkstra FA, Morgan JA, Blumenthal D, Follett RF (2010b) Water limitation and plant inter-specific competition reduce rhizosphere-induced C decomposition and plant N uptake. Soil Biology and Biochemistry, 42, 1073-1082.
- Feng XJ, Simpson AJ, Wilson KP, Williams DD, Simpson MJ (2008) Increased cuticular carbon sequestration and lignin oxidation in response to soil warming. Nature Geoscience, 1, 836-839.
- Fog K (1988) The effect of added nitrogen on the rate of decomposition of organicmatter. Biological Reviews of the Cambridge Philosophical Society, 63, 433-462.
- Fontaine S, Bardoux G, Abbadie L, Mariotti A (2004) Carbon input to soil may decrease soil carbon content. Ecology Letters, 7, 314-320.
- Fontaine S, Barot S (2005) Size and functional diversity of microbe populations control plant persistence and long-term soil carbon accumulation. Ecology Letters, 8, 1075-1087
- Heimann M, Reichstein M (2008) Terrestrial ecosystem carbon dynamics and climate feedbacks. Nature, 451, 289-292.
- Hobbie SE (1996) Temperature and plant species control over litter decomposition in Alaskan tundra. Ecological Monographs, 66, 503-522.

- Hovenden MJ, Miglietta F, Zaldei A, Vander Schoor JK, Wills KE, Newton PCD (2006) The TasFACE climate-change impacts experiment: design and performance of combined elevated CO₂ and temperature enhancement in a native Tasmanian grassland. Australian Journal of Botany, 54, 1–10.
- Hovenden MJ, Newton PCD, Carran RA *et al.* (2008a) Warming prevents the elevated CO₂-induced reduction in available soil nitrogen in a temperate, perennial grassland. *Global Change Biology*, **14**, 1018–1024.
- Hovenden MJ, Wills KE, Chaplin RE, Schoor JKV, Williams AL, Osanai Y, Newton PCD (2008b) Warming and elevated CO₂ affect the relationship between seed mass, germinability and seedling growth in *Austrodanthonia caespitosa*, a dominant Australian grass. *Global Change Biology*, **14**, 1633–1641.
- Hughes L (2003) Climate change and Australia: trends, projections and impacts. Austral Ecology, 28, 423–443.
- Keeler BL, Hobbie SE, Kellogg LE (2009) Effects of long-term nitrogen addition on microbial enzyme activity in eight forested and grassland sites: implications for litter and soil organic matter decomposition. Ecosystems, 12, 1–15.
- Knops JMH, Naeemw S, Reich PB (2007) The impact of elevated CO₂, increased nitrogen availability and biodiversity on plant tissue quality and decomposition. Global Change Biology, 13, 1960–1971.
- Kuzyakov Y, Friedel JK, Stahr K (2000) Review of mechanisms and quantification of priming effects. Soil Biology and Biochemistry, 32, 1485–1498.
- Lagomarsino A, De Angelis P, Moscatelli MC, Grego S (2009) The influence of temperature and labile C substrates on heterotrophic respiration in response to elevated CO₂ and nitrogen fertilization. *Plant and Soil*, 317, 223–234.
- Langley JA, McKinley DC, Wolf AA, Hungate BA, Drake BG, Megonigal JP (2009) Priming depletes soil carbon and releases nitrogen in a scrub-oak ecosystem exposed to elevated CO₂. Soil Biology and Biochemistry, 41, 54–60.
- Luo Y (2007) Terrestrial carbon-cycle feedback to climate warming. Annual Review of Ecology. Evolution and Systematics. 38, 683–712.
- Luo Y, Gerten D, Le Maire G et al. (2008) Modelled effects of precipitation on ecosystem carbon and water dynamics in different climatic zones. Global Change Biology, 14, 2365–2379.
- Luo Y, Hui D, Zhang D (2006) Elevated CO₂ stimulates net accumulations of carbon and nitrogen in land ecosystems: a meta-analysis. *Ecology*, **87**, 53–63.
- Melillo JM, Steudler PA, Aber JD et al. (2002) Soil warming and carbon-cycle feedbacks to the climate system. Science, 298, 2173–2176.
- Milchunas DG, Mosier AR, Morgan JA, Le Cain DR, King JY, Nelson JA (2005) Elevated CO₂ and defoliation effects on a shortgrass steppe: forage quality vs. quantity for ruminants. Agriculture, Ecosystems and Environment, 111, 166–184.
- Morgan J, LeCain D, Mosier A, Milchunas D (2001) Elevated CO₂ enhances water relations and productivity and affects gas exchange in C₃ and C₄ grasses of the Colorado shortgrass steppe. Global Change Biology, 7, 451–466.
- Morgan J, Mosier A, Milchunas D, LeCain D, Nelson J, Parton B (2004a) CO₂ enhances productivity, alters species composition and reduces digestibility of shortgrass steppe vegetation. *Ecological Applications*, 14, 208–219.
- Morgan JA, Pataki DE, Korner C *et al.* (2004b) Water relations in grassland and desert ecosystems exposed to elevated atmospheric CO₂. *Oecologia*, **140**, 11–25.
- Owensby CE, Ham J, Knapp A, Auen L (1999) Biomass production and species composition changes in a tallgrass prairie ecosystem after long-term exposure to elevated atmospheric CO₂. Global Change Biology, 5, 497–506.
- Parton WJ, Morgan JA, Wang GM, Del Grosso S (2007) Projected ecosystem impact of the Prairie heating and CO₂ enrichment experiment. New Phytologist, 174, 823–834.
- Pendall E, Del Grosso S, King JY et al. (2003) Elevated atmospheric CO₂ effects and soil water feedbacks on soil respiration components in a Colorado grassland. Global Biogeochemical Cycles, 17, doi: 10.1029/2001GB001821.
- Pendall E, King JY (2007) Soil organic matter dynamics in grassland soils under elevated CO₂: insights from long-term incubations and stable isotopes. Soil Biology and Biochemistry, 39, 2628–2639.
- Pendall E, Mosier AR, Morgan JA (2004) Rhizodeposition stimulated by elevated CO₂ in a semi-arid grassland. New Phytologist, 162, 447–458.
- Robertson GP, Paul E (2000) Decomposition and soil organic matter dynamics. In: Methods in Ecosystem Science (eds Sala OE, Jackson R, Mooney H, Howarth R), pp. 104–116. Springer-Verlag, New York.
- Rustad LE, Campbell JL, Marion GM et al. (2001) A meta-analysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental ecosystem warming. Oecologia, 126, 542–562.

- Sage RF, Kubien DS (2003) Quo vadis C-4? An ecophysiological perspective on global change and the future of C₄ plants. Photosynthesis Research, 77, 209–225.
- Saleska SR, Shaw MR, Fischer ML, Dunne JA, Still CJ, Holman ML, Harte J (2002) Plant community composition mediates both large transient decline and predicted long-term recovery of soil carbon under climate warming. Global Biogeochemical Cycles, 16, 1055, doi: 10.1029/2001GB001573.
- Sardans J, Penuelas J, Estiarte M, Prieto P (2008) Warming and drought alter C and N concentration, allocation and accumulation in a Mediterranean shrubland. Global Change Biology, 14, 2304–2316.
- SAS Institute Inc. (2003) SAS/STAT Software for Windows, Version 9.1. SAS Institute Inc., Carv. NC.
- Shaw M, Zavaleta E, Chiariello N, Cleland E, Mooney H, Field C (2002) Grassland responses to global environmental changes suppressed by elevated CO₂. Science, 298, 1987–1990.
- Townsend AR, Vitousek PM, Desmarais DJ, Tharpe A (1997) Soil carbon pool structure and temperature sensitivity inferred using CO₂ and ¹³CO₂ incubation fluxes from five Hawaiian soils. *Biogeochemistry*, **38**, 1–17.
- Vinton MA, Burke IC (1997) Contingent effects of plant species on soils along a regional moisture gradient in the Great Plains. Oecologia, 110, 393–402.
- Vivanco L, Austin AT (2006) Intrinsic effects of species on leaf litter and root decomposition: a comparison of temperate grasses from North and South America. Oecologia, 150, 97–107.
- Wan S, Norby RJ, Ledford J, Weltzin JF (2007) Responses of soil respiration to elevated CO₂, air warming, and changing soil water availability in a model old-field grassland. Global Change Biology, 13, 2411–2424.
- Wan S, Norby RJ, Pregitzer KS, Ledford J, O'Neill EG (2004) CO₂ enrichment and warming of the atmosphere enhance both productivity and mortality of maple tree fine roots. New Phytologist, 162, 437–446.
- Wan SQ, Hui DF, Wallace L, Luo YQ (2005) Direct and indirect effects of experimental warming on ecosystem carbon processes in a tallgrass prairie. Global Biogeochemical Cycles, 19, doi:10.1029/2004GB002315.
- Williams AL, Wills KE, Janes JK, Schoor JKV, Newton PCD, Hovenden MJ (2007) Warming and free-air CO₂ enrichment alter demographics in four co-occurring grassland species. New Phytologist, 176, 365–374.
- Zhang W, Parker KM, Luo Y, Wan S, Wallace LL, Hu S (2005) Soil microbial responses to experimental warming and clipping in a tallgrass prairie. Global Change Biology, 11, 266–277.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

- **Figure S1.** Intrinsic microbial respiration rate over the long-term incubation experiment. A. C_3 soil, 0–5 cm depth; B. C_4 soil, 0–5 cm depth; C. C_3 soil, 5–10 cm depth; D. C_4 soil, 5–10 cm depth.
- **Figure S2.** Cumulative intrinsic microbial respiration over the long-term incubation experiment. A. C_3 soil, 0–5 cm depth; B. C_4 soil, 0–5 cm depth; C. C_3 soil, 5–10 cm depth; D. C_4 soil, 5–10 cm depth.
- **Table S1.** Mean (standard error) of soil organic matter (OM) concentrations and decomposition rate of the resistant SOM pool (r in Eqn. 1). FT functional type. Results of factorial ANOVA are shown below each column.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.