

Plants Mediate the Sensitivity of Soil Respiration to Rainfall Variability

Zachary T. Aanderud,^{1,2} Donald R. Schoolmaster Jr.,¹ and Jay T. Lennon^{1,3*}

¹W. K. Kellogg Biological Station, Michigan State University, 3700 East Gull Lake Drive, Hickory Corners, Michigan 49060, USA;

²Department of Plant and Wildlife Sciences, Brigham Young University, 489 WIDB, Provo, Utah 84602, USA; ³Department of Microbiology and Molecular Genetics, Michigan State University, Hickory Corners, Michigan 49060, USA

ABSTRACT

Soil respiration from grasslands plays a critical role in determining carbon dioxide (CO₂) feedbacks between soils and the atmosphere. In these often mesic systems, soil moisture and temperature tend to co-regulate soil respiration. Increasing variance of rainfall patterns may alter aboveground–belowground interactions and have important implications for the sensitivity of soil respiration to fluctuations in moisture and temperature. We conducted a set of field experiments to evaluate the independent and interactive effects of rainfall variability and plant–soil processes on respiration dynamics. Plant removal had strong effects on grassland soils, which included altered CO₂ flux owing to absence of root respiration; increased soil moisture and temperature; and reduced availability of dissolved organic carbon (DOC) for heterotrophic respiration by microorganisms. These plant-mediated effects interacted with our rainfall variability treatments to determine the sensitivity

of soil respiration to both moisture and temperature. Using time-series multiple regression, we found that plants dampened the sensitivity of respiration to moisture under high variability rainfall treatments, which may reflect the relative stability of root contributions to total soil respiration. In contrast, plants increased the sensitivity of respiration to temperature under low variability rainfall treatment suggesting that the environmental controls on soil CO₂ dynamics in mesic habitats may be context dependent. Our results provide insight into the aboveground–belowground mechanisms controlling respiration in grasslands under variable rainfall regimes, which may be important for predicting CO₂ dynamics under current and future climate scenarios.

Key words: CO₂; heterotrophic respiration; pulse; root respiration; time-series; sensors; microbial; climate change; precipitation.

INTRODUCTION

A major goal of terrestrial ecosystem ecology is to understand how biotic and abiotic factors control

the turnover of soil carbon (C). Soils represent a large and dynamic C pool that has the potential to mitigate or amplify trends associated with global climate change. Nearly 10% of the atmospheric carbon dioxide (CO₂) passes through soil on a yearly basis (Raich and Potter 1995), and this terrestrial reservoir holds more C than the atmosphere and vegetation combined (Jobbagy and Jackson 2000; Schlesinger 1997). The mineralization of soil organic C is regulated to a large extent by respiratory processes, which are sensitive to changes in environmental conditions (Jenkinson and others 1991; Atkin and others 2005; Davidson and

Received 4 October 2010; accepted 1 November 2010;
published online 25 November 2010

Electronic supplementary material: The online version of this article (doi:10.1007/s10021-010-9401-y) contains supplementary material, which is available to authorized users.

Author Contributions: ZTA and JTL designed the study; ZTA performed the research; DRS, ZTA, and JTL analyzed and interpreted the data; ZTA, DRS, and JTL wrote the paper.

*Corresponding author; e-mail: lennonja@msu.edu

Janssens 2006). In particular, moisture is considered a master variable that drives patterns of soil respiration in many terrestrial ecosystems (Bartholomew and Broadbent 1950; Schimel and others 2007; Gu and others 2008). Although soil respiration tends to increase with average soil moisture content (for example, Cook and Orchard 2008), recent studies have demonstrated that the temporal variability of soil moisture has strong and sometimes independent effects on the dynamics and cumulative production of CO₂ (Sponseller 2007; Fay and others 2008; Borken and Matzner 2009).

Rainfall patterns play a central role in determining the temporal variability of moisture and respiratory activity in soils. For example, rainfall events in grassland soils generate short-term pulses of soil CO₂ following prolonged dry periods (Fay and others 2000; Knapp and others 2002; Xu and Baldocchi 2004). Within hours of a rewetting event, CO₂ production can achieve rates that are 500% higher than pre-wetting conditions (Fierer and Schimel 2003; Sponseller 2007). When scaled over longer time periods, rainfall variability can have mixed effects on soil respiration. Some studies have shown that rainfall induced-pulses of soil respiration can account for 5–10% of annual net ecosystem production in mid-latitude forests (Lee and others 2004) and up to 90% of the late-season ecosystem respiration in semi-arid grasslands (Xu and Baldocchi 2004). In contrast, altered rainfall regimes in a tall grass prairie have been shown to reduce summer soil CO₂ flux (Harper and others 2005). Ultimately, rainfall-induced fluxes may determine whether ecosystems function as sources or sinks of atmospheric CO₂ (Shim and others 2009). Such findings have important implications for predicting soil CO₂ dynamics because the schedule, magnitude, and timing of rainfall are expected to become increasingly variable under future climate change scenarios (Weltzin and others 2003; Zhang and others 2007; Knapp and others 2008).

The coupling of aboveground and belowground processes may help explain the dynamic responses of soil respiration to rainfall variability (van der Putten and others 2009). This coupling is determined to a large extent by the direct and indirect effects that plants have on soil moisture dynamics, soil physiochemical characteristics, and soil food webs. First, plants are capable of enhancing soil respiration through the delivery of labile carbon substrates via roots to heterotrophic microorganisms (Cardon and Gage 2006; Scott-Denton and others 2006). Second, plants can indirectly alter soil respiration by modifying soil moisture and

temperature. Although plants minimize evaporative water loss by reducing soil temperature through shading, more often they deplete soil moisture through transpiration (Lauenroth and Bradford 2006). Also, plants may directly alter the amount of rainfall that reaches the soil through interception and stem flow processes. Lastly, plant roots directly contribute to soil respiration, in some cases accounting for more than half of total soil CO₂ flux (Hanson and others 2000; Tang and others 2005; Crow and Wieder 2005). There are some reports, however, that this source of respiration may be less responsive to rainfall variability than heterotrophic respiration (Cisneros-Dozal and others 2006; Zobitz and others 2008; Carbone and others 2008), presumably because the abundance and activity of microorganisms can rapidly change in response to environmental fluctuations. It appears that at least some of these aboveground–belowground linkages may be decoupled by drying–wetting cycles that are associated with rainfall variability (Yepez and others 2007). However, few studies have evaluated the sensitivity of respiration to moisture (Noormets and others 2008) or investigated how interactions between rainfall and plants regulate soil respiration (Liu and others 2002, 2009; Cable and others 2008).

In this study, we conducted a set of field experiments to determine how soil respiration responds to the interactive effects of rainfall variability and plant–soil processes. Specifically, we quantified the sensitivity of respiration to moisture and temperature (that is, CO₂ evolved per unit change in moisture) in a successional grassland in the presence and absence of plants under low and high variability rainfall regimes. In this mesic habitat, soil respiration may be co-regulated by temperature and moisture. For example, soil respiration in temperate grasslands is influenced by seasonal and diel fluctuations in temperature, but also responds to moisture, which tends to covary with temperature during spring and summer months (Davidson and others 1998, 2006; Conant and others 2004; Almagro and others 2009). Although there is evidence that soil respiration in mesic ecosystems can be controlled by moisture and/or temperature, it is unclear whether the strength of these environmental drivers is contingent upon rainfall variability. Therefore, we also evaluated the sensitivity of soil respiration to temperature under natural and manipulated rainfall regimes. The first part of the study quantified how plants modified soil CO₂ flux and environmental conditions (that is, moisture, temperature, and resource availability) under natural rainfall variability. The second part of the

study directly manipulated rainfall variability to quantify the sensitivity of soil respiration to fluctuations in moisture and temperature. Thus, results from our study shed light on the environmental factors that influence soil respiration dynamics in mesic ecosystems under variable climate regimes.

METHODS

Overview of Experiments

To assess the effects of aboveground–belowground linkages on soil respiration dynamics, we manipulated plant communities in replicate grasslands at the W. K. Kellogg Biological Station (KBS) Long Term Ecological Research (LTER) site. This manipulation was used for two related experiments. The first experiment monitored CO₂ flux and additional soil characteristics under ambient rainfall conditions. This replicated experiment allowed us to test the effects of our plant manipulation and assess the spatial variability of soil respiration across a 42 ha LTER landscape. The second experiment crossed the plant manipulation with a rainfall variability treatment. We deployed one set of environmental sensors in each of the treatment combinations to quantify the sensitivity of soil respiration to rapid fluctuations in moisture and temperature. We used multiple regression time-series regression procedures to draw conclusions from this unreplicated experiment. We discuss the advantages and challenges of our two experimental approaches in the “Discussion”.

Site Description

We conducted our study in the successional grassland treatment (T7) at the KBS-LTER site located in southwestern Michigan, USA. This land-use treatment consists of replicate 1 ha replicated fields containing a mixture of perennial grasses and forbs that established after the field was abandoned from agricultural activity in 1989 (Robertson and others 2000). Dominant plant species include *Solidago canadensis* (L.), *Apocynum cannabinum* (L.), and *Phleum pratense* (L.). Average annual precipitation at the KBS-LTER is 890 mm (± 148.0 SD, $n = 21$) with half falling as snow, and the mean annual temperature is 9.0°C (± 0.81 SD, $n = 21$) (<http://www.kbs.msu.edu/databases>). We performed our experiment from June to September 2007. During this time, mean monthly rainfall was 376 mm (± 120.3 SD) and mean monthly temperature was 19.2°C (± 1.95 SD). All soils are fine-loamy, mixed, mesic Typic Hapludalfs with a total soil C of 1.3%

and nitrogen (N) of 0.13%, pH of 5.5, and cation exchange capacity of 5.5 cmol kg⁻¹.

Plant Manipulation

The first part of our study involved the initiation and monitoring of a plant manipulation, which ultimately allowed us to assess the importance of aboveground–belowground coupling to the sensitivity of respiration under variable rainfall treatments. We manipulated plant communities in a 10 m × 10 m area at the north end of each of the T7 replicates ($n = 5$) on the KBS-LTER site. We subdivided each of these areas into four 5 m × 5 m plots. The plant community was left intact in two of the randomly chosen plots (+P) and removed from the remaining two plots (–P). We created and maintained the –P treatment across the entire 5 m × 5 m plot by clipping and removing all plants, spraying herbicide (glyphosate) on the stubble, and subsequently weeding all emergent seedlings on a weekly basis. We left a 1.5 m buffer strip between the +P and –P treatments to control for edge effects.

We evaluated the direct and indirect effects of plant manipulations on soil CO₂ flux in all experimental plots on a weekly basis from June through September 2007 using in situ closed-cover flux chambers (Livingston and Hutchinson 1995; Ambus and Robertson 2006). The chambers consisted of a PVC collar (with a beveled edge for ease of soil insertion) and a fitted cap. We attached a piece of 8 cm tygon tubing, fitted with a three-way septum, to the top of the cap to allow for gas headspace sampling. All soil collars were driven 5 cm into the soil at the center of each plot 2 weeks prior to data collection to minimize potential soil disturbance effects. To quantify CO₂ flux, we sampled the headspace of the chamber (diameter = 25 cm, height = 8 cm) four times over approximately 2 h (that is, time zero, 40 min, 80 min, and 120 min). At each sampling time we attached a 10 ml syringe to the septum, opened the valve, plunged the syringe five times to mix the headspace in the chamber and tube, pulled a 8 ml gas sample, and placed it in an evacuated 5.9-ml septum-sealed glass vial. Owing to the relatively large volume of the headspace in the chamber (4 l) and the short sampling duration, we assume that the negative pressure or concentration effects on our CO₂ fluxes was negligible (Ambus and Robertson 2006). CO₂ concentrations in the vials were measured with a LI-820 infrared gas analyzer (LI-COR Biosciences, Lincoln, NE, USA). The overpressure in the glass vials was released prior to each CO₂ measurement. We used

these four CO₂ measurements and the ideal gas law to calculate CO₂ flux.

In addition, we quantified the direct and indirect effects of plant manipulations on a suite of soil characteristics from June through September. Specifically, we measured soil moisture and temperature each week for 14 weeks; dissolved organic carbon (DOC), inorganic N concentrations, total C, and total N were measured once at the beginning of each month. Soil characteristics were determined on 2 mm-sieved soil samples composited from five soil cores (diameter = 2 cm, height 10 cm) that were extracted using a soil probe. After each soil core was removed we placed a 12 cm piece of PVC pipe, capped with a rubber stopper, to prevent preferential water flow from rainfall events. We measured soil moisture gravimetrically on 30 g of the composited soil sample. The soil sample was weighed, dried at 105°C for 48 h, and re-weighed to determine water content. We converted these gravimetric water contents to volumetric water contents using an average bulk density for each treatment. Bulk density was calculated from ten 10 cm diameter × 8 cm deep PVC cores that were sampled at the end of the experiment. Soil temperature measurements were taken adjacent to the permanent gas chambers to a depth of 10 cm during the time zero CO₂ sampling points using a soil thermometer (Forestry Suppliers Inc., Jackson, MS, USA). For DOC, soils were extracted within 24 h of sampling via a distilled water extraction (1:2 w/v), passed through a 0.2 μm nylon filter, and measured on a TOC analyzer (Shimadzu, Columbia MD, USA). For inorganic N, soils were extracted within 48 h via a 1 M KCl extraction (1:10 w/v), passed through a Whatman #1 filter, and measured on an OI Analytical Flow Solution IV analyzer (OI Analytical, College Station TX, USA). We quantified total C and total N from soils using a TruSpec C/H/N/S/O analyzer (Leco Inc, St. Joseph, MI, USA). We conducted all of our field sampling between 10:00 and 14:00 to minimize variation associated with diel fluctuations of soil conditions. We tested for the effect influence of the plant manipulations (+P versus -P, fixed effect) and time (random effect) on our response variables (that is, CO₂ flux, soil moisture, temperature, DOC, inorganic N, total C, and total N) prior to rainfall treatments (see “[Rainfall Variability Treatments](#)” section) using repeated measures (RM) ANOVA (SAS PROC MIXED) with covariance structures selected using Bayesian Information Criterion (Wolfinger and Chang 1999).

Rainfall Variability Treatments

After the plant manipulations had been established for 14 weeks, we initiated our second study, which consisted of a factorial experiment that exposed +P and -P plots in one randomly selected T7 replicate to a low (LV) rainfall variability treatment or a high variability (HV) rainfall treatment. During this unreplicated experiment, we protected soils from ambient rainfall by erecting rainout shelters (2 m length × 2 m width × 1.5 m high) over our plot, which consisted of PVC frames (4 m²) covered with polyethylene plastic. Using a watering can, we delivered the same amount of water (60 l) to each plot over a 26-day period (August 29–Sept 23, 2007). The HV treatment received three simulated events of 20 mm every 7 days, whereas the LV treatment received a rainfall event of 2.3 mm each day of the experiment. These treatments were chosen to reflect patterns of rainfall variability observed on the KBS-LTER over the past two decades. For example, the average number of rainfall events occurring during the summer (June–September) is 37 (±8.9 SD, *n* = 21), but most of these are small with only 33% of events measuring over 10 mm and 14% of the events measuring over 20 mm. Further, the more extreme (>20 mm) events are highly variable and occur on average every 15 days (±17 SD, *n* = 21). Therefore, the LV treatment simulates stable rainfall conditions consisting of frequent and minor events, whereas the HV treatment captures more extreme events that are typical of precipitation regimes in our study area.

Soil Respiration Sensitivity

We quantified the sensitivity of soil respiration to moisture and temperature by applying time-series regression models to CO₂, moisture, and temperature data that were continuously measured using environmental sensors over the 26-days rainfall variability experiment in one randomly selected T7 replicate plot. We measured CO₂ concentrations (ppmv) at a depth of 2 cm in each treatment using non-dispersive infrared absorption with 3% CO₂ GMT222 sensors (Vaisala, Helsinki, Finland). By placing the sensor close to the soil surface we were able to capture an integrated CO₂ response to the rainfall treatments before the CO₂ was released to the atmosphere. The CO₂ concentrations measured by the sensors were corrected for variations in temperature and pressure (Tang and others 2003), converted to a mole concentration (Tang and others 2003, 2005), and corrected for rapid changes in volumetric soil air content induced by rainfall to

estimate the amount of CO₂ generated from the soil profile. We converted the volume fraction C_v ($\mu\text{mol mol}^{-1}$) from the sensors to mole concentration of C ($\mu\text{mol m}^{-3}$) using $C = C_v P/RT$, where P is the air pressure (Pa), T the soil absolute temperature (K), and R the universal gas constant ($8.3144 \text{ J mol}^{-1} \text{ K}^{-1}$) (Tang and others 2005). Finally, we multiplied C by the volumetric soil air content (air-filled porosity, m^{-3}). This correction accounts for the potential to overestimate C due to the condensing of soil air that occurs during a rainfall event independent of respiration. For example, if the soil C was $10,000 \mu\text{mol m}^{-3}$ before a rainfall event and the event reduced the volume of soil air by 50% the resulting C at the next time step would be elevated to $20,000 \mu\text{mol m}^{-3}$ regardless of changes in soil respiration. This overestimation would skew inferences of soil respiration sensitivity to moisture. Using the same set of sensors, Daly and others (2008) found that near-surface CO₂ concentrations were significantly and positively correlated with more traditional chamber CO₂ flux measurements. Moreover, they found that the correlation between sensor-based CO₂ concentrations and chamber-based CO₂ fluxes were robust under variable soil moisture conditions induced by precipitation events. Based on these results, we treated the corrected CO₂ time-series measured with near-surface sensors as an integrated measure of heterotrophic and root respiration. In addition, we monitored soil moisture ($\text{m}^3 \text{ H}_2\text{O m}^{-3}$ soil) and temperature ($^{\circ}\text{C}$) over a soil depth of 2–7 cm in each treatment with ECH₂O-TM sensors (Decagon Devices, Pullman WA, USA). Sensor data (CO₂, soil moisture, and soil temperature) were generated every 10 s, averaged to create a 30 min value, and stored on field dataloggers (CR1000, Campbell Scientific, Inc., Logan UT, USA). Each treatment combination had one CO₂ sensor, one soil moisture sensor, and one soil temperature sensor that were placed within 5 cm of each other. We expressed and modeled all our data on a daily time-step, calculated from 30 min intervals. This daily averaging removed diel variability, which may have masked the impact of our treatments on respiration sensitivity (Riveros-Iregui and others 2007; Carbone and others 2008). We used our plant manipulations to approximate root and heterotrophic respiration. Specifically, we assumed that respiration in the +P soils was due to root and heterotrophic respiration, whereas respiration in the –P soils was due primarily to heterotrophic respiration (Anderson 1973; Tang and others 2005).

We estimated soil respiration sensitivity using time-series multiple regression models that included both soil moisture and temperature as predictor variables. Specifically, we defined sensitivity as the slopes from linear regressions where corrected CO₂ concentrations through time were fit as a function of soil moisture (moles CO₂/m³ H₂O m^{–3} soil) or temperature (moles CO₂/ $^{\circ}\text{C}$). To meet the assumptions of the procedures, we examined the residuals of the time-series regressions for autocorrelation using the Ljung–Box test (Ljung and Box 1978). The residuals for some of the treatment combinations (+P, LV and –P, HV) were marginally significant suggesting that the confidence intervals around these slopes might be larger than what was calculated from the regression. However, when we modeled the errors as an autoregressive processes with lag equal to 1 (that is, AR1) autocorrelation in the residuals was removed, but the estimated sensitivity parameters were similar and the relationship among model parameter estimates (and thus our conclusions) were unchanged. Therefore, we report results from the non-autoregressive analysis, which are simpler, more conservative, and easier to interpret. The details of the time-series regression and results of the autoregressive analyses can be found in the Appendix in the Supplemental Materials. All models generated an adjusted R^2 and standard errors. From these standard errors, we constructed 95% confidence intervals around the parameter estimates. Because differences among parameters cannot accurately be judged using confidence intervals alone, we performed t -tests to determine significant differences ($P < 0.05$) in CO₂ sensitivity between the four treatment combinations (–P LV, –P HV, +P LV, +P HV). Interactions were identified as cases where differences between sensitivities of one treatment significantly changed relative to another treatment.

RESULTS

Plant Manipulation

The plant manipulation had strong effects on the temporal dynamics of nearly all measured soil response variables prior to initiating the rainfall variability treatments. With the exception of total C and total N, results from RM-ANOVA revealed that there were significant time \times treatment (that is, plant manipulation) interactions for all of measured soil response variables (Table 1; Figure 1). We used the marginal means (or least square means [LSM]) generated from the RM-ANOVA

Table 1. Repeated Measures ANOVA Table for Soil Characteristics Through Time in Response to a Plant Manipulation (+P versus -P) in a Mesic Grassland at the Kellogg Biological Station (KBS) Long-Term Ecological Research (LTER) Site

Source	CO ₂ flux ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	Moisture (g g^{-1})	Temperature (°C)	DOC ($\mu\text{g C g soil}^{-1}$)	NH ₄ ⁺ (mg N g soil ⁻¹)	NO ₃ ⁻ (mg N g soil ⁻¹)	Total C (%)	Total N (%)
Plant	$F_{1,8} = 0.77$ $P = 0.41$	$F_{1,8} = 27.34$ $P = 0.0008$	$F_{1,8} = 5.78$ $P = 0.043$	$F_{1,8} = 49.91$ $P = 0.0001$	$F_{1,8} = 11.21$ $P = 0.0101$	$F_{1,8} = 67.65$ $P = 0.0003$	$F_{1,8} = 18.89$ $P = 0.908$	$F_{1,8} = 5.70$ $P = 0.044$
Time	$F_{17,136} = 51.28$ $P < 0.0001$	$F_{17,136} = 61.13$ $P < 0.0001$	$F_{17,132} = 76.99$ $P < 0.0001$	$F_{3,24} = 112.76$ $P < 0.0001$	$F_{3,8} = 21.22$ $P = 0.0004$	$F_{3,8} = 21.54$ $P < 0.0001$	$F_{3,8} = 18.89$ $P < 0.0001$	$F_{3,24} = 8.49$ $P = 0.0005$
Plant × Time	$F_{17,136} = 3.14$ $P = 0.0001$	$F_{17,136} = 6.57$ $P = 0.0001$	$F_{17,132} = 2.31$ $P = 0.004$	$F_{3,24} = 40.30$ $P < 0.0001$	$F_{3,8} = 28.12$ $P = 0.001$	$F_{3,8} = 24.36$ $P = 0.0002$	$F_{3,8} = 0.02$ $P = 0.897$	$F_{3,24} = 1.92$ $P = 0.153$

models to assess the magnitude and direction of soil responses to the plant manipulation. CO₂ flux was 14% higher in the +P versus -P plot. This reduction in soil CO₂ flux in the -P plots was accompanied by a 42% increase in soil moisture and a 1°C increase in soil temperature. In addition, plant removal significantly altered resource availability. For example, DOC concentrations were 44% lower in the -P plots (27.1 $\mu\text{g g}^{-1}$) compared to the +P plots (48.3 $\mu\text{g g}^{-1}$). In contrast, inorganic N was higher in the -P versus +P plots. Plant removal more than doubled NH₄⁺ concentrations and resulted in a 30-fold increase in NO₃⁻ availability. Although total C changed through time, it was unaffected by the plant manipulation. Total N was approximately 20% higher in the +P versus -P treatment at the beginning of the manipulation, but this effect attenuated with time.

Respiration Sensitivity to Moisture

The plant manipulation determined baseline soil moisture levels in the experimental plot, whereas the rainfall variability treatment controlled short-term fluctuations in soil moisture (Figure 2). Under LV rainfall, soil moisture levels were relatively stable through time, but were consistently lower in the +P versus -P plot (Figure 2). Under HV rainfall, simulated precipitation events generated large fluctuations in soil moisture that masked the effect of the plant manipulation observed in the LV treatment (Figure 2). Total C in the +P and -P treatment were not different prior to the rainfall manipulations (Table 1; Figure 1) suggesting that dead roots in the -P contributed minimally to soil respiration. This assumption allowed us to estimate that root respiration in the +P treatment accounted for approximately 55% and 68% of the total soil CO₂ under HV and LV rainfall, respectively.

The plant manipulation also modified the sensitivity of respiration to fluctuations in soil moisture, but only under HV rainfall (Figure 3). Plants significantly dampened the sensitivity of respiration to moisture in the HV rainfall treatment ($P < 0.001$), but not in the LV rainfall treatment ($P = 0.15$). Sensitivity to soil moisture was almost four times lower in the presence versus the absence of plants under HV rainfall. Further, respiration sensitivity to moisture was two-and-a-half times lower under HV than LV rainfall in the presence of plants ($P = 0.03$). Overall, plants decreased the statistical variability associated with respiration sensitivity to moisture and improved the fit of the time-series data. For example, the 95% confidence intervals for the sensitivity of respiration to moisture in the

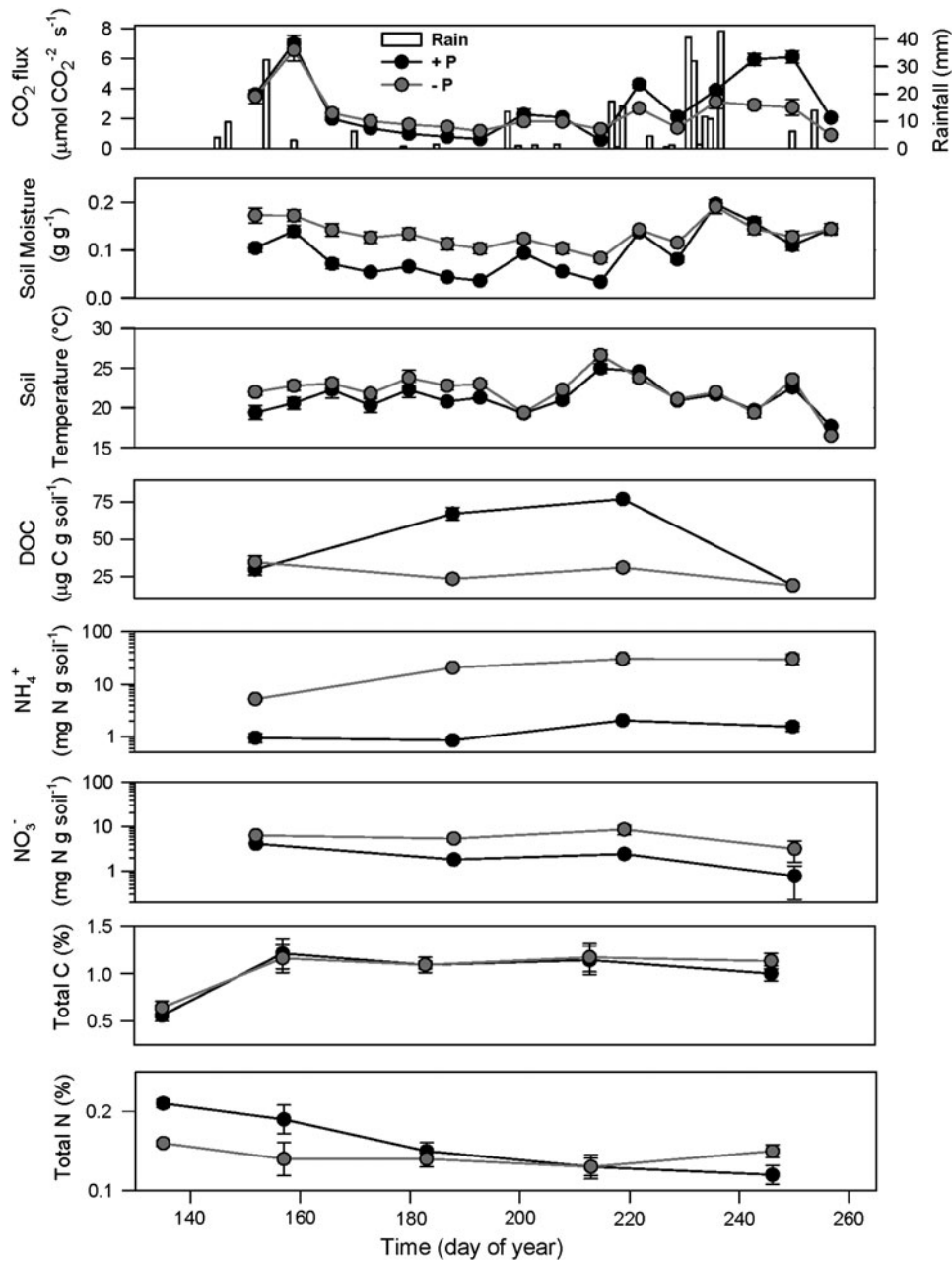


Figure 1. The effects of an experimental plant manipulation on soil characteristics in a mesic grassland at the Kellogg Biological Station (KBS) Long-Term Ecological Research (LTER) site. Plants removal = $-P$ (gray circles), plants present = $+P$ (black circles). The values are means \pm SEM for five replicates prior to initiating the rainfall variability treatments. The statistical effects of the plant manipulations on soil characteristics were determined with RM-ANOVA (see Table 1).

$+P$ treatment were at least three times smaller than those for $-P$ treatment. In addition, the fits for the time-series in the $+P$ treatment were higher (adjusted $R^2 > 0.86$) than the fits for the $-P$ treatments (adjusted $R^2 < 0.62$).

Respiration Sensitivity to Temperature

Plant removal increased temperatures by 0.9–5.3°C under LV rainfall and by 0.1–3.6°C under HV rainfall (Figure 2). Although not directly manipulated in this study, we were able to assess whether our experimental treatments altered the sensitivity

of respiration to indirect changes and natural variability in temperature. Soil moisture and soil temperature did not covary during our rainfall variability experiment ($r = -0.08$, $P = 0.40$). Plants enhanced the sensitivity of respiration to temperature, but only in the LV rainfall treatment (Figure 3). Specifically, in the LV rainfall treatment, sensitivity to temperature was five times greater in $+P$ versus $-P$ soils ($P = 0.02$). With the exception of the $-P$ LV treatment ($P = 0.71$), soil temperature was positively correlated with respiration ($P < 0.05$).

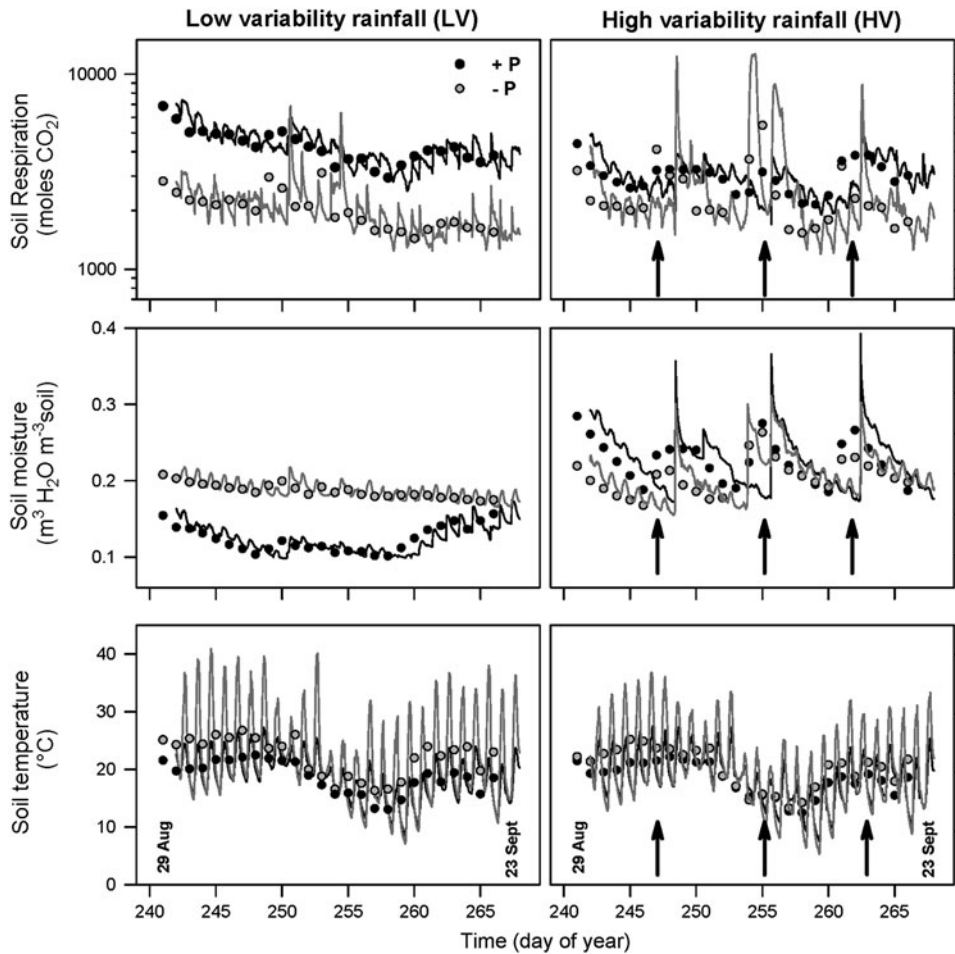


Figure 2. The impact of low variability (LV) and high variability (HV) rainfall treatments and the presence (+P) and absence (–P) of plants on soil CO₂ concentration, soil moisture, and soil temperature. The HV rainfall treatment consisted of three simulated rainfall events every 7 days of 20 mm (upward-pointing arrows), whereas the LV rainfall treatment consisted of 26 daily rainfall events of 2.3 mm. Solid gray and black lines represent real-time sensor data that were generated every 10 s and averaged to create a 30 min value. Circles represent the daily means of these data, which were used to quantify respiration sensitivity.

DISCUSSION

The overarching factors that govern soil respiration are well known, but much of the detail describing how root and heterotrophic components of respiration respond to environmental variables, such as rainfall variability is lacking (Trumbore 2006). We addressed these issues by experimentally relating soil respiration dynamics to manipulations of rainfall variability in the presence and absence of roots, which couple aboveground–belowground processes. Overall, the plant removal had dramatic effects on the grassland soils, which included altered CO₂ flux, reduced DOC, and increased soil moisture and N availability (Figure 1). These plant-mediated effects interacted with our rainfall variability treatments to determine the sensitivity of respiration to both moisture and temperature. Together, our results suggest the direct and indirect effects involved in aboveground–belowground coupling should buffer soil respiration sensitivity under more variable rainfall conditions predicted for grasslands under future climate-change scenarios (IPCC 2007; Zhang and others 2007).

Soil Respiration Sensitivity to Moisture

Plants diminished the effects of rainfall variability on the sensitivity of respiration to moisture (Figure 3). The plant dampening effect measured under high variability rainfall may have stemmed from root respiration. For example, growing evidence suggests that root respiration is less variable and responsive to environmental change than heterotrophic respiration (Cisneros-Dozal and others 2006; Scott-Denton and others 2006; Carbone and others 2008). In our rainfall treatments, root respiration accounted for approximately one-half to two-thirds of the total measured soil CO₂. Based on the assumption that root respiration is less variable and constituted a large portion of soil respiration, we can make two inferences. First, we may have observed reduced respiration sensitivity in the +P treatments by including the less responsive root component in our CO₂ measurements. Second, because root contributions to soil CO₂ were roughly comparable between the HV and LV treatments, our results suggest that differences in respiration sensitivity may be due largely to het-

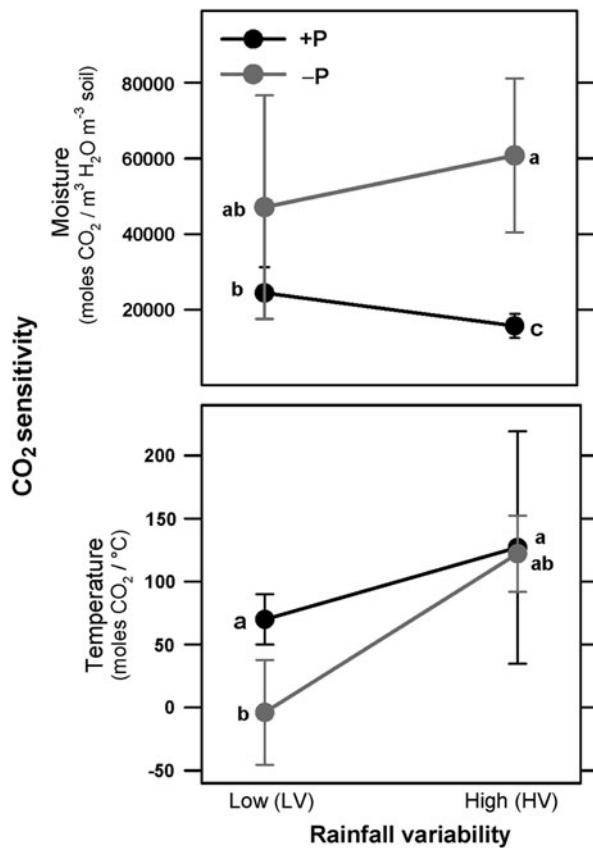


Figure 3. Sensitivity of soil respiration to fluctuations in moisture and temperature under a low variability (LV) and high variability (HV) rainfall treatment in +P and -P soils. Sensitivity is defined as the change in CO_2 per unit change of an environmental variable (that is, soil moisture or soil temperature). All values are parameter estimates from time-series multiple regression models with attending 95% confidence intervals. Different letters indicate significant differences between treatments ($P < 0.05$) based on pairwise t -tests.

erotrophic organisms. Here, we briefly discuss potential plant-mediated mechanisms that could contribute to a decline in respiration sensitivity to moisture by heterotrophic organisms.

First, plants may have dampened respiration sensitivity to moisture by altering the availability of resources to heterotrophic organisms (primarily bacteria and fungi). For example, DOC was higher and inorganic N was lower in +P than -P soils (Table 1; Figure 1). Such changes in resource availability could potentially influence the responsiveness of respiration to changes in soil moisture. We found some evidence for this in +P soils where higher DOC concentrations did not correspond with more CO_2 being produced following high variability rainfall events. Instead, CO_2 pulses following rain events were 200% higher in

-P than +P soils. These distinctions in the rapid CO_2 responses between plant treatments may have resulted from differences in the quality and quantity of C or N sources available to soil microbes. In the absence of plants, labile C may be derived primarily from intracellular solutes that are released as microbial biomass turns over. Further, the amount of these substances may vary due to sudden changes in soil moisture (Halverson and others 2000; Fierer and Schimel 2003). Therefore, following rainfall events, intracellular solutes may be rapidly mineralized and assimilated leading to large short-term pulses of CO_2 that heighten heterotrophic respiration sensitivity.

Plants may have also influenced respiration sensitivity by creating drier soil conditions through evapotranspiration. Under high variability rainfall regimes soils experience prolonged dry-down periods that are punctuated by episodic rainfall events. Under such conditions high variability rainfall generally enhances respiratory responses (Fay and others 2000, 2008; Harper and others 2005). We did not observe this pattern in our mesic system where the magnitude and frequency of rainfall events were relatively small. In contrast, soils under our LV rainfall treatment were the driest, whereas soils under HV rainfall never substantially dried out between the weekly events (Figure 2). Under these conditions the relatively drier soil under LV rainfall may have stimulated heterotrophic respiration and caused respiration sensitivity to be enhanced. Therefore, respiration sensitivity reflected changes in the dryness of the soil more so than what was predicted based on the nominal rainfall treatment.

Co-regulation of Respiration Sensitivity: The Importance of Temperature and Moisture

Our results indicate there are strong interactions between rainfall variability and aboveground-belowground processes on the sensitivity of soil respiration to moisture and temperature. For example, under LV rainfall the sensitivity of respiration to temperature significantly increased by a factor of five when plants were present in the soil (Figure 3). In contrast, there was no affect of temperature fluctuations on respiration in -P soils under the LV rainfall. Respiration was also sensitive to temperature under HV rainfall, but was unaffected by the plant manipulation. As we discussed above, the sensitivity of respiration to moisture was predominantly positive and significant. Thus, our results generally agree with other studies

demonstrating that soil respiration in mesic ecosystems is co-regulated by moisture and temperature (Davidson and others 1998; Conant and others 2004; Almagro and others 2009). Additional manipulative studies are needed, however, to better understand how temperature and moisture variability independently affect the sensitivity of soil respiration both on a daily and seasonal time-step.

The enhanced respiration sensitivity to temperature under low variability rainfall may have resulted from multiple plant effects. First, shading by plants may reduce temperature stress (Lloyd and Taylor 1994), which could in turn enhance the sensitivity of respiration. Under low variability rainfall, temperatures in the +P plot did not rise above 25°C and were 2–5°C cooler relative to bare soil for 85% of the experiment, whereas soil temperatures in the –P plots were greater than 25°C for 27% of the experiment. Second, respiration sensitivity to temperature may be enhanced if rising soil temperatures occur when C substrate availability is high. In the absence of moisture limitation, soil respiration tends to be enhanced if rising soil temperatures are synchronized with high resource availability (Boone and others 1998; Cleveland and others 2007; Yuste and others 2007). Our results are consistent with this resource-temperature synchrony because DOC concentrations were often higher in the presence than the absence of plants and sensitivity was elevated in these soils (Figure 1).

Experimental Design: Caveats and Inference Space

Ecologists are commonly challenged by the optimal design of field experiments (see Cottingham and others 2005). One such challenge involves trade-offs between replication, sampling frequency, and the number of factors that can be simultaneously explored in a given study. For example, our first experiment examined the effects of a plant manipulation (+P versus –P) on soil respiration in plots within replicate 1-ha grassland fields (Figure 1). This study had the advantage of statistical power and strong spatial inference, but with bi-weekly sampling, we were unable to resolve the sensitivity of soil respiration to rapid fluctuations in moisture and temperature (Figure 2). We addressed this shortcoming by conducting a second manipulative experiment that generated high-resolution temporal data using environmental sensors. In this case, however, we were unable to replicate the treatment combinations owing to the costs of the sensor network. Similar constraints arise when ecologists conduct whole-ecosystem

experiments or when unplanned events interrupt long-term studies (Miao and Carstenn 2006). Fortunately, there is a suite of statistical techniques that allow ecologists to make robust inferences about unreplicated time-series data (Carpenter 1990; Diggle 1990; Pole and others 1994; Bence 1995). In this study, we used a relatively simple set of multiple regression time-series models to test the effects of plant and precipitation manipulations on soil respiration dynamics. Given the rapid development and application of environmental sensor technology, it seems likely that ecosystem ecologists will increasingly need to consider using similar statistical approaches. Even though time-series statistics provide an opportunity to analyze unreplicated experiments, it is important to acknowledge the limitation of these analyses. For example, soil organisms and their metabolic functions are notoriously heterogeneous (Frey 2007). In this particular study, the spatial variability of CO₂ flux in the replicated +P and –P treatments across the 42-ha site was relatively low (coefficient of variation = 0.22–0.31), but this pattern may not hold for other processes or in different ecosystems. Therefore, it is important to exercise caution when extrapolating findings from unreplicated experiment across the landscape.

CONCLUSION

Grasslands comprise approximately 40% of the terrestrial surface of the earth (Wang and Fang 2009). Due to the extent of this biome, fluxes of C from these systems may play a critical role in determining CO₂ feedbacks between the soil and the atmosphere. We found that the coupling of aboveground and belowground processes via plants was important for predicting the dynamics of soil respiration to rainfall variability. In our mesic grassland, respiration was co-regulated by moisture and temperature even as rainfall became more variable. To more fully understand our results, it is essential to determine how the sensitivity of respiration is partitioned between root and heterotrophic respiration, if these plant-mediating effects are consistent across grasslands in different bioregions with different vegetation communities, and the role of root deposited C sources in promoting sensitivity.

ACKNOWLEDGEMENTS

We thank the KBS LTER field technicians for helping to maintain experimental plots, C. McMinn and B. Phillips for assistance with soil sampling,

B. Lehmkuhl for logistical support, and S.E. Jones and A.S. Hartshorn for critical comments on an earlier version of this manuscript. We acknowledge support from the Rackham Research Endowment and the Michigan Agricultural Experiment Station (MAES). In addition, this project was supported by National Research Initiative Grants (2006-35107-16725 and 2008-35107-04481) from the USDA National Institute of Food and Agriculture. Kellogg Biological Station contribution # 1527.

REFERENCES

- Almagro M, Lopez J, Querejeta JI, Martinez-Mena M. 2009. Temperature dependence of soil CO₂ efflux is strongly modulated by seasonal patterns of moisture availability in a Mediterranean ecosystem. *Soil Biol Biochem* 41:594–605.
- Ambus P, Robertson GP. 2006. The effect of increased N deposition on nitrous oxide, methane, and carbon dioxide fluxes from unmanaged forest and grassland communities in Michigan. *Biogeochemistry* 79:315–37.
- Anderson JM. 1973. Carbon dioxide evolution from two temperate, deciduous woodland soils. *J Appl Ecol* 10:361–78.
- Atkin OK, Bruhn D, Hurry VM, Tjoelker MG. 2005. The hot and the cold: unravelling the variable response of plant respiration to temperature. *Funct Plant Biol* 32:87–105.
- Bartholomew WV, Broadbent FE. 1950. Apparatus for control of moisture, temperature, and air composition in microbiological respiration experiments. *Soil Sci Soc Am J* 14:156–60.
- Bence JR. 1995. Analysis of short time series: correcting for autocorrelation. *Ecology* 76:628–39.
- Boone RD, Nadelhoffer KJ, Canary JD, Kaye JP. 1998. Roots exert a strong influence on the temperature sensitivity of soil respiration. *Nature* 396:570–2.
- Borken W, Matzner E. 2009. Reappraisal of drying and wetting effects on C and N mineralization and fluxes in soils. *Glob Chang Biol* 15:808–24.
- Cable JM, Ogle K, Williams DG, Weltzin JF, Huxman TE. 2008. Soil texture drives responses of soil respiration to precipitation pulses in the Sonoran Desert: implications for climate change. *Ecosystems* 11:961–79.
- Carbone MS, Winston GC, Trumbore SE. 2008. Soil respiration in perennial grass and shrub ecosystems: linking environmental controls with plant and microbial sources on seasonal and diel timescales. *J Geophys Res Biogeosci* 113:G02022.
- Cardon ZG, Gage DJ. 2006. Resource exchange in the rhizosphere: molecular tools and the microbial perspective. *Annu Rev Ecol Syst* 37:459–88.
- Carpenter SR. 1990. Large-scale perturbations: opportunities for innovation. *Ecology* 71:2038–43.
- Cisneros-Dozal LM, Trumbore S, Hanson PJ. 2006. Partitioning sources of soil-respired CO₂ and their seasonal variation using a unique radiocarbon tracer. *Glob Chang Biol* 12:194–204.
- Cleveland CC, Nemergut DR, Schmidt SK, Townsend AR. 2007. Increases in soil respiration following labile carbon additions linked to rapid shifts in soil microbial community composition. *Biogeochemistry* 82:229–40.
- Conant RT, Dalla-Betta P, Klopatek CC, Klopatek JA. 2004. Controls on soil respiration in semiarid soils. *Soil Biol Biochem* 36:945–51.
- Cook FJ, Orchard VA. 2008. Relationships between soil respiration and soil moisture. *Soil Biol Biochem* 40:1013–18.
- Cottingham KL, Lennon JT, Brown BL. 2005. Designing more informative ecological experiments. *Front Ecol Environ* 3:145–52.
- Crow SE, Wieder RK. 2005. Sources of CO₂ emission from a northern peatland: root respiration, exudation, and decomposition. *Ecology* 86:1825–34.
- Daly E, Oishi CA, Porporato A, Katul GG. 2008. A stochastic model for daily subsurface CO₂ concentration and related soil respiration. *Adv Water Resour* 31:987–94.
- 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. *Glob Chang Biol* 4:217–27.
- Davidson EA, Janssens IA. 2006. Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. *Nature* 440:165–73.
- Davidson EA, Janssens IA, Luo YQ. 2006. On the variability of respiration in terrestrial ecosystems: moving beyond Q₁₀. *Glob Chang Biol* 12:154–64.
- Diggle PJ. 1990. *Time series: a biostatistical introduction*. Oxford: Oxford University Press.
- Fay PA, Carlisle JD, Knapp AK, Blair JM, Collins SL. 2000. Altering rainfall timing and quantity in a mesic grassland ecosystem: design and performance of rainfall manipulation shelters. *Ecosystems* 3:308–19.
- Fay PA, Kaufman DM, Nippert JB, Carlisle JD, Harper CW. 2008. Changes in grassland ecosystem function due to extreme rainfall events: implications for responses to climate change. *Glob Chang Biol* 14:1600–8.
- Fierer N, Schimel JP. 2003. A proposed mechanism for the pulse in carbon dioxide production commonly observed following the rapid rewetting of a dry soil. *Soil Sci Soc Am J* 67:798–805.
- Frey SD. 2007. Spatial distribution of soil organisms. In: *Ecology Biochemistry*, Paul EA, Eds. Soil microbiology. London: Academic Press.
- Gu LH, Hanson PJ, Mac Post W, Liu Q. 2008. A novel approach for identifying the true temperature sensitivity from soil respiration measurements. *Glob Biogeochem Cycles* 22:GB4009.
- Halverson LJ, Jones TM, Firestone MK. 2000. Release of intracellular solutes by four soil bacteria exposed to dilution stress. *Soil Sci Soc Am J* 64:1630–7.
- 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* 48:115–46.
- Harper CW, Blair JM, Fay PA, Knapp AK, Carlisle JD. 2005. Increased rainfall variability and reduced rainfall amount decreases soil CO₂ flux in a grassland ecosystem. *Glob Chang Biol* 11:322–34.
- IPCC. 2007. *Climate change 2007: the scientific basis*. Cambridge, UK: Cambridge Press.
- Jenkinson DS, Adams DE, Wild A. 1991. Model estimates of CO₂ emissions from soil in response to global warming. *Nature* 351:304–6.
- Jobbagy EG, Jackson RB. 2000. The vertical distribution of soil organic carbon and its relation to climate and vegetation. *Ecol Appl* 10:423–36.
- Knapp AK, Fay PA, Blair JM, Collins SL, Smith MD, Carlisle JD, Harper CW, Danner BT, Lett MS, McCarron JK. 2002. Rainfall

- variability, carbon cycling, and plant species diversity in a mesic grassland. *Science* 298:2202–5.
- Knapp AK, Beier C, Briske DD, Classen AT, Luo Y, Reichstein M, Smith MD, Smith SD, Bell JE, Fay PA, Heisler JL, Leavitt SW, Sherry R, Smith B, Weng E. 2008. Consequences of more extreme precipitation regimes for terrestrial ecosystems. *BioScience* 58:811–21.
- Lauenroth WK, Bradford JB. 2006. Ecohydrology and the partitioning AET between transpiration and evaporation in a semiarid steppe. *Ecosystems* 9:756–67.
- Lee X, Wu HJ, Sigler J, Oishi C, Siccama T. 2004. Rapid and transient response of soil respiration to rain. *Glob Chang Biol* 10:1017–26.
- Liu XZ, Wan SQ, Su B, Hui DF, Luo YQ. 2002. Response of soil CO₂ efflux to water manipulation in a tallgrass prairie ecosystem. *Plant Soil* 240:213–23.
- 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. *Glob Chang Biol* 15:184–95.
- Ljung GM, Box EP. 1978. On a measure of lack of fit in time series models. *Biometrika* 65:297–303.
- Livingston GP, Hutchinson GL. 1995. Enclosure-based measurement of trace gas exchange: applications and sources of error. In: Matson PA, Harriss RC, Eds. *Biogenic trace gases: measuring emissions from soil, water*. Osney Mead, Oxford, UK: Blackwell Science. p 14–51.
- Lloyd J, Taylor JA. 1994. On the temperature-dependence of soil respiration. *Funct Ecol* 8:315–23.
- Miao S, Carstenn S. 2006. A new direction for large-scale experimental design and analysis. *Front Ecol Environ* 4:227.
- Noormets A, Desai AR, Cook BD, Euskirchen ES, Ricciuto DM, Davis KJ, Bolstad PV, Schmid HP, Vogel CV, Carey EV, Su HB, Chen J. 2008. Moisture sensitivity of ecosystem respiration: comparison of 14 forest ecosystems in the Upper Great Lakes Region, USA. *Agric For Meteorol* 148:216–30.
- Pole A, West M, Harrison J. 1994. *Applied Bayesian forecasting and time series analysis*. New York: Chapman-Hall.
- Raich JW, Potter CS. 1995. Global patterns of carbon-dioxide emissions from soils. *Glob Biogeochem Cycles* 9:23–36.
- Riveros-Iregui DA, Emanuel RE, Muth DJ, McGlynn BL, Epstein HE, Welsch DL, Pacific VJ, Wraith JM. 2007. Diurnal hysteresis between soil CO₂ and soil temperature is controlled by soil water content. *Geophys Res Lett* 34:L17404.
- Robertson GP, Paul EA, Harwood RR. 2000. Greenhouse gases in intensive agriculture: contributions of individual gases to the radiative forcing of the atmosphere. *Science* 289:1922–5.
- Schimel J, Balsler TC, Wallenstein M. 2007. Microbial stress-response physiology and its implications for ecosystem function. *Ecology* 88:1386–94.
- Schlesinger WH. 1997. *Biogeochemistry, an analysis of global change*. San Diego: Academic Press.
- Scott-Denton LE, Rosenstiel TN, Monson RK. 2006. Differential controls by climate and substrate over the heterotrophic and rhizospheric components of soil respiration. *Glob Chang Biol* 12:205–16.
- Shim JH, Pendall E, Morgan JA, Ojima DS. 2009. Wetting and drying cycles drive variations in the stable carbon isotope ratio of respired carbon dioxide in semi-arid grassland. *Oecologia* 160:321–33.
- Sponseller RA. 2007. Precipitation pulses and soil CO₂ flux in a Sonoran Desert ecosystem. *Glob Chang Biol* 13:426–36.
- Tang JW, Baldocchi DD, Qi Y, Xu LK. 2003. Assessing soil CO₂ efflux using continuous measurements of CO₂ profiles in soils with small solid-state sensors. *Agric For Meteorol* 118:207–20.
- Tang JW, Misson L, Gershenson A, Cheng WX, Goldstein AH. 2005. Continuous measurements of soil respiration with and without roots in a ponderosa pine plantation in the Sierra Nevada Mountains. *Agric For Meteorol* 132:212–27.
- Trumbore S. 2006. Carbon respired by terrestrial ecosystems—recent progress and challenges. *Glob Chang Biol* 12:141–53.
- van der Putten WH, Bardgett RD, de Ruiter PC, Hol WHG, Meyer KM, Bezemer TM, Bradford MA, Christensen S, Eppinga MB, Fukami T, Hemerik L, Molofsky J, Schadler M, Scherber C, Strauss SY, Vos M, Wardle DA. 2009. Empirical and theoretical challenges in aboveground-belowground ecology. *Oecologia* 161:1–14.
- Wang W, Fang JY. 2009. Soil respiration and human effects on global grasslands. *Glob Planet Change* 67:20–8.
- Weltzin JF, Loik ME, Schwinning S, Williams DG, Fay PA, Haddad BM, Harte J, Huxman TE, Knapp AK, Lin GH, Pockman WT, Shaw MR, Small EE, Smith MD, Smith SD, Tissue DT, Zak JC. 2003. Assessing the response of terrestrial ecosystems to potential changes in precipitation. *BioScience* 53:941–52.
- Wolfinger R, Chang M. 1999. Comparing the SAS GLM and MIXED Procedures for repeated measures. Cary, NC: SAS Institute, Inc.
- Xu LK, Baldocchi DD. 2004. Seasonal variation in carbon dioxide exchange over a Mediterranean annual grassland in California. *Agric For Meteorol* 123:79–96.
- Yepez EA, Scott RL, Cable WL, Williams DG. 2007. Intraseasonal variation in water and carbon dioxide flux components in a semiarid riparian woodland. *Ecosystems* 10:1100–15.
- Yuste JC, Baldocchi DD, Gershenson A, Goldstein A, Misson L, Wong S. 2007. Microbial soil respiration and its dependency on carbon inputs, soil temperature and moisture. *Glob Chang Biol* 13:2018–35.
- Zhang XB, Zwiers FW, Hegerl GC, Lambert FH, Gillett NP, Solomon S, Stott PA, Nozawa T. 2007. Detection of human influence on twentieth-century precipitation trends. *Nature* 448:461–464.
- Zobitz JM, Moore DJP, Sacks WJ, Monson RK, Bowling DR, Schimel DS. 2008. Integration of process-based soil respiration models with whole-ecosystem CO₂ measurements. *Ecosystems* 11:250–69.