

Effects of soil moisture on the temperature sensitivity of heterotrophic respiration vary seasonally in an old-field climate change experiment

VIDYA SUSEELA*, RICHARD T. CONANT†‡, MATTHEW D. WALLENSTEIN† and JEFFREY S. DUKES*§¶

*Department of Forestry and Natural Resources, Purdue University, West Lafayette, IN 47907, USA, †Natural Resource Ecology Laboratory, Colorado State University, Fort Collins, CO 80523, USA, ‡Institute for Sustainable Resources, Queensland University of Technology, Brisbane, Qld 4001, Australia, §Department of Biological Sciences, Purdue University, West Lafayette, IN 47907, USA, ¶Department of Biology, University of Massachusetts Boston, Boston, MA 02125, USA

Abstract

Microbial decomposition of soil organic matter produces a major flux of CO₂ from terrestrial ecosystems and can act as a feedback to climate change. Although climate-carbon models suggest that warming will accelerate the release of CO₂ from soils, the magnitude of this feedback is uncertain, mostly due to uncertainty in the temperature sensitivity of soil organic matter decomposition. We examined how warming and altered precipitation affected the rate and temperature sensitivity of heterotrophic respiration (R_h) at the Boston-Area Climate Experiment, in Massachusetts, USA. We measured R_h inside deep collars that excluded plant roots and litter inputs. In this mesic ecosystem, R_h responded strongly to precipitation. Drought reduced R_h , both annually and during the growing season. Warming increased R_h only in early spring. During the summer, when R_h was highest, we found evidence of threshold, hysteretic responses to soil moisture: R_h decreased sharply when volumetric soil moisture dropped below ~15% or exceeded ~26%, but R_h increased more gradually when soil moisture rose from the lower threshold. The effect of climate treatments on the temperature sensitivity of R_h depended on the season. Apparent Q_{10} decreased with high warming (~3.5 °C) in spring and fall. Presumably due to limiting soil moisture, warming and precipitation treatments did not affect apparent Q_{10} in summer. Drought decreased apparent Q_{10} in fall compared to ambient and wet precipitation treatments. To our knowledge, this is the first field study to examine the response of R_h and its temperature sensitivity to the combined effects of warming and altered precipitation. Our results highlight the complex responses of R_h to soil moisture, and to our knowledge identify for the first time the seasonal variation in the temperature sensitivity of microbial respiration in the field. We emphasize the importance of adequately simulating responses such as these when modeling trajectories of soil carbon stocks under climate change scenarios.

Keywords: apparent Q_{10} , microbial respiration, precipitation, soil respiration, warming

Received 22 January 2011 and accepted 24 June 2011

Introduction

While it is well known that both temperature and moisture are strong controls on soil heterotrophic respiration (Schimel *et al.*, 1994; Ise & Moorcroft, 2006), limited understanding of their interactive effects constrains our ability to predict ecosystem carbon fluxes under future climate regimes. Warmer temperatures accelerate heterotrophic respiration (R_h) by increasing the activity of extracellular enzymes that degrade polymeric organic matter in soils, by increasing rates of microbial uptake of soluble substrates, and by increasing microbial respi-

ration rates (Wallenstein *et al.*, 2010). Results from many models have suggested that climatic warming will accelerate the release of carbon dioxide from soils, leading to additional warming (Cox *et al.*, 2000; Dufresne *et al.*, 2002; Friedlingstein *et al.*, 2003). Many studies have examined the responses of the temperature sensitivities of labile and recalcitrant pools of soil organic matter to warming (e.g. Giardina & Ryan, 2000; Knorr *et al.*, 2005; Fang *et al.*, 2006; Conant *et al.*, 2008) as well as the mechanisms underlying those responses, such as the degree of acclimation of microbes (Luo *et al.*, 2001; Bradford *et al.*, 2008; Allison *et al.*, 2010) or depletion of substrates (Kirschbaum, 2004; Eliasson *et al.*, 2005; Hartley & Ineson, 2008). However, the temperature sensitivity of soil organic matter decomposition is not only governed by the molecular structure of the substrate but also by the environmental constraints

Correspondence: Vidya Suseela, Department of Forestry and Natural Resources, Purdue University, West Lafayette, IN 47907, USA, tel. +1 765 494 3531, fax +1 765 494 9461, e-mail: vsuseela@purdue.edu

that will be altered by changing climatic conditions (Davidson & Janssens, 2006; Conant *et al.*, 2011).

Many studies have shown that warming accelerates microbial respiration, but most of these studies have examined soil in laboratory conditions, eliminating many environmental constraints that affect the temperature sensitivity of organic matter decomposition. To date, most of the ecosystem-scale experiments that used trenching to separate R_h have focused on the contribution of R_a (autotrophic respiration) and R_h to total soil respiration (Gaumont-Guay *et al.*, 2008; Ruehr & Buchmann, 2010; Subke *et al.*, 2011), rather than the effects of climate change on the components of soil respiration. A recent trenching experiment conducted in a Norway spruce forest found that experimental warming increased R_h (Schindlbacher *et al.*, 2009). Warming of 2°C also increased R_h in a tallgrass prairie ecosystem (Zhou *et al.*, 2007). However, Lavigne *et al.* (2003) found that annual R_h varied little across a natural climatic gradient. These inconsistent responses could be due to interactions with other environmental constraints on decomposition, such as soil moisture, which will be affected by changing precipitation patterns and evapotranspiration (Weltzin *et al.*, 2003).

Moisture limitation can suppress, microbial activity, regardless of temperature, which should decrease the temperature sensitivity of R_h (Davidson & Janssens, 2006). Currently, most biogeochemical models represent the relationship between soil organic matter decomposition and warming using a temperature function with a fixed Q_{10} (Davidson *et al.*, 2006; Zhou *et al.*, 2009), with a reducing function for moisture (Bauer *et al.*, 2008). However, a recent laboratory study found that the temperature sensitivity of R_h is also affected by soil moisture, and the nature of this interaction varied between two different soils (Craine & Gelderman, 2011). Within an ecosystem, we do not know if these interactions are consistent through time, or whether they vary seasonally. One might expect seasonal variation; for instance, microbial community composition and exo-enzyme production could vary with seasonal changes in substrate and nutrient availability, and studies have shown that the temperature sensitivity of microbial enzymes varies with seasons (Wallenstein *et al.*, 2009).

It is difficult to examine the effect of moisture on the temperature sensitivity of R_h using field-based data because other factors such as substrate supply and microbial biomass vary throughout a season (Davidson *et al.*, 2006), and because of the difficulty in separating heterotrophic and autotrophic respiration (Ryan & Law, 2005). Ecosystem-scale experiments that manipulate both soil temperature and moisture provide an opportunity to simultaneously measure R_h under a range of abiotic conditions. To date, very few ecosys-

tem-scale experiments have studied the combined effects of warming and precipitation on soil respiration (Wu *et al.*, 2011) and even fewer have attempted to isolate the heterotrophic component of respiration. We are not aware of any previous such studies in mesic systems, where we might expect moisture to have a less important role in controlling R_h than in arid ecosystems.

Ecosystem responses to climate change can be non-additive or nonlinear (Gill *et al.*, 2002; Burkett *et al.*, 2005), and both plants and microbes can exhibit threshold responses (Porporato *et al.*, 2004). Identifying these nonlinearities and thresholds could reduce some uncertainties associated with climate change decision-making (Zhou *et al.*, 2008). To date, most climate change experiments have examined only two levels of each climatic factor, but nonlinear and threshold responses can be determined most convincingly when experiments have multiple levels of each climatic factor.

The main objectives of this study were to characterize the diel, seasonal and annual responses of microbial respiration in a mesic, old-field ecosystem to a gradient of warming and altered precipitation. We identify the main and interactive effects of multiple levels of warming and altered precipitation on the short-term (seasonal) and long-term (annual) temperature sensitivity of R_h . We hypothesized that altered precipitation would modify the rate and temperature sensitivity of R_h . More specifically, we expected warming to decrease R_h and its temperature sensitivity more dramatically under drought conditions.

Materials and methods

Site description

The study took place within the Boston-Area Climate Experiment (BACE), located at the University of Massachusetts Suburban Experiment Station in Waltham, Massachusetts (42°23.1'N, 71°12.9'W). In nearby Boston, long-term means for annual precipitation and temperature are 1063 mm and 10.3°C. Historical monthly average rainfall in Bedford, MA (1952–2008) reveals large interannual variation in precipitation patterns (Fig. S1). The soil at BACE is a Mesic Typic Dystrudept (Haven series) with loamy topsoil (45% sand, 46% silt, 9% clay; gravel content: 7%) and a gravelly sandy loam subsoil. The old-field plant community harbors about 38 species of grasses and forbs, most of which are non-native. During the peak growing period (June 2009), mean percent cover of grasses and forbs in the unwarmed ambient treatment was 29% and 52%, respectively. In addition to the herbaceous community, the 2 m by 2 m plots at the BACE contained seedlings of four tree species (*Acer rubrum*, *Betula lenta*, *Quercus rubra*, *Pinus strobus*) in 0.5 m by 0.5 m subplots. Mean aboveground

biomass production in the unwarmed, ambient precipitation treatment was 425 g m^{-2} in 2009. The average leaf area index (May–September 2009) of the unwarmed, ambient precipitation treatment was 1.55.

Experimental design

The BACE infrastructure exposed each plot to one of 12 different treatments: four levels of warming (referred to as unwarmed, low, medium, and high warming) were crossed with three levels of precipitation [ambient, wet (+50%) and drought (−50%)] in a factorial design (Tharayil *et al.*, 2011). Precipitation treatments were applied to a zone within each of the three experimental blocks using rainout shelters, storage tanks, and sprinklers. The drought zone lay under a shelter with 15 cm-wide clear polycarbonate slats spaced 15 cm apart, which removed 50% of the incoming rainfall and transferred it to a storage tank. In the non-freezing months, this water was immediately pumped to sprinklers above the wet zone. A group of four plots lay within each zone, with one plot subjected to each of the four warming treatments. Warming of the plant canopy and soil was achieved using ceramic infrared heaters mounted 1 m above the ground at each corner of each plot. Feedback control was achieved within each group of four plots based on the difference in temperature between the plant canopies of the warmest and ambient plots, which was monitored every 10 s. Different wattage heaters (200, 600, 1000) were used to achieve different levels of warming. The system limited warming of the plant canopy to a maximum of 4°C above ambient in the warmest plots. There were a total of 36 experimental plots (three replicates of each of the 12 treatments). Plots were arranged linearly within each block, and were spaced 1 m apart. In 2007 a 0.6 m deep trench was dug around each plot, and lined with layers of polyethylene sheets to prevent the lateral movement of water and nutrients between the plots. All treatments were in effect as of July 2008.

Soil responses

To create an area without plant roots, we installed a 25 cm diameter PVC collar to a depth of 30 cm in each experimental plot in November 2007. We weeded plants from the collar at the time of installation. To prevent regrowth, we kept the collar covered with brown non-woven spunbound DeWitt weed fabric (Gemplers, LLC, Madison, WI, USA) that prevented light transmission but was permeable to water and air, and checked the collars regularly for weeds. Thus, weed growth was completely prevented in the collars. Root growth below 30 cm was minimal in these plots. The average root production in the unwarmed, ambient precipitation plots (2009) at 0–10 cm and 10–40 cm depth was $153 \text{ g m}^{-2} \text{ yr}^{-1}$ (79%) and $40 \text{ g m}^{-2} \text{ yr}^{-1}$ (20%), respectively. The 'B' horizon (observed during trenching the plots at the beginning of the experiment) did not extend below 25–30 cm in any of the plots, and the soil profile was gravelly after 30 cm. The contribution of roots from tree seedlings was also likely minimal beyond 30 cm, since the seedlings had been planted in late summer of 2008.

Measuring heterotrophic respiration (R_h)

Separation of soil respiration into its components (autotrophic and heterotrophic) is not a straightforward task and each method has its own problems. Studies have found that inserting deep collars to exclude plant roots is an effective technique to separate R_h (Zhou *et al.*, 2007) although it has some drawbacks. Any method used to determine R_h by excluding plants will significantly alter soil moisture due to lack of plant uptake (Hanson *et al.*, 2000), but to our knowledge the actual moisture content from such collars has not been measured in previous climate change experiments. At BACE, root exclusion collars increased soil moisture, but did not affect the fidelity of the drought treatments.

Another challenge of using deep collars to estimate microbial respiration is the potential for measurements to include decomposition of pre-existing roots. Measurements presented here were taken from March 2009 to 2010, at least 15 months after the installation of deep collars in November 2007. This should have allowed sufficient time for labile compounds from any roots in the collar to decompose, thereby minimizing the influence of old roots in our measurements. The diffusion of CO_2 from outside the collar area into the plant exclusion collar may be another concern with deep collars. While we cannot rule out the possibility of some diffusion, we think any contribution would be minimal. In our old-field system we did not have much root growth below 30 cm and because the moisture content of plant exclusion collar was always greater than the rest of the plot (data not shown), we would expect slower diffusion of CO_2 through soil in the collars than through soil in the vegetated areas.

Since the collars excluded plant roots and fresh litter input, we attribute respiration measured within the collars entirely to the non-rhizosphere associated microbial decomposition of soil organic matter. We measured respiration monthly, using a LI-COR 6400-09 soil CO_2 flux chamber attached to a 6400 portable photosynthetic system. Respiration was measured between 10:00 and 16:00 hours (local time) in a small PVC collar (10 cm in diameter and 5 cm in height) installed 2–3 cm into the soil within the larger collar. During winter, snow was removed from the collars before measurements to avoid the influence of snow on soil CO_2 efflux. Soil temperature at 5 cm depth was measured simultaneously during respiration measurements using a thermocouple probe. We also measured volumetric moisture in the top 10 cm of soil on the same day of the respiration measurements, using time domain reflectometry waveguides installed vertically in the plant exclusion collars. In August 2009 and April 2010, we also measured respiration at two-hour intervals for 24-hour periods in the unwarmed and high warming plots within all three precipitation treatments.

Data analysis

We used a mixed-model restricted maximum likelihood (REML) analysis with repeated measures to identify any main and interactive effects of warming and altered precipitation on R_h , soil temperature and soil moisture. The four levels of

warming and three levels of precipitation were designated numerically in the statistical design. To identify seasonal effects, we also conducted separate mixed-model analyses for the spring (March–May), summer (June–August), fall (September–November) and winter (December–February). We used Tukey's HSD multicomparison test to detect differences among individual treatments. Statistical analyses were performed using SAS version 9.2 (SAS Institute, Inc., 2002–2008, North Carolina, USA).

We calculated annual, growing season (April–September) and non-growing season (October–March) cumulative respiration using the method followed by Bremer *et al.* (1998). Our measurements were assumed to represent daily maximum soil CO₂ efflux. We calculated the daily minimum efflux as a percentage of maximum based on the diel data taken periodically. We then multiplied the corrected daily flux with the number of days between each measurement and calculated the cumulative flux over the entire year, as well as during the growing and non-growing seasons.

We assessed the temperature sensitivity of R_h by fitting data collected over time (April 2009–March 2010) within each individual treatment to the exponential function

$$R_h = ae^{bT} \quad (1)$$

where R_h is the measured CO₂ efflux ($\mu\text{mol m}^{-2} \text{s}^{-1}$), T is the soil temperature ($^{\circ}\text{C}$) at 5 cm depth, a is the basal respiration (intercept of soil CO₂ efflux when temperature is zero) and b is the temperature sensitivity of soil CO₂ efflux. Respiratory quotient (Q_{10}) is calculated as:

$$Q_{10} = e^{10b} \quad (2)$$

Seasonal variation in plant activity strongly influences the Q_{10} of total soil respiration (Wang *et al.*, 2010). Since our collars excluded litter and labile carbon inputs, we assumed that seasonal variation in plant activity would not influence our measurements of R_h . However, seasonal changes in temperature and soil moisture could exert a major control over temperature sensitivity of R_h . Therefore, Q_{10} is referred to as apparent temperature sensitivity or apparent Q_{10} hereafter. In addition to annual apparent Q_{10} , we calculated the apparent Q_{10} for individual seasons, and refer to this hereafter as seasonal apparent Q_{10} . We used monthly measurements within each season to calculate the seasonal apparent Q_{10} for each plot. We then used mixed model REML analysis to determine whether warming and precipitation treatments affected the annual and seasonal apparent Q_{10} of R_h .

To account for variation in R_h due to soil moisture, both alone and in combination with temperature, we used separate functions to model respiration. Measured respiration rates were fit to soil moisture content using the quadratic function

$$R_h = y_0 + ax + bx^2 \quad (3)$$

where x is the volumetric moisture content (V/V) in the top 10 cm of soil, and y_0 , a and b are constants. All curves were fit with SigmaPlot version 11 (Systat Software, Inc., San Jose, CA, USA).

We fit respiration measurements to both soil temperature and soil water content with a combined exponential and

quadratic function used by Mielnick & Dugas (2000):

$$R_h = (ae^{bT})^* [2.12 * (\theta v - \min \theta v) * (\max \theta v - \theta v)^c] \quad (4)$$

where θv is the volumetric moisture content, the minimum volumetric water content of our data set was 5.7% and the maximum was 38.7%.

We used Matlab (The MathWorks, Inc., 1994–2010, Natick, MA, USA) to generate a response surface based on the Mielnick–Dugas equation. We determined coefficients for soil temperature and soil moisture for each treatment, in both annual and seasonal analyses.

Results

Soil microclimate

The precipitation treatments effectively altered the amount of daily rainfall (Fig. S2) in the drought and wet treatments. The effect of the precipitation treatments on soil moisture varied by month, with the largest effects generally occurring during the growing season (Fig. 1a; Table S1; $P < 0.0001$). For example, at the end of spring (May), soil moisture in the drought treatment was 31% lower than in ambient plots ($P = 0.0171$) and 41% lower than in wet treatments ($P < 0.0001$). This pattern was again observed during the latter half of fall (October–November). Drought treatment soils were drier than wet treatment soils from summer (36%; $P = 0.0292$) until the beginning of fall (43% drier in September; $P < 0.0001$).

Warming treatments altered soil temperature throughout the year ($P < 0.0001$) except for January (Fig. 1b; Table S2; $P < 0.0001$), when soils in the unwarmed, ambient treatment were frozen to about 12 cm depth, with an average soil temperature of 0.6°C at 10 cm. All levels of warming increased soil temperature during the spring, summer, and fall relative to the unwarmed treatment ($P < 0.0001$). Soils were warmed by an annual average of 1, 2.3, and 3.5°C in the low, medium and high warming treatments, respectively, compared to unwarmed plots. During August and September, drought treatment soils were warmer than wet treatment soils (by 2°C ; $P < 0.05$).

Heterotrophic respiration: annual and seasonal pattern

Seasonal patterns of R_h generally followed the seasonal variation of soil temperature, ranging from a minimum of $0.21 \mu\text{mol m}^{-2} \text{s}^{-1}$ in February to a maximum of $5.09 \mu\text{mol m}^{-2} \text{s}^{-1}$ in June in the unwarmed, ambient precipitation treatment (Fig. 2a). Both drought and supplemental precipitation decreased respiration rates across all warming treatments, primarily during the growing season (Fig. 2b, c). Over the year (April 2009–

March 2010) and within seasons, the effect of warming and precipitation treatments on R_h varied by month (Table S3; $P < 0.0001$).

During early spring (March), high (43%) and medium (55%) warming treatments had greater R_h than the unwarmed treatment (Fig. 3a; $P < 0.01$). In April, the medium warming treatment increased R_h by 36% relative to the unwarmed treatment ($P = 0.0257$). This pattern reversed in late spring (May), when medium and high warming treatments suppressed R_h relative to unwarmed and low warming treatments. Warming did not affect R_h during summer and fall. In winter, the effect of warming varied by month ($P = 0.0475$) with high and medium warming accelerating respiration in December and February.

During spring, summer, and fall, precipitation treatments affected R_h (Fig. 3b). During late spring (May) drought suppressed respiration relative to the ambient precipitation treatment (by 22%; $P = 0.03$). During June, drought decreased respiration compared to both ambient (by 29% June; $P = 0.0135$) and wet treatments (by 35%; $P = 0.0007$). At that time, soil volumetric water content dropped below 15% in the drought treatment (Fig. 4). Although soils in the drought treatment had become wetter by August, microbial respiration did not increase correspondingly. R_h in the supplemental precipitation treatment peaked in June, when soil volumetric

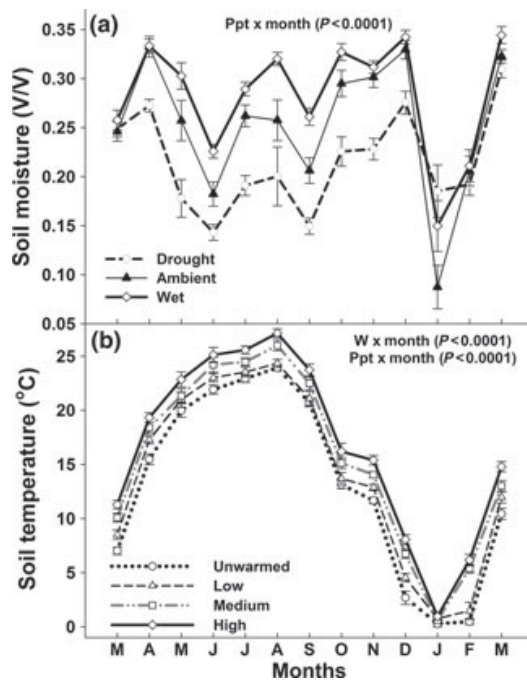


Fig. 1 Seasonal variation in (a) volumetric soil moisture ($n = 12$) and (b) soil temperature at 5 cm depth ($n = 9$) in the plant exclusion collar from March 2009 to March 2010. Values represent means \pm SE. Key: W, warming; Ppt, precipitation.

water content remained between 20% and 25% (Fig. 4). In July, when water content increased above 26% in the supplemental precipitation treatment, R_h declined sharply. During late summer and early fall, drought reduced respiration compared to ambient precipitation (August: 31%; $P = 0.0062$; September: 27%; $P = 0.0006$). During mid-fall (October) drought again decreased respiration compared to both ambient (by 24%; $P = 0.0044$) and supplemental precipitation treatments (by 21%; $P = 0.0230$).

Heterotrophic respiration: diel pattern

The diel pattern of R_h did not closely follow the diel increase in soil temperature at either measurement date

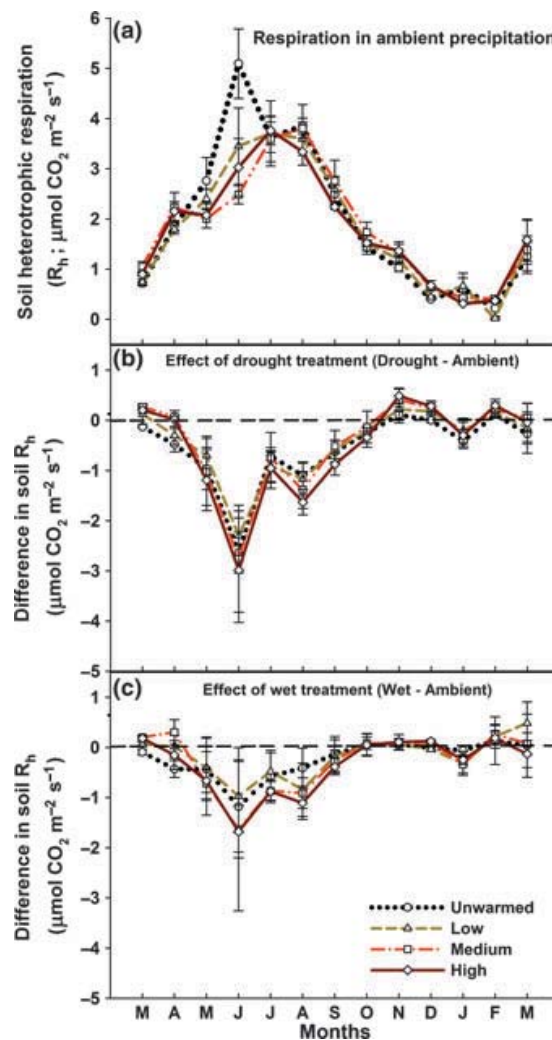


Fig. 2 (a) Seasonal variation in R_h in the ambient precipitation treatment from March 2009 to March 2010 (b) Response of R_h to the drought treatment, measured as the difference between R_h in the droughted treatments and that in the unwarmed ambient precipitation (control) treatments. (c) Effect of the wet treatment on R_h , calculated as in (b). Values represent means ($n = 3$) \pm SE.

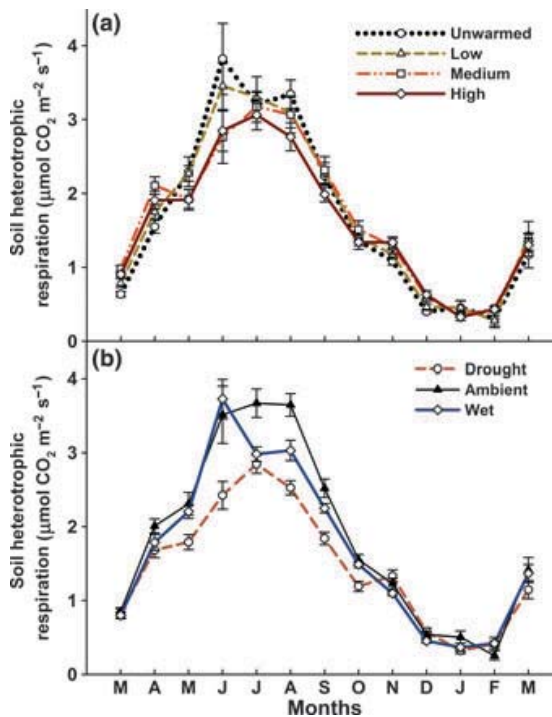


Fig. 3 (a) Seasonal variation in R_h averaged by warming treatment ($n = 9$) and (b) precipitation treatment ($n = 12$) from March 2009 to March 2010. Values represent means \pm SE. Key: W, warming; Ppt, precipitation.

(Figs S3 and S4), although precipitation and warming treatments affected mean daily R_h in August. On that day, high warming suppressed mean daily R_h ($4.8 \mu\text{mol m}^{-2} \text{s}^{-1}$) by 20% compared to the unwarmed treatment ($6.0 \mu\text{mol m}^{-2} \text{s}^{-1}$; $P = 0.0036$). Drought also suppressed mean daily R_h relative to the ambient treatment ($P = 0.0077$) but not relative to the wet treatments. In April 2010, climate treatments did not significantly affect mean daily R_h .

Heterotrophic respiration: cumulative measures

The precipitation treatments affected both annual and growing season cumulative R_h ($P < 0.001$; Fig. 5; Table S4). Drought ($452 \pm 15 \text{ g C m}^{-2} \text{ yr}^{-1}$) suppressed annual cumulative R_h relative to both ambient ($575 \pm 25 \text{ g C m}^{-2} \text{ yr}^{-1}$; $P = 0.0003$; Fig. 5a) and wet treatments ($535 \pm 13 \text{ g C m}^{-2} \text{ yr}^{-1}$; $P = 0.0083$). During the growing season, drought similarly decreased cumulative R_h compared to ambient (30%; $P < 0.0001$; Fig. 5b) and wet treatments (22%; $P = 0.0004$). During the non-growing season, precipitation did not affect R_h ($P = 0.3979$). Warming treatments did not affect annual ($P = 0.7946$) or growing season cumulative R_h ($P = 0.3231$), but marginally increased respiration in the non-growing season (23%; $P = 0.0723$).

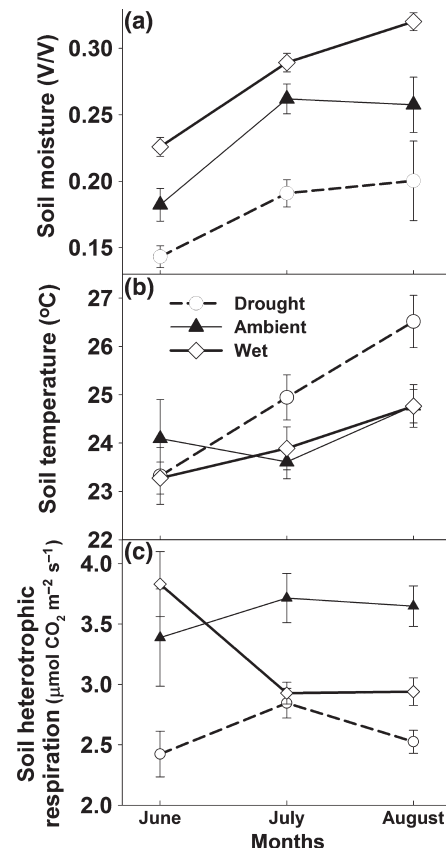


Fig. 4 Effect of precipitation treatments on soil moisture at 10 cm depth (a), soil temperature at 5 cm depth (b) and R_h (c) during summer 2009. Values represent means ($n = 12$) \pm SE.

Heterotrophic respiration: temperature sensitivity

Model fits using the exponential function in Eqn (1) showed that R_h was strongly related to soil temperature at 5 cm depth (Fig. 6 and Table 1). When analyzed by treatment, the annual apparent Q_{10} of R_h ranged from 1.57 (drought + high warming) to 2.09 (ambient + high warming). However, mixed model analysis of annual Q_{10} values found no effect of warming treatments on apparent Q_{10} ($P = 0.439$). The drought treatment marginally decreased apparent Q_{10} (1.78; $P = 0.0692$) compared to ambient precipitation (2.07).

In spring, warming affected the apparent Q_{10} of R_h (Fig. 7a; Table S5; $P = 0.0009$). High and medium warming suppressed the apparent Q_{10} relative to the unwarmed treatment ($P < 0.01$). During the summer, apparent Q_{10} did not vary with warming and precipitation treatments (Fig. 7b).

In the fall, both precipitation ($P = 0.0008$) and warming ($P = 0.0255$) affected apparent Q_{10} , with drought decreasing Q_{10} below that of ambient and wet treatments (Fig. 7c). The high warming treatment decreased

apparent Q_{10} relative to the unwarmed (Fig. 7c; $P = 0.0458$) and low warming treatments ($P = 0.0425$). In the winter, neither warming nor precipitation treatments affected apparent Q_{10} of R_h .

We found a positive correlation of annual, spring and fall apparent Q_{10} to soil volumetric moisture content at 10 cm depth (Fig. 8a; Table 2) and a negative correlation to soil temperature at 5 cm depth (Fig. 8b; Table 2). However, in summer apparent Q_{10} was not related to soil water content or soil temperature.

Heterotrophic respiration: characterizing sensitivity to moisture and temperature

Although more than 50% of the variation in annual R_h in our study was explained by soil temperature at 5 cm depth (Eqn 1), the combined soil temperature and moisture model best captured annual variability in 8 out of 12 treatments (Eqn 3; Table S6). In spring and fall most of the variability was explained by the temperature function, but in summer the moisture function alone provided the best fit. The surface response curve produced by fitting the Mielnick–Dugas equation combining both temperature and moisture to the unwarmed, ambient precipitation treatment (Fig. 9) illustrated that respiration proceeded fastest at the

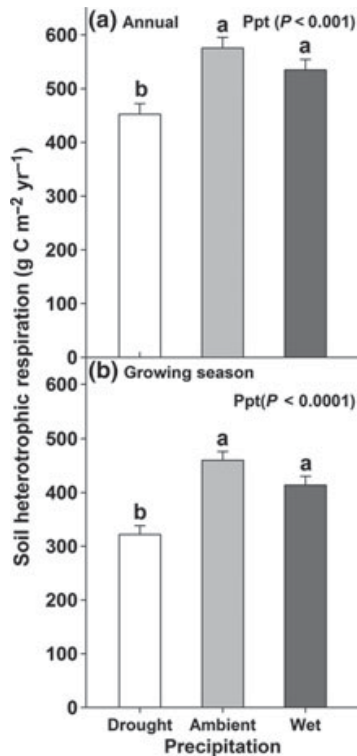


Fig. 5 Annual (a) and growing season (b) cumulative R_h . Values represent means ($n = 12$) \pm SE.

warmest temperatures when soils contained 20–30% volumetric water content.

Discussion

To our knowledge, this is the first study to examine the temperature sensitivity of heterotrophic respiration both annually and seasonally, as affected by experimental warming and altered precipitation. Our results clearly suggest that the effect of warming on R_h and its temperature sensitivity varied seasonally. In contrast to warming, altered precipitation strongly affected R_h , with cumulative R_h decreasing in drought. Even in this mesic ecosystem, where water might be expected to play only a weak role in controlling R_h , respiration

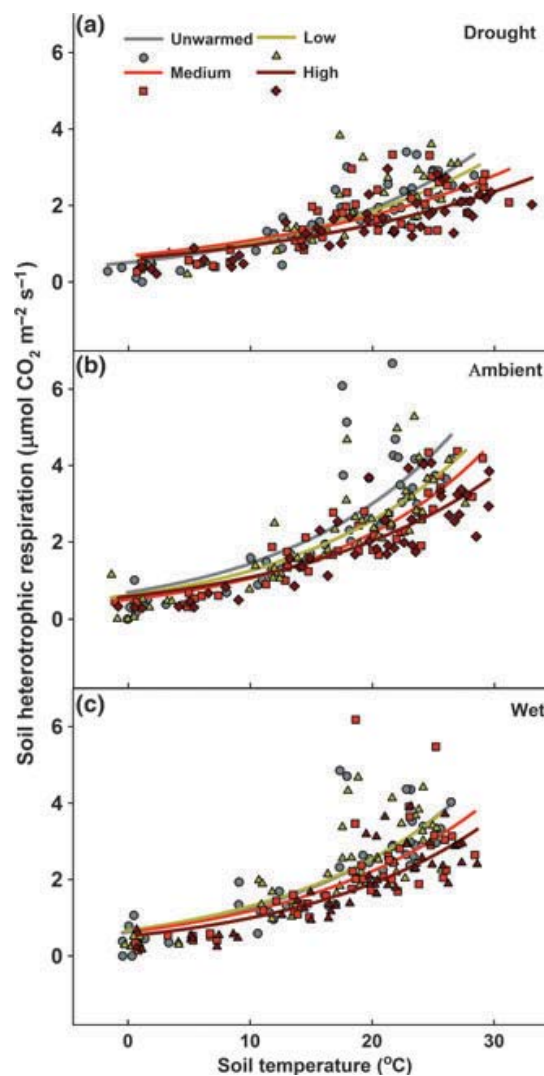


Fig. 6 Exponential relationship between R_h and soil temperature (April 2009–March 2010) in drought (a) ambient (b) and wet (c) precipitation treatments. Points represent individual plot measurements. Details in Table 1.

Table 1 Exponential relationship between R_h and soil temperature (April 2009–March 2010) and apparent Q_{10} values

| Warming | Drought | Ambient | Wet |
|----------|--|--|--|
| Unwarmed | $R_h = 0.59e^{0.06T}$, $r^2 = 0.60$, $Q_{10} = 1.77$ | $R_h = 0.70e^{0.07T}$, $r^2 = 0.61$, $Q_{10} = 2.07$ | $R_h = 0.67e^{0.07T}$, $r^2 = 0.66$, $Q_{10} = 1.96$ |
| Low | $R_h = 0.51e^{0.07T}$, $r^2 = 0.77$, $Q_{10} = 1.94$ | $R_h = 0.63e^{0.07T}$, $r^2 = 0.69$, $Q_{10} = 2.03$ | $R_h = 0.62e^{0.07T}$, $r^2 = 0.66$, $Q_{10} = 2.01$ |
| Medium | $R_h = 0.60e^{0.05T}$, $r^2 = 0.61$, $Q_{10} = 1.57$ | $R_h = 0.51e^{0.07T}$, $r^2 = 0.82$, $Q_{10} = 2.09$ | $R_h = 0.62e^{0.06T}$, $r^2 = 0.50$, $Q_{10} = 1.89$ |
| High | $R_h = 0.68e^{0.05T}$, $r^2 = 0.57$, $Q_{10} = 1.60$ | $R_h = 0.60e^{0.06T}$, $r^2 = 0.69$, $Q_{10} = 1.84$ | $R_h = 0.52e^{0.06T}$, $r^2 = 0.68$, $Q_{10} = 1.91$ |

dropped sharply at both lower and upper soil moisture thresholds. The observed hysteresis in R_h following drought (see Fig. 10, and below) may have been due to changes in soil physical properties and lags in microbial activity. Further research to characterize the mechanisms driving these patterns could contribute to a generalizable framework for reducing uncertainty in predictions of climate-carbon feedbacks.

Responses of heterotrophic respiration to warming

Based on kinetic theory (Arrhenius, 1889), it is widely assumed that microbial decomposition of soil organic matter increases with temperature. Thus, climate warming is expected to stimulate heterotrophic respiration and produce a positive feedback by increasing atmospheric CO_2 . Consistent with this expectation, many studies have found positive relationships between temperature and R_h within ecosystems over time (Lin *et al.*, 1999; Zhou *et al.*, 2007; Schindlbacher *et al.*, 2009). In contrast, we found that experimental warming did not consistently stimulate R_h in this mesic old-field ecosystem. Warming stimulated R_h only during the early spring. Early spring coincides with freeze-thaw cycles, which may have provided a flush of labile carbon at a time of optimal soil moisture for microbial activity (Matzner & Borken, 2008). The labile carbon could have come from microbial biomass (Skogland *et al.*, 1988) or the release of physically protected organic matter due to changes in aggregation following a freeze-thaw cycle (Herrmann & Witter, 2002). By late spring, the warming effect disappeared as warming intensified soil moisture limitation, which likely triggered substrate limitation of microbial respiration. Previous studies in arid and semiarid ecosystems have also shown such that the indirect effects of warming on soil moisture can outweigh the thermal stimulation of microbial activity (Almagro *et al.*, 2009; Liu *et al.*, 2009). Our results suggest that this can occur even in mesic systems where moisture is not generally thought to limit microbial activity.

During summer and fall, R_h did not respond to warming even in the ambient and wet treatments, where soil moisture was unlikely to be limiting. We speculate that the lack of a temperature response dur-

ing this period could have been due to reductions in microbial biomass or changes in community composition resulting from (unmeasured) changes in substrate availability. Our results suggest that there are many conditions under which warming will not stimulate microbial respiration; microbes can experience substrate limitation due to limited enzyme activity or limited diffusion under dry conditions, and some conditions might induce stress responses or even reduce microbial biomass.

Responses of heterotrophic respiration to altered precipitation

In contrast to the limited effects of experimental warming, our results showed that altered precipitation can strongly influence R_h , not only through well-known direct effects, but also by altering the apparent temperature sensitivity of R_h . Annual cumulative R_h decreased by 21% with drought, but did not change in the wet treatments relative to ambient. These results are consistent with those of a girdling experiment conducted in a pine forest, where drought significantly suppressed R_h (Scott-Denton *et al.*, 2006). Other studies in old-field systems have found total soil respiration (including root respiration) unresponsive to warming, but sensitive to soil moisture (Wan *et al.*, 2007; Garten *et al.*, 2009). Soil water content can alter R_h by changing substrate availability and by altering the composition and activity of decomposer microbes (Williams, 2007). In drier soils, a thinner film of water coats the soil particles, slowing the diffusion of labile substrates and reducing the activity of exo-enzymes needed for the decomposition of organic matter (Stark & Firestone, 1995).

Threshold, hysteretic responses of heterotrophic respiration to summer soil moisture

In contrast to many other studies, and to moisture functions incorporated into some biogeochemical models, maximum R_h occurred at intermediate moisture levels (Fig. 9). Soil water content below a threshold of about 15% may have imposed desiccation stress on microbial decomposers, and may have limited diffusion of soluble substrates that are necessary for microbial growth

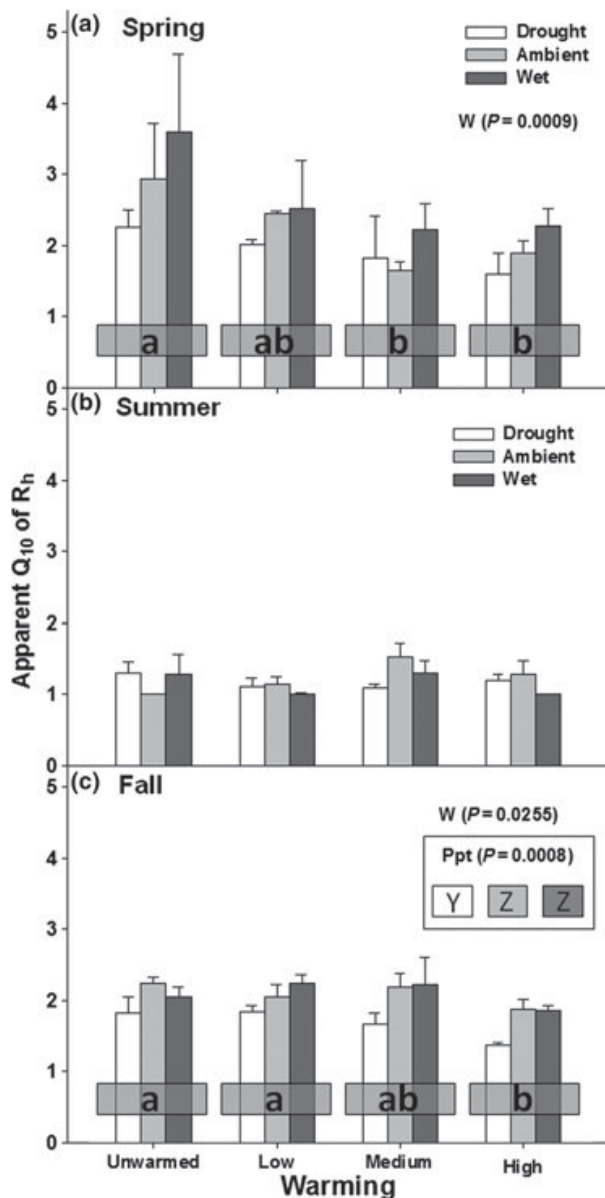


Fig. 7 Apparent Q_{10} of R_h in spring (a), summer (b), and fall (c). Values represent means \pm SE ($n = 3$). Letters a and b indicates difference (Tukey's HSD) between the warming treatments and y and z between precipitation treatments.

and metabolic activity. In the drought treatment, we saw evidence for hysteresis: although soil water content increased from $<15\%$ in June to 20% in August in the drought treatment, R_h remained low (Fig. 10). We suspect that the microbial community was stressed by the drought, causing a decline in biomass and activity. Following rewetting, there may have been a lack of heterotrophic activity prior to population recovery. Microbes may have also allocated more assimilated C to growth than respiration during the recovery period, resulting in increased carbon use efficiency, which may be a criti-

cal control on R_h rates (Allison *et al.*, 2010). The hysteresis in R_h could also be explained by changes in soil structural properties during drought that further affect soil microbes and the mobility of enzymes and substrates. Soil properties such as water repellency and aggregate structure can change with soil drying, affecting soil water holding capacity, surface tension, and other properties upon rewetting (Derjaguin & Churaev, 1986; Bauters *et al.*, 1998; Goebel *et al.*, 2011). Water repellency due to prolonged drying can prevent the homogenous rewetting of the organic horizon, which could delay the recovery of respiration following rewetting of dried soils (Muhr & Borken, 2009). These results are important in the context of changing precipitation patterns; future projections suggest less frequent, more intense rainfall, increasing the severity of drought in some systems (Easterling *et al.*, 2000; Neelin *et al.*, 2006).

Heterotrophic respiration declined above a threshold of about 26% soil moisture. Soil water content above this threshold could reduce the diffusion of oxygen and thus impede microbial activity (Skopp *et al.*, 1990; Luo & Zhou, 2006). At soil water contents between 15% and 25% , heterotrophic activity maintained a nearly constant rate in the ambient precipitation treatment.

Studies to date have highlighted the potential of reduced precipitation to affect carbon balance in semi-arid ecosystems, but mesic systems are thought to be less prone to changes in moisture availability (Wu *et al.*, 2011). Our study site is a mesic system with intermediate water levels where R_h strongly responded to the alteration in the quantity of rainfall. Mesic systems such as ours could have nonlinear responses to microbial respiration with distinct soil moisture thresholds (Knapp *et al.*, 2008) such as those we found evidence for. If similar thresholds separate the upper and lower limit of optimum soil moisture for soil microbes in other systems, responses similar to the framework in Fig. 10 could be implemented in models to improve predictions of the consequences of projected climatic scenarios (Porporato *et al.*, 2004) for soil organic matter decomposition. It is possible that hysteresis could be better captured in models of soil respiration when the measure of water content is replaced by water potential. Hysteresis is commonly observed in the relationship between soil water content and water potential when a given soil is dried and then rewetted, and can be caused by a variety of changes in soil properties (Haines, 1930; Klausner, 1991). After drying, soil holds water with greater tenacity at a given water content; this phenomenon has been simulated with a variety of hysteresis models (Pham *et al.*, 2005). Studies have shown that soil matric potential is a better index of soil water availability for plant roots and microbes than vol-

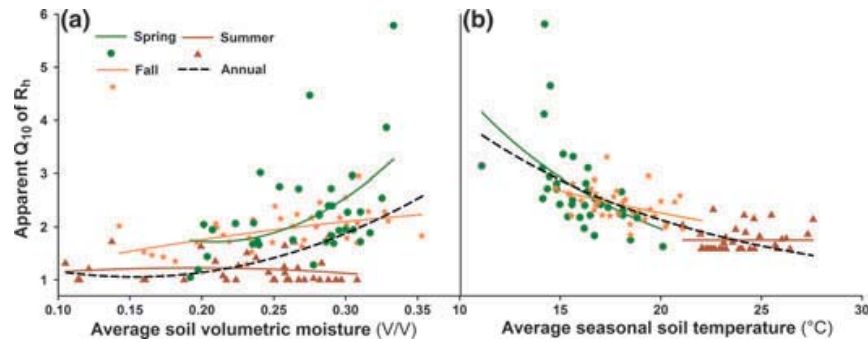


Fig. 8 The relationship between apparent Q_{10} of R_h to soil volumetric moisture at 10 cm depth (a) and soil temperature at 5 cm depth (b). Points represent seasonal apparent Q_{10} values of individual plots estimated using Eqn (1). Details in Table 2.

Table 2 Correlation of apparent Q_{10} of R_h to soil temperature (5 cm) and soil volumetric moisture content (10 cm)

| Season | Correlation with soil temperature | Correlation with soil moisture |
|--------|--|---|
| Spring | $Q_{10} = 13.6564 \exp(-0.1127 x); r^2 = 0.30$ | $Q_{10} = 6.0846 - 41.7753 x + 99.9902 x^2; r^2 = 0.24$ |
| Summer | $Q_{10} = 1.1886 \exp(2.3622E-011 x); r^2 = 0$ | $Q_{10} = 0.8973 + 3.4273 x - 8.8545 x^2; r^2 = 0.01$ |
| Fall | $Q_{10} = 4.5528 \exp(-0.0472 x); r^2 = 0.22$ | $Q_{10} = 0.7018 + 6.5199 x - 6.1747 x^2; r^2 = 0.27$ |
| Annual | $Q_{10} = 8.6787 \exp(-0.0838 x); r^2 = 0.50$ | $Q_{10} = 2.1655 - 10.8743 x + 35.7325 x^2; r^2 = 0.20$ |

umetric or gravimetric water content, particularly at lower water contents (Davidson *et al.*, 1998).

Our study also highlights the importance of seasonal variability in precipitation for the response of soil carbon to warming. Periods of rain during the growing season may lead to carbon losses through increased R_h , whereas periods of drought will decrease microbial activity and hence R_h . However, some of the carbon stored due to decrease in R_h due to drought could be released during subsequent wet periods or years (Borken *et al.*, 2006). Most of the recent debate on the carbon

feedback to climate has focused on the temperature effect on soil organic matter decomposition (Melillo *et al.*, 2002; Kirschbaum, 2004; Reichstein *et al.*, 2005). However, we found that the absolute rate of R_h responded more strongly to altered precipitation than warming in all three growing seasons. This suggests that projected changes in rainfall patterns during these growing seasons could strongly influence carbon storage or loss.

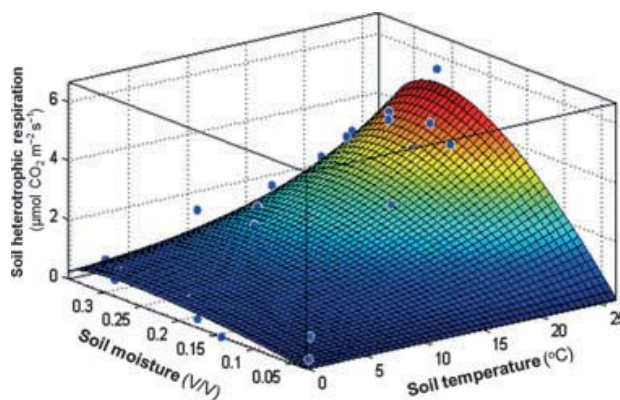


Fig. 9 Response surface curve formed using the output from Mielnick and Dugas model for the unwarmed, ambient precipitation treatment. The model combines both soil temperature data (5 cm depth) and volumetric moisture content (10 cm depth) for the entire year (2009–2010) to generate the response surface.

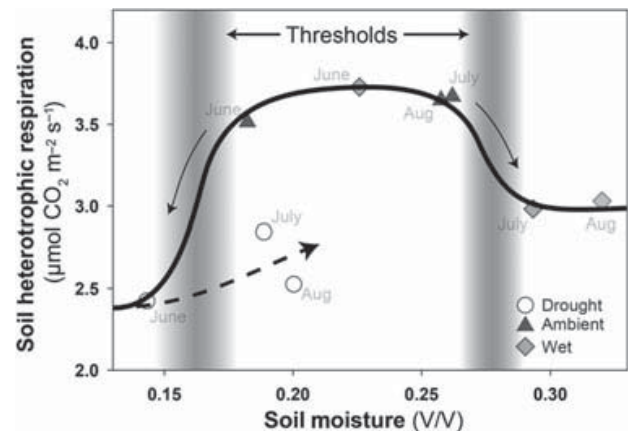


Fig. 10 Conceptual diagram illustrating threshold, hysteretic responses of R_h to soil moisture during summer. Shaded areas represent the lower and upper thresholds. Solid arrows show the trajectory of R_h as soil moisture slips out of the optimum zone. The broken arrow shows the trajectory of R_h as soil moisture increases from below the lower threshold. Data points are means of monthly R_h .

Interactive effects of temperature and moisture on R_h : implications for climate-carbon feedbacks

The magnitude of any soil carbon feedback to climate change depends on the temperature sensitivity (Q_{10}) of soil organic matter decomposition (Kirschbaum, 2010). We believe the respiration we measured primarily resulted from the decomposition of humified organic matter, since our root exclusion technique prevented labile carbon inputs through root exudates or plant litter. [Root exclusion techniques are more widely used to separate autotrophic and heterotrophic components of soil respiration than isotope labeling methods because they are more straightforward (Epron, 2009). However, they can be subject to some error; issues related to our deep-collar technique are discussed above, in the Methods section.] Compared to the annual apparent Q_{10} of total soil respiration and rhizospheric respiration (V. Suseela & J. S. Dukes, unpublished results), the annual apparent temperature sensitivity of R_h was unresponsive to warming treatments. Similar results have been reported by other studies in which root respiration responded more strongly to warming, but respiration from areas in which roots and new carbon inputs were excluded had the lowest Q_{10} values (Boone *et al.*, 1998; Zhou *et al.*, 2007). Studies have also shown that microbially mediated soil respiration had lower temperature sensitivity in the absence of labile carbon (Arevalo *et al.*, 2010; Yuste *et al.*, 2010). However, we found that the effects of warming and precipitation on the apparent Q_{10} of R_h varied by season. Irrespective of soil moisture content, our high warming treatment decreased apparent Q_{10} in both spring and fall. We suspect substrate availability decreased and became a limiting factor in warmed soils, leading to lower apparent Q_{10} (Davidson *et al.*, 2006). Our results support the suggestion by Yuste *et al.* (2003) that models with seasonally varying parameters may be more accurate than those with constant parameters.

The reduction in apparent Q_{10} in the drought treatment during fall confirms the potential of soil water content to modify the temperature sensitivity of R_h . Low soil moisture in the drought treatment during the fall might have contributed to this pattern. These findings have potential implications for climate-carbon modeling, as uncertainty remains regarding environmental controls over microbial decomposition of soil organic matter. While much controversy surrounds the effect of warming on microbial respiration (e.g. Luo *et al.*, 2001; Knorr *et al.*, 2005; Fang *et al.*, 2006; Conant *et al.*, 2008; Hartley & Ineson, 2008) our results highlight the relative importance of soil moisture and seasonal variation in determining the responses of R_h to temperature and moisture. Many ecosystem processes

respond rapidly to short-term weather variation, and models that do not include this high-frequency environmental variability may produce misleading conclusions (Heimann & Reichstein, 2008; Medvigy *et al.*, 2010).

Our results suggest that the use of algorithms that allow temperature sensitivity to change with seasonal variations in temperature and moisture could improve model predictions of R_h for some locations, but the spatial and temporal scales at which models operate may complicate the implementation of such algorithms. Our results were observed in a temperate ecosystem with strong seasonality; tropical ecosystems have much smaller seasonal temperature variability and could conceivably have little variation in temperature sensitivity, although we are not aware of studies that have tested this. Our study clearly suggests that annual apparent Q_{10} of R_h is negatively related to temperature and positively to moisture. However, seasonal apparent Q_{10} values varied temporally with soil moisture and soil temperature (Fig. 8a, b). The apparent Q_{10} values of R_h from this study and from future work in other biomes could be used to develop a temporally variable Q_{10} function (Tjoelker *et al.*, 2001) that responds to abiotic conditions.

Acknowledgements

We thank Carol Goranson and Hollie Emery for help with the fieldwork and for keeping the BACE running. We also thank Brita Jessen, Nicole Fallon and the many others who helped build and maintain the infrastructure of the BACE, Harmony Dagleish, who provided statistical advice for the mixed model and Susanne Hoepfner, who coordinated the biomass data. Two anonymous reviewers provided constructive and thoughtful comments that improved the quality of the manuscript. This research was financially supported by the NSF (DEB-0546670, to JSD), and the U.S. Department of Energy's Office of Science (BER), through the Northeastern Regional Center of the National Institute for Climatic Change Research. This is paper No. 1122 of the Purdue Climate Change Research Center.

References

- Allison SD, Wallenstein MD, Bradford MA (2010) Soil-carbon response to warming dependent on microbial physiology. *Nature Geoscience*, **3**, 336–340.
- Almagro M, Lopez J, Querejeta JJ, Martinez-Mena M (2009) Temperature dependence of soil CO₂ efflux is strongly modulated by seasonal patterns of moisture availability in a Mediterranean ecosystem. *Soil Biology & Biochemistry*, **41**, 594–605.
- Arevalo CBM, Bhatti JS, Chang SX, Jassal RS, Sidders D (2010) Soil respiration in four different land use systems in north central Alberta, Canada. *Journal of Geophysical Research-Biogeosciences*, **115**, G01003, 12 pp.
- Arrhenius S (1889) On the reaction rate of the inversion of non-refined sugar upon souring. *Zeitschrift für Physikalische Chemie*, **4**, 226–248.
- Bauer J, Herbst M, Huisman JA, Weiermuller L, Vereecken H (2008) Sensitivity of simulated soil heterotrophic respiration to temperature and moisture reduction functions. *Geoderma*, **145**, 17–27.
- Bauters TWJ, DiCarlo DA, Steenhuis TS, Parlange JY (1998) Preferential flow in water-repellent sands. *Soil Science Society of America Journal*, **62**, 1185–1190.

- Boone RD, Nadelhoffer KJ, Canary JD, Kaye JP (1998) Roots exert a strong influence on the temperature sensitivity of soil respiration. *Nature*, **396**, 570–572.
- Borken W, Davidson EA, Savage K, Sundquist ET, Steudler P (2006) Effect of summer throughfall exclusion, summer drought, and winter snow cover on methane fluxes in a temperate forest soil. *Soil Biology & Biochemistry*, **38**, 1388–1395.
- Bradford MA, Davies CA, Frey SD *et al.* (2008) Thermal adaptation of soil microbial respiration to elevated temperature. *Ecology Letters*, **11**, 1316–1327.
- Bremer DJ, Ham JM, Owensby CE, Knapp AK (1998) Responses of soil respiration to clipping and grazing in a tallgrass prairie. *Journal of Environmental Quality*, **27**, 1539–1548.
- Burkett VR, Wilcox DA, Stottlemeyer R *et al.* (2005) Nonlinear dynamics in ecosystem response to climatic change: case studies and policy implications. *Ecological Complexity*, **2**, 357–394.
- Conant RT, Drijber RA, Haddix ML *et al.* (2008) Sensitivity of organic matter decomposition to warming varies with its quality. *Global Change Biology*, **14**, 868–877.
- Conant RT, Ryan MG, Agren GI *et al.* (2011) Temperature and soil organic matter decomposition rates – synthesis of current knowledge and a way forward. *Global Change Biology*, doi: 10.1111/j.1365-2486.2011.02496.x.
- Cox PM, Betts RA, Jones CD, Spall SA, Totterdell JJ (2000) Acceleration of global warming due to carbon-cycle feedbacks in a coupled climate model. *Nature*, **408**, 184–187.
- Craine JM, Gelderman TM (2011) Soil moisture controls on temperature sensitivity of soil organic carbon decomposition for a mesic grassland. *Soil Biology and Biochemistry*, **43**, 455–457.
- Davidson EA, Belk E, Boone RD (1998) Soil water content and temperature as independent or confounded factors controlling soil respiration in a temperate mixed hardwood forest. *Global Change Biology*, **4**, 217–227.
- Davidson EA, Janssens IA (2006) Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. *Nature*, **440**, 165–173.
- Davidson EA, Janssens IA, Luo YQ (2006) On the variability of respiration in terrestrial ecosystems: moving beyond Q(10). *Global Change Biology*, **12**, 154–164.
- Derjaguin BV, Churaev NV (1986) Properties of water layers adjacent to interfaces. In: *Fluid Interfacial Phenomena* (ed. Croxton CA), pp. 663–738. John Wiley and Sons, New York.
- Dufresne JL, Friedlingstein P, Berthelot M *et al.* (2002) On the magnitude of positive feedback between future climate change and the carbon cycle. *Geophysical Research Letters*, **29**, 1405, 4 pp.
- Easterling DR, Meehl GA, Parmesan C, Changnon SA, Karl TR, Mearns LO (2000) Climate extremes: observations, modeling, and impacts. *Science*, **289**, 2068–2074.
- Eliasson PE, McMurtrie RE, Pepper DA, Stromgren M, Linder S, Agren GI (2005) The response of heterotrophic CO₂ flux to soil warming. *Global Change Biology*, **11**, 167–181.
- Epron D (2009) Separating autotrophic and heterotrophic components of soil respiration: lessons learned from trenching and related root-exclusion experiments. In: *Soil Carbon Dynamics An integrated methodology* (eds Kutsch WL, Bahn M, Heinemeyer A), pp. 157–168. Cambridge University Press, UK.
- Fang C, Smith P, Smith JU (2006) Is resistant soil organic matter more sensitive to temperature than the labile organic matter? *Biogeosciences*, **3**, 65–68.
- Friedlingstein P, Dufresne JL, Cox PM, Rayner P (2003) How positive is the feedback between climate change and the carbon cycle? *Tellus Series B-Chemical and Physical Meteorology*, **55**, 692–700.
- Giardina CP, Ryan MG (2000) Evidence that decomposition rates of organic carbon in mineral soil do not vary with temperature. *Nature*, **404**, 858–861.
- Garten CT, Classen AT, Norby RJ (2009) Soil moisture surpasses elevated CO₂ and temperature as a control on soil carbon dynamics in a multi-factor climate change experiment. *Plant and Soil*, **319**, 85–94.
- Gaumont-Guay D, Black TA, Barr AG, Jassal RS, Nesic Z (2008) Biophysical controls on rhizospheric and heterotrophic components of soil respiration in a boreal black spruce stand. *Tree Physiology*, **28**, 161–171.
- Gill RA, Polley HW, Johnson HB, Anderson LJ, Maherali H, Jackson RB (2002) Nonlinear grassland responses to past and future atmospheric CO₂. *Nature*, **417**, 279–282.
- Goebel M, Bachmann J, Reichstein M, Janssens IA, Guggenberger G (2011) Soil water repellency and its implications for organic matter decomposition – is there a link to extreme climatic events? *Global Change Biology*, **17**, 2640–2656.
- Haines WB (1930) Studies in the physical properties of soil V. The hysteresis effect in capillary properties, and the modes of moisture distribution associated therewith. *Journal of Agricultural Science*, **20**, 97–116.
- Hanson PJ, Edwards NT, Garten CT, Andrews JA (2000) Separating root and soil microbial contributions to soil respiration: a review of methods and observations. *Biogeochemistry*, **48**, 115–146.
- Hartley IP, Ineson P (2008) Substrate quality and the temperature sensitivity of soil organic matter decomposition. *Soil Biology & Biochemistry*, **40**, 1567–1574.
- Heimann M, Reichstein M (2008) Terrestrial ecosystem carbon dynamics and climate feedbacks. *Nature*, **451**, 289–292.
- Herrmann A, Witter E (2002) Sources of C and N contributing to the flush in mineralization upon freeze-thaw cycles in soils. *Soil Biology & Biochemistry*, **34**, 1495–1505.
- Ise T, Moorcroft PR (2006) The global-scale temperature and moisture dependencies of soil organic carbon decomposition: an analysis using a mechanistic decomposition model. *Biogeochemistry*, **80**, 217–231.
- Kirschbaum MUF (2004) Soil respiration under prolonged soil warming: are rate reductions caused by acclimation or substrate loss? *Global Change Biology*, **10**, 1870–1877.
- Kirschbaum MUF (2010) The temperature dependence of organic matter decomposition: seasonal temperature variations turn a sharp short-term temperature response into a more moderate annually averaged response. *Global Change Biology*, **16**, 2117–2129.
- Klausner Y (1991) *Fundamentals of Continuum Mechanics of Soils*. Springer-Verlag, New York.
- Knapp AK, Beier C, Briske DD *et al.* (2008) Consequences of more extreme precipitation regimes for terrestrial ecosystems. *BioScience*, **58**, 811–821.
- Knorr W, Prentice IC, House JI, Holland EA (2005) Long-term sensitivity of soil carbon turnover to warming. *Nature*, **433**, 298–301.
- Lavigne MB, Boutin R, Foster RJ, Goodine G, Bernier PY, Robitaille G (2003) Soil respiration responses to temperature are controlled more by roots than by decomposition in balsam fir ecosystems. *Canadian Journal of Forest Research-Revue Canadienne De Recherche Forestiere*, **33**, 1744–1753.
- Lin GH, Ehleringer JR, Rygielwicz PT, Johnson MG, Tingey DT (1999) Elevated CO₂ and temperature impacts on different components of soil CO₂ efflux in Douglas-fir terracosms. *Global Change Biology*, **5**, 157–168.
- Liu WX, Zhang Z, Wan SQ (2009) Predominant role of water in regulating soil and microbial respiration and their responses to climate change in a semiarid grassland. *Global Change Biology*, **15**, 184–195.
- Luo Y, Zhou X (2006) *Soil Respiration and the Environment*. Academic Press/Elsevier, San Diego, CA, USA.
- Luo YQ, Wan SQ, Hui DF, Wallace LL (2001) Acclimatization of soil respiration to warming in a tall grass prairie. *Nature*, **413**, 622–625.
- Matzner E, Borken W (2008) Do freeze-thaw events enhance C and N losses from soils of different ecosystems? A review. *European Journal of Soil Science*, **59**, 274–284.
- Medvigy D, Wofsy SC, Munger JW, Moorcroft PR (2010) Responses of terrestrial ecosystems and carbon budgets to current and future environmental variability. *Proceedings of the National Academy of Sciences of the United States of America*, **107**, 8275–8280.
- Melillo JM, Steudler PA, Aber JD *et al.* (2002) Soil warming and carbon-cycle feedbacks to the climate system. *Science*, **298**, 2173–2176.
- Mielnick PC, Dugas WA (2000) Soil CO₂ flux in a tallgrass prairie. *Soil Biology & Biochemistry*, **32**, 221–228.
- Muhr J, Borken W (2009) Delayed recovery of soil respiration after wetting of dry soil further reduces C losses from a Norway spruce forest soil. *Journal of Geophysical Research-Biogeosciences*, **114**, G04023, 11 pp.
- Neelin JD, Munnich M, Su H, Meyerson JE, Holloway CE (2006) Tropical drying trends in global warming models and observations. *Proceedings of the National Academy of Sciences of the United States of America*, **103**, 6110–6115.
- Pham HQ, Fredlund DG, Barbour SL (2005) A study of hysteresis models for soil-water characteristic curves. *Canadian Geotechnical Journal*, **42**, 1548–1568.
- Porporato A, Daly E, Rodriguez-Iturbe I (2004) Soil water balance and ecosystem response to climate change. *American Naturalist*, **164**, 625–632.
- Reichstein M, Katterer T, Andren O *et al.* (2005) Temperature sensitivity of decomposition in relation to soil organic matter pools: critique and outlook. *Biogeosciences*, **2**, 317–321.
- Ruehr NK, Buchmann N (2010) Soil respiration fluxes in a temperate mixed forest: seasonality and temperature sensitivities differ among microbial and root-rhizosphere respiration. *Tree Physiology*, **30**, 165–176.
- Ryan MG, Law BE (2005) Interpreting, measuring, and modeling soil respiration. *Biogeochemistry*, **73**, 3–27.
- Schimel DS, Braswell BH, Holland EA *et al.* (1994) Climatic, edaphic, and biotic controls over storage and turnover of carbon in soils. *Global Biogeochemical Cycles*, **8**, 279–293.
- Schindlbacher A, Zechmeister-Boltenstern S, Jandl R (2009) Carbon losses due to soil warming: do autotrophic and heterotrophic soil respiration respond equally? *Global Change Biology*, **15**, 901–913.
- Scott-Denton LE, Rosenstiel TN, Monson RK (2006) Differential controls by climate and substrate over the heterotrophic and rhizospheric components of soil respiration. *Global Change Biology*, **12**, 205–216.

- Skogland T, Lomeland S, Goksoyr J (1988) Respiratory burst after freezing and thawing of soil – experiments with soil bacteria. *Soil Biology & Biochemistry*, **20**, 851–856.
- Skopp J, Jawson MD, Doran JW (1990) Steady-state aerobic microbial activity as a function of soil-water content. *Soil Science Society of America Journal*, **54**, 1619–1625.
- Stark JM, Firestone MK (1995) Mechanisms for soil-moisture effects on activity of nitrifying bacteria. *Applied and Environmental Microbiology*, **61**, 218–221.
- Subke JA, Voke NR, Leronni V, Garnett MH, Ineson P (2011) Dynamics and pathways of autotrophic and heterotrophic soil CO₂ efflux revealed by forest girdling. *Journal of Ecology*, **99**, 186–193.
- Tharayil N, Suseela V, Triebwasser DJ, Preston CM, Gerard PD, Dukes JS (2011) Changes in the structural composition and reactivity of *Acer rubrum* leaf litter tannins exposed to warming and altered precipitation: climatic stress-induced tannins are more reactive. *New Phytologist*, **191**, 132–145.
- Tjoelker MG, Oleksyn J, Reich PB (2001) Modeling respiration of vegetation: evidence for a general temperature-dependent Q(10). *Global Change Biology*, **7**, 223–230.
- Wallenstein MD, McMahon SK, Schimel JP (2009) Seasonal variation in enzyme activities and temperature sensitivities in Arctic tundra soils. *Global Change Biology*, **15**, 1631–1639.
- Wallenstein MD, Allison S, Ernakovich J, Steinweg JM, Sinsabaugh R (2010) Controls on the temperature sensitivity of soil enzymes: a key driver of in-situ enzyme activity rates. In: *Soil Enzymology* (eds Shukla G, Varma A), pp. 245–258. Springer-Verlag, Berlin, Heidelberg.
- 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.
- Wang XH, Piao SL, Ciais P, Janssens IA, Reichstein M, Peng SS, Wang T (2010) Are ecological gradients in seasonal Q(10) of soil respiration explained by climate or by vegetation seasonality? *Soil Biology & Biochemistry*, **42**, 1728–1734.
- Weltzin JF, Loik ME, Schwinning S *et al.* (2003) Assessing the response of terrestrial ecosystems to potential changes in precipitation. *BioScience*, **53**, 941–952.
- Williams MA (2007) Response of microbial communities to water stress in irrigated and drought-prone tallgrass prairie soils. *Soil Biology & Biochemistry*, **39**, 2750–2757.
- Wu Z, Dijkstra P, Koch GW, Penuelas J, Hungate BA (2011) Responses of terrestrial ecosystems to temperature and precipitation change: a meta-analysis of experimental manipulation. *Global Change Biology*, **17**, 927–942.
- Yuste JC, Janssens IA, Carrara A, Meiresonne L, Ceulemans R (2003) Interactive effects of temperature and precipitation on soil respiration in a temperate maritime pine forest. *Tree Physiology*, **23**, 1263–1270.
- Yuste JC, Ma S, Baldocchi DD (2010) Plant-soil interactions and acclimation to temperature of microbial-mediated soil respiration may affect predictions of soil CO₂ efflux. *Biogeochemistry*, **98**, 127–138.
- Zhou T, Shi PJ, Hui DF, Luo YQ (2009) Global pattern of temperature sensitivity of soil heterotrophic respiration (Q10) and its implications for carbon-climate feedback. *Journal of Geophysical Research-Biogeosciences*, **114**, G02016, 9 pp.
- Zhou X, Wan SQ, Luo YQ (2007) Source components and interannual variability of soil CO₂ efflux under experimental warming and clipping in a grassland ecosystem. *Global Change Biology*, **13**, 761–775.
- Zhou XH, Weng ES, Luo YQ (2008) Modeling patterns of nonlinearity in ecosystem responses to temperature, CO₂, and precipitation changes. *Ecological Applications*, **18**, 453–466.

Supporting Information

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

Figure S1. Monthly average rainfall (1952–2008) from Bedford weather station, Massachusetts. The upper and lower error bars indicate 90th and 10th percentile, respectively.

Figure S2. Daily rainfall in the ambient treatment (vertical bars) and cumulative rainfall in the drought, ambient and wet treatments from March 2009 to March 2010. The filled stars represent the day of measurement of microbial respiration.

Figure S3. Diurnal variation in R_h on April 19, 2010 in drought (a), ambient (b), and wet (c) treatments, and corresponding soil temperature in drought (d), ambient (e), and wet (f) treatments. (g) Soil moisture content on the measurement day.

Figure S4. Diurnal variation in R_h on August 17, 2009 in drought (a), ambient (b), and wet (c) treatments, and corresponding soil temperature in drought (d), ambient (e), and wet (f) treatments. (g) Soil moisture content on the measurement day.

Table S1. Results of the mixed model restricted maximum likelihood analysis of the responses of soil moisture (10 cm depth) to warming and precipitation treatments and time of sampling. Key: W, warming; Ppt, precipitation; NDF, numerator degrees of freedom; DDF, denominator degrees of freedom.

Table S2. Results of the mixed model restricted maximum likelihood analysis of the responses of soil temperature (5 cm depth) to warming and precipitation treatments and time of sampling. Key: W, warming; Ppt, precipitation; NDF, numerator degrees of freedom; DDF, denominator degrees of freedom.

Table S3. Results of the mixed model restricted maximum likelihood analysis of the responses of R_h to warming and precipitation treatments and time of sampling. Key: W, warming; Ppt, precipitation; NDF, numerator degrees of freedom; DDF, denominator degrees of freedom.

Table S4. Results of the mixed model restricted maximum likelihood analysis of the responses of cumulative R_h to warming and precipitation treatments. Key: W, warming; Ppt, precipitation; NDF, numerator degrees of freedom; DDF, denominator degrees of freedom.

Table S5. Results of the mixed model restricted maximum likelihood analysis of the responses of apparent temperature sensitivity of R_h to warming and precipitation treatments. Key: W, warming; Ppt, precipitation; NDF, numerator degrees of freedom; DDF, denominator degrees of freedom.

Table S6. Correlation (r^2) of measured microbial respiration values to environmental variables using temperature ($y = ae^{bx}$), moisture ($y = y_0 + ax + bx^2$) and combined exponential and quadratic (Melnick–Dugas) functions. Numbers in bold indicate the best fit of the three functions in cases where the fit is greater than 0.2.

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.