

Sensitivity of soil respiration and microbial communities to altered snowfall

Zachary T. Aanderud^{a,1}, Stuart E. Jones^{a,2}, Donald R. Schoolmaster Jr.^a, Noah Fierer^{b,c}, Jay T. Lennon^{a,d,e,*}^a W.K. Kellogg Biological Station,³ Michigan State University, Hickory Corners, MI 49060, USA^b Department of Ecology and Evolutionary Biology, University of Colorado, Boulder, CO 80309, USA^c Cooperative Institute for Research in Environmental Sciences, University of Colorado, Boulder, CO 80309, USA^d Department of Microbiology and Molecular Genetics, Michigan State University, Hickory Corners, MI 49060, USA^e Department of Biology, Indiana University, Bloomington, IN 47405, USA

ARTICLE INFO

Article history:

Received 2 March 2012

Received in revised form

17 July 2012

Accepted 26 July 2012

Available online 22 August 2012

Keywords:

Bacteria

Dissolved organic carbon

Freeze–thaw cycles

Fungi

Legacy

Spring–thaw

Recovery

Seasonally snow-covered ecosystems

Seed banks

Sub-zero conditions

Winter CO₂ flux

ABSTRACT

Winter respiration is a quantitatively important, yet variable flux of carbon dioxide (CO₂) from soils to the atmosphere. Variability in winter soil respiration may be influenced by the effects of snowfall on microbial communities and their metabolic activities. In this study, we evaluated the importance of snowpack depth on soil respiration and microbial communities in a temperate deciduous forest. Snow removal created relatively dry, frequently frozen, and carbon substrate-poor soils, while snow additions led to wetter, warmer, and relatively carbon substrate-rich soils. Using time-series multiple regression, we observed enhanced sensitivity of respiration to moisture under ambient snow and snow removal; however, this effect was accompanied by a temporal lag suggesting that microorganisms had a delayed response to increases in free-water during soil thawing events. Conversely, soil respiration was only sensitive to temperature in the snow addition treatment when soil temperatures were consistently above 0 °C. The snow-induced respiration dynamics were accompanied by shifts in the structure of wintertime fungal and bacterial communities. We detected an impact of altered snowpack on bacterial richness during the growing season, but our manipulation did not have legacy effects on other features of the soil microbial community at spring thaw. Our results suggest that microbial communities may be “reset” during seasonal transitions from winter to spring, and that soil microorganisms are likely adapted to annual fluctuations in snowpack depth. As snowpack becomes more variable in mid-latitude systems due to climate change, our findings suggest that soil moisture and temperature will co-regulate wintertime respiration through a non-linear relationship surrounding soil freeze–thaw cycles, with snow-mediated changes in microbial community structure likely influencing wintertime respiration dynamics.

© 2012 Elsevier Ltd. All rights reserved.

1. Introduction

The exchange of carbon dioxide (CO₂) between soils and the atmosphere is a major component of the global carbon (C) cycle (Raich and Potter, 1995). While most of the C influx to terrestrial ecosystems can be attributed to photosynthesis during the spring and summer months, mineralization processes, such as soil

respiration, occur throughout the year. As a result, wintertime soil respiration can be quantitatively important when estimating annual carbon budgets. For example, winter respiration can account for more than half of the C sequestered by higher plants during the growing season (Sommerfeld et al., 1993; Winston et al., 1997; Monson et al., 2002). However, winter respiration is highly variable and may be regulated by fluctuations in environmental variables that covary with the timing and accumulation of a snowpack (Brooks et al., 1997, 2004; Mikan et al., 2002; Liptzin et al., 2009).

Through its effects on temperature and moisture, the depth of a snowpack is an important environmental characteristic that controls wintertime soil respiration. Under a deep snowpack, soils are insulated from colder air temperatures, which can increase heterotrophic respiration (Mariko et al., 1994; Brooks et al., 1997; Rey et al., 2002). Although soil respiration is reduced under

* Corresponding author. Current address. Department of Biology, Indiana University, 1001 E. 3rd St., Bloomington, IN 47405, USA.

E-mail address: lennonj@indiana.edu (J.T. Lennon).

¹ Current address: Department of Plant and Wildlife Sciences, Brigham Young University, Provo, UT 84602, USA.

² Current address: Department of Biological Sciences, University of Notre Dame, South Bend, IN 46556, USA.

³ Kellogg Biological Station contribution #1592.

a shallow snowpack, microorganisms are capable of maintaining catabolic (CO₂ production) and anabolic (biomass synthesis) processes under sub-zero temperatures (Panikov et al., 2006; Drotz et al., 2010; McMahon et al., 2011). Snow-mediated temperature effects on respiration, however, are simultaneously influenced by soil moisture. For example, under a deep snowpack warmer soil temperatures are often associated with wetter conditions, which can promote respiration (Liptzin et al., 2009). Conversely, under a shallow snowpack, soils commonly undergo freeze–thaw cycles (FTCs) where shifts in temperature regulate transitions of water between solid (ice) and liquid phases. As frozen soils thaw, heterotrophic respiration is stimulated by warmer soil temperatures, more free water, and the release of labile C substrates from lysed plant and microbial cells (Brooks et al., 2004; Schimel et al., 2007; Borken and Matzner, 2008).

Winter conditions may influence the sensitivity of soil respiration to moisture and temperature by affecting the composition and activity of soil microbial communities. A number of studies have documented differences in the composition of soil microbial communities collected during summer and winter seasons (Lipson and Schmidt, 2004; Lipson et al., 2009; McMahon et al., 2011). In some instances, these compositional differences correspond with changes in temperature sensitivity. For example, wintertime microbial communities from the soils of a subalpine forest exhibited exponential growth at 0 °C, while summertime microbial communities were unable to grow below 4 °C (Monson et al., 2006). Seasonal shifts in the composition and function of microbial communities may be due in part to the physiological stress associated with FTCs (Schimel et al., 2007), which are thought to select for taxa that can tolerate freezing soil conditions (Sharma et al., 2006; Walker et al., 2006). It remains to be determined if the environmental effects of variable snowfall on microbial communities are transient or if they have the ability to persist into the growing season.

In this study, we evaluated the sensitivity of soil respiration to snowpack-induced fluctuations in temperature and moisture in a temperate deciduous forest. We compared soil respiration sensitivity (i.e., CO₂ evolved per unit change in moisture or temperature) in a replicated field experiment where snowpack levels were directly manipulated. In addition to measuring soil properties, including organic C availability, which are often influenced by a snowpack (Groffman et al., 2001; Schimel et al., 2004; Brooks et al., 2004), we evaluated the effects of our manipulations on the structure of soil microbial communities. Bacterial and fungal responses measured throughout winter season allowed us to identify potential links between microbial communities, snow, and our observed winter CO₂ dynamics. Furthermore, we tested whether or not snowpack induced shifts in bacterial community structure persisted beyond spring–thaw, which could potentially have legacy effects on springtime soil processes.

2. Materials and methods

2.1. Site description

We conducted our study at the W. K. Kellogg Biological Station (KBS) Long-Term Ecological Research (LTER) site located in southwestern Michigan, USA. The experiment was performed in a native maple–hickory deciduous forest (KBS LTER treatment DF3) (DeGryze et al., 2004). Dominant tree species include *Acer saccharum* (Marsh.), *Carya glabra* (Mill.), and *Quercus alba* (L.). Average annual precipitation at the site is 890 mm (± 148.0 SD, $n = 21$) with approximately half falling as snow, and the mean annual temperature is 9.0 °C (± 0.81 SD, $n = 21$) (<http://www.kbs.msu.edu/>

databases). All soils are fine-loamy, mixed, mesic Typic Hapludalfs with a total C of 1.6%, total soil N of 0.10%, and pH of 5.3.

2.2. Snow manipulation

To investigate the effects of a variable snowpack on ecosystem processes and soil microbial structure, we created three treatments where snow was removed (Snow removal, –S), added (Snow addition, +S) or left in place (Ambient). Treatments were assigned in a complete randomized block design ($n = 3$) for a total of nine experimental units. We constructed three blocks (5 m \times 13 m), subdivided the blocks into three plots (3 m \times 3 m), and randomly assigned one treatment to each plot. We left a 1 m buffer strip around each plot to control for edge effects. The treatments were created and maintained over four months (20 December–5 April 2008) by removing the snowfall from the –S treatment with a shovel and redistributing this removed snow evenly across replicates in the +S treatment. Snow was removed from the –S treatment following any snowfall event greater than 20 mm. When removing the snow from the –S treatment, we left approximately 20 mm of snowfall to minimize physical disturbance to the litter and soil surface.

2.3. C substrate availability and chemistry

We quantified soil dissolved organic carbon (DOC) and inorganic nitrogen (N) concentrations in the snow treatments, as these variables can affect soil respiration and microbial communities. DOC (mg C g soil^{–1}), dissolved organic N (DON, mg C g soil^{–1}), and inorganic N (NH₄⁺ and NO₃[–], $\mu\text{g N g soil}^{-1}$) were measured on at least a monthly basis (3 snow manipulations \times 3 replicates \times 6 time points = 36 samples) over the four-month experiment. Each soil sample represented a composite of three subsamples removed from each plot with a soil probe (diameter = 2 cm, 0–5 cm depth). After each soil core was removed, we placed a 12 cm piece of PVC pipe, capped with a rubber stopper, into the hole made by the soil probe to prevent preferential water flow from snow melt. Within 24 h of sampling, soils were water-extracted (1:2 w/v), passed through a 0.2- μm nylon filter (Millipore, Billerica MA, USA), and analyzed for DOC and DON using a total organic C and total N 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). DON was estimated as the difference between total N and total inorganic N. All soil variables were expressed on a soil dry-weight basis by correcting for gravimetric water content (subsample dried at 105 °C for 48 h). We tested for the effect of the snow treatments on our response variables (i.e., DOC, DON, NH₄⁺ and NO₃[–] concentrations) using repeated measures (RM) ANOVA (SAS PROC MIXED) with covariance structures selected using Bayesian Information Criterion (Kelman et al., 1999).

2.4. Soil CO₂ concentrations, temperature, and moisture

To determine the effects of a snowpack on respiration, we measured real-time soil CO₂ concentrations, soil moisture, and soil temperature in a single replicate of each snow treatment. We measured near-surface CO₂ concentrations (ppmv) as a proxy for soil respiration. It has been shown that near-surface CO₂ concentrations generated with our sensor technology provide accurate estimates of soil respiration (Daly et al., 2008). By placing the sensor close to the soil surface, one is able to capture an integrated CO₂ response before the gas is released to the atmosphere (Daly et al., 2008; Aanderud et al., 2011). Specifically, we placed sensors at

a depth of 2 cm in the center of each treatment with a 3% CO₂ GMT222 sensor using non-dispersive infrared absorption (Vaisala, Helsinki, Finland). A detailed description of GMT222 sensors and the pressure and temperature corrections that we applied to our CO₂ measurements can be found elsewhere (Tang et al., 2003). Volumetric soil moisture (m³ H₂O m⁻³ soil) and soil temperature (°C) were also measured over a soil depth of 0–5 cm in each treatment using ECH₂O-TM sensors (Decagon Devices, Pullman WA, USA). The sensors measured CO₂ concentrations, temperature, and moisture every 10 s and dataloggers stored and integrated these values into 30-min means (CR1000, Campbell Scientific, Inc., Logan UT, USA). Data were radio-transmitted back to the laboratory (RF401 900-MHz spread-spectrum radio, Campbell Scientific, Inc., Logan UT, USA). The data were expressed on an averaged daily time-step to remove diel variability, which may have masked the impact of our treatments on soil respiration (Riveros-Iregui et al., 2007; Carbone et al., 2008).

We documented FTC during the winter by analyzing the fluctuations in volumetric soil moisture measured with ECH₂O-TM sensors (Decagon Devices, Pullman WA, USA) in one replicate of each snow treatment. We defined a FTC based on changes in soil moisture using the following criteria: first, moisture had to decline as free liquid water became ice (i.e., freeze); and second, moisture had to then increase (i.e., thaw) back to similar values prior to the freeze.

2.5. Respiration sensitivity to snow-induced environmental fluctuations

We evaluated the sensitivity of soil respiration to environmental fluctuations using time-series multiple regression. Sensitivity was defined as the rate of CO₂ evolved per unit change in moisture or temperature based on parameter estimates from the following linear regression model that fit the time-series data from each treatment:

$$\text{CO}_2(t) = \text{CO}_2(t-1) + \text{temp}(t) + \text{moisture}(t) + \text{temp}(t-1) + \text{moisture}(t-1) \quad (1)$$

where t indicates time. Thus, the fitted parameters associated with $\text{temp}(t)$ and $\text{moisture}(t)$ represent the sensitivity of $\text{CO}_2(t)$ at time t , to fluctuations in temperature and moisture at that time step. The parameters associated with $\text{CO}_2(t-1)$, $\text{moisture}(t-1)$ and $\text{temp}(t-1)$ represent the sensitivity of $\text{CO}_2(t)$ to fluctuations in CO₂, moisture, and temperature at the previous time step (i.e., $t-1$). We tested the residuals of the time-series regressions for autocorrelation using a Ljung–Box test (Ljung and Box, 1978) and found the residuals of all models to be independently distributed ($P > 0.05$), indicating no temporal autocorrelation. Adjusted R^2 and standard errors were estimated for all models. We tested for the significance of sensitivity parameters by constructing 95% confidence intervals (estimate $\pm [1.99_{(t,0.975, n=88)} \times \text{standard error}]$) around each parameter and performing pairwise t -tests to determine significant differences ($P < 0.05$) in CO₂ sensitivity between the treatments. In addition, we displayed the standardized regression coefficients from the models for each treatment using causal networks. All modeling was performed with the stats package from the R Statistics Environment (R Development Core Team, 2008).

We examined the relationship between temperature and moisture to better understand their interactive effects on respiration. We fit the following logistic function to the daily means of temperature and moisture data collected from sensors in the three snow treatments:

$$\text{moisture}(t) = \beta_0 + \frac{\beta_1}{1 + e^{-(\beta_2 + \beta_3 \text{temp}(t))}} \quad (2)$$

The residuals of the model had a positive first-order autocorrelation, which had the effect of reducing the effective sample size used to estimate the standard errors of the parameter estimates. To address this issue, we estimated the effective sample size as,

$$N_{\text{adj}} = N \frac{1 - r_1}{1 + r_1} \quad (3)$$

where N is the sample size and r_1 is the first-order autocorrelation coefficient. Accordingly, we adjusted the standard errors of the parameter estimates as follows:

$$\text{SE}_{\text{adj}} = \frac{\sqrt{N\text{SE}}}{\sqrt{N_{\text{adj}}}} \quad (4)$$

2.6. Wintertime bacterial and fungal community structure

To evaluate the effects of our snow manipulations on bacterial and fungal communities throughout the winter season, we used terminal restriction fragment length polymorphism (T-RFLP), a DNA fingerprinting technique. Although this technique does not yield any phylogenetic information, it is a cost-effective way to assess broad-scale responses of microbial communities to environmental change. Soils for these analyses were removed at approximately two-week intervals over the four-month experimental period at a soil depth of 0–5 cm resulting in a total of 81 samples (3 snow manipulations \times 3 replicates \times 9 time points = 81 samples). Each sample represented a composite of three subsamples similar to the samples used for our soil chemistry analyses. Total genomic DNA was extracted from approximately 1 g of soil using the PowerSoil DNA Isolation Kit (MoBio, Carlsbad, CA, USA). For fungi, we PCR-amplified DNA using a fluorescently (FAM-6) labeled ITS1-F forward primer, an unlabeled ITS4 reverse primer, and the thermal cycler pattern described by Avis et al. (2006). For bacteria, we amplified DNA using a fluorescently (FAM-6) labeled 8F forward primer, an unlabeled 1492R reverse primer, and the thermal cycler pattern described in Lennon and Martiny (2008). Following PCR cleanup with a Qiagen Qiaquick PCR purification kit (Qiagen Inc., Valencia, CA, USA), 20–30 ng of the fluorescently labeled products were digested for 24 h with 5 units of *Hae*III restriction enzyme following the manufacturers protocol (Promega Co., Madison, WI, USA). Finally, a cleanup of the restriction digest products was conducted using a Qiagen Qiaquick Nucleotide Removal Kit (Qiagen Inc., Valencia, CA, USA) prior to fragment analysis by capillary electrophoresis on an ABI PRISM[®] 3100 Genetic Analyzer at the Research Technology Support Facility at Michigan State University. Restriction fragment profiles were aligned with an internal ROX size-standard using custom scripts implemented in the R Statistics Environment. Briefly, migration times were converted to base pair lengths using the internal standard and the Local Southern method (Elder and Southern, 1983). An overlay of profiles from all samples was used to define T-RFLP operational taxonomic units (OTU). Finally, profiles were converted to an OTU \times sample matrix that was used in subsequent statistical analyses. For each sample, the sum of OTU peak heights was standardized to one. We used permutational multivariate analysis of variance (PERMANOVA; Anderson, 2001) to assess the effects of our snow manipulation on the temporal dynamics of the bacterial and fungal T-RFLP data. PERMANOVA was implemented with the function *adonis* in the vegan package of the R Statistics Environment (R Development Core Team, 2008). The PERMANOVA models included the main

effects of our treatment (snow manipulation), block, and time, along with a treatment \times time interaction term.

We also evaluated the effects of our manipulations on soil microbial communities using fungal to bacterial ratios (F:B). We used a qPCR approach similar to that described in Fierer et al. (2005) to estimate the abundance of bacteria and fungi on the same soils and DNA that were used in the T-RFLP analyses. We performed qPCR assays with a Mastercycler EP Realplex qPCR machine (Eppendorf, Hamburg, Germany) using SYBRGreen to detect the abundance of fungal and bacterial DNA using primers that target the internal transcribed spacer (ITS) region of fungal nuclear DNA and the 16S rRNA gene of bacteria. Each 30- μ L reaction contained the following: 13.5 μ L of 2.5 \times RealMasterMix SYBR ROX (5 Prime, Inc., Gaithersburg, MD, USA); 0.5 μ L of forward and reverse primer; 1 μ L of DNA template; and 14.5 μ L of nuclease free H₂O. We used the forward primer Eub338 and reverse primer Eub518 for bacteria and used the forward primer ITS1-F and reverse primer 5.8S for fungi, which are both universal primer sets (Fierer et al., 2005). Using microbial isolates from the KBS LTER, we generated qPCR standards from a bacterium (*Micrococcus* sp.) and fungus (*Trichosporon* sp.) with the TOPO TA Cloning[®] Kit (Invitrogen). We extracted plasmids from transformed cells and used M13 forward and reverse primers from the cloning kit to generate PCR products that we used for our standard curve, which captured a range of 10¹–10⁸ gene copies μ L⁻¹. The coefficients of determination (r^2) for our assays ranged from 0.93 to 0.99, while amplification efficiencies fell between 0.95 and 0.99. Based on melting curve analyses, we found no evidence for primer dimers.

2.7. Bacterial community structure during spring–thaw

We tested whether or not the effects of manipulated snowpack influenced certain microbial attributes beyond spring–thaw using a combination of T-RFLP and bar-coded pyrosequencing. Pyrosequencing is much more sensitive than T-RFLP and provides phylogenetic information that can be used for identifying groups of microorganisms that may be responsive to wintertime snow manipulations. For the pyrosequencing efforts, we focused on bacteria instead of fungi because they have been reported to be more dynamic under winter conditions (Buckeridge and Grogan, 2008; Zinger et al., 2009). We selected April 3rd (Julian day = 94) samples a snapshot date for evaluating the effects of snow manipulations on springtime microbial communities. By this time, all of the snow had melted, soils had not experienced a FTC for approximately one month, and soil temperatures were beginning to increase (4–6 °C) in all of our treatments. Soils and genomic DNA for these analyses were obtained as described in the section, *Bacterial and fungal community structure during winter*. Details of the pyrosequencing procedures are described in detail elsewhere (Hamady et al., 2008; Fierer et al., 2009). Briefly, 16S rRNA genes were amplified using the bacterial specific primer set 27F and 338R with unique 12-nt error correcting Golay barcodes. For this amplification we used the following thermal cycle conditions: an initial denaturation step at 94 °C for 3 min followed by 35 cycles of denaturation at 94 °C for 45 s, annealing at 50 °C for 30 s, and an extension at 72 °C for 90 s. All PCR amplicons from each sample were pooled at approximately equimolar concentrations prior to pyrosequencing. Samples were sequenced at the Environmental Genomics Core Facility at the University of South Carolina in a 454 Life Sciences genome sequence FLX (Roche, Branford, CT, USA) instrument. We analyzed all sequences following procedures that are described in greater detail elsewhere (Hamady et al., 2008; Lauber et al., 2009). Only sequences that were >200 bp in length were included in the analysis to assure the accuracy and quality of pyrosequencing (Huse et al., 2007). OTU identification was

performed using Megablast at a minimum coverage of 99%, and minimum pairwise identity of 97%. The phylogenetic identity of sequences was determined with BLAST against the Greengenes database (<http://greengenes.lbl.gov/>) (DeSantis et al., 2006) using an E-value cutoff of 1e⁻¹⁰ and the Hugenholtz taxonomic scheme.

To assess the effects of our snowpack manipulation on spring–thaw bacterial communities, we compared bacterial OTU richness along with the relative recovery of taxa at different taxonomic resolutions. First, OTU richness was estimated using Chao-1 estimation (Chao, 1984; Aanderud and Lennon, 2011). We then used ANOVA and Tukey's HSD (SAS PROC GLM) to determine how richness was affected by the snow manipulations. Second, we assessed whether or not the relative recovery of bacteria at higher taxonomic levels (i.e., phyla and subclasses) was influenced by variable snowpack. Relative recovery was estimated by dividing the number of sequences belonging to each phylogenetic group by the total number of sequences in a given sample. We then tested for the effects of treatment (i.e., snow manipulation) and block as main effects using PERMANOVA. Further, to visualize the microbial communities, we performed dual hierarchical clustering and constructed a heatmap with the heatmap.2 function in the gplot package of the R Statistics Environment (R Development Core Team, 2008). Briefly, following rarefaction of sequences to a set sequencing depth of 1100 reads per sample, we estimated the mean relative recovery of taxa belonging to thirteen bacterial phyla or proteobacterial classes across the three replicate plots. We then standardized the relative recoveries within each phylum or subclass to emphasize the occurrence patterns across treatments. These data were then hierarchically clustered using an Unweighted Pair Group Method with Arithmetic Mean (UPGMA).

3. Results

3.1. Snowpack manipulation and the soil environment

During the four-month experiment, snowfall levels in the deciduous forest were typical for winters in southwestern Michigan and adequate to achieve differences among snowpack treatments. Specifically, the Ambient treatment received 1311 mm of snowfall, which equaled 82% of the total snowfall for the winter (2007–2008) and 89% of mean annual snowfall from 2000 to 2008 (1471 mm, \pm 267 SD, $n = 9$). The +S treatment received almost double this amount (approximately 2151 mm), while the –S treatment remained relatively bare of snow. The Ambient treatment also went through periods of time that were relatively bare of snow, especially toward the end of the winter season. There were 28 snowfall events ranging from 3 to 116 mm (Fig. 1a). The last snowfall occurred on March 22nd (Julian day = 82). Spring–thaw started in mid-March; after this time, soil temperatures began to increase, there were no more FTCs, and there was no accumulation of snowfall in any of the treatments.

We observed a strong non-linear relationship between temperature and moisture, which reflected the phase transition of H₂O between free liquid and ice (Fig. 2). The snow treatments did not significantly alter the parameter estimates (β_1 , upper asymptote) of this temperature–moisture relationship (AICs = –876.84 vs –876.78). The fitted parameters of the logistic model were found to be $\beta_0 = 0.034 (\pm 0.019)$, $\beta_1 = 0.258 (\pm 0.022)$, $\beta_2 = 1.533 (\pm 0.396)$ and $\beta_3 = 6.718 (\pm 1.673)$, where the standard error of the estimate is given in parentheses. Nevertheless, soil temperatures in the Ambient treatment were intermediate to those measured in the –S and +S treatments. Soil temperatures in the Ambient treatment remained below zero for most of the winter, but on average were 0.23 °C warmer than the –S treatment and 0.60 °C colder than +S treatment (Fig. 1c). The coldest temperature measured in Ambient

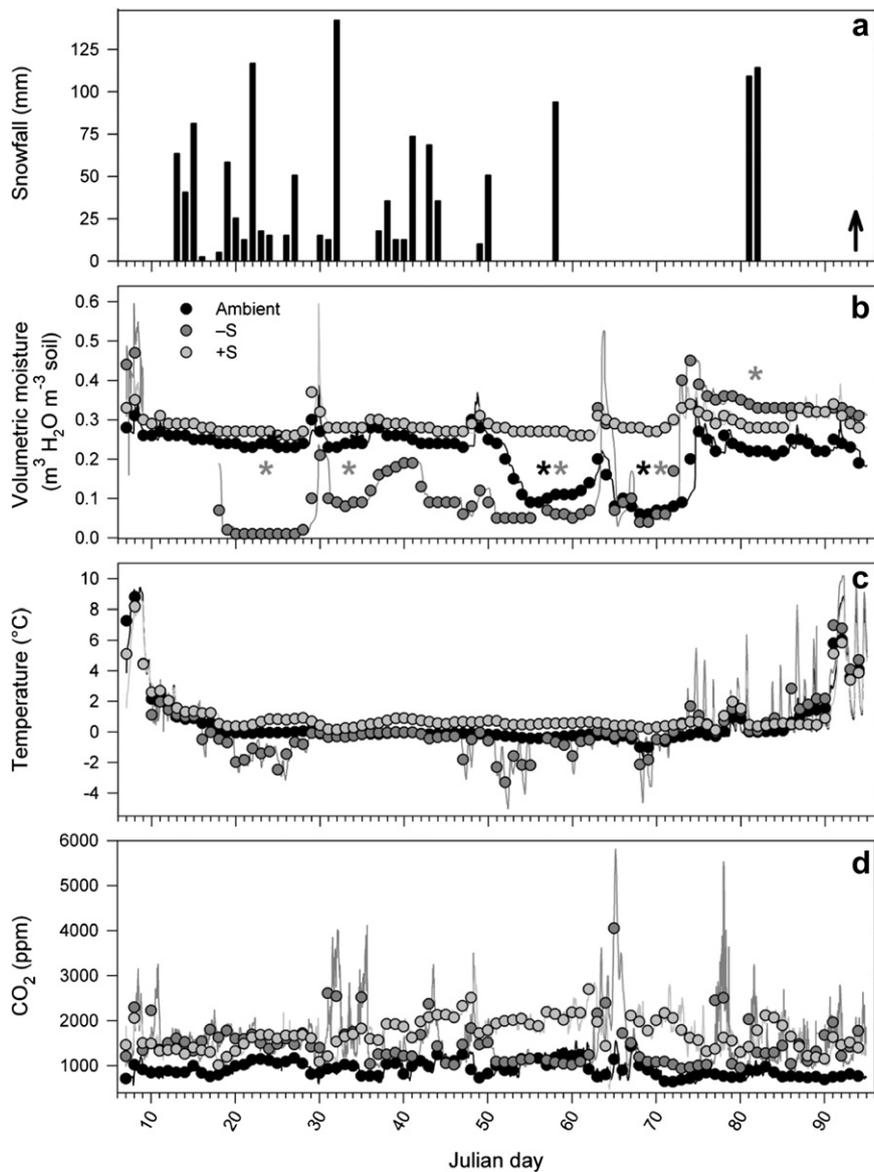


Fig. 1. Snowfall events (vertical bars in **a**) and the effects of snow manipulations on soil moisture (**b**), temperature (**c**), and CO₂ concentration (**d**). Treatments included an ambient snowfall (Ambient), snow removal (–S), and snow addition (+S). The solid lines in **b**, **c**, and **d** represent real-time sensor data that was generated every 10 s and averaged to create a 30 min value. Circles in **b**, **c**, and **d** represent the daily means of these data that were used in our time-series regression analyses to quantify respiration sensitivity. Lines and circles are color-coded (ambient snowfall = black, snow removal = dark gray, and snow addition = light gray). CO₂ values were measured at a depth of 2 cm and soil moisture and temperature were measured over depth of 0–5 cm. Asterisks in **b** indicate a freeze–thaw cycle (FTC) in the ambient (black) and/or –S treatments (dark gray); there were no FTCs in the +S treatment. An upward pointing arrow in **a** identifies the date (April 3rd, Julian day = 94) for the bacterial community analysis during spring–thaw. Data from days 3–12 in the –S were removed due to a malfunction with the CO₂ sensor.

soils was $-1.0\text{ }^{\circ}\text{C}$, while –S soils dropped to $-3.3\text{ }^{\circ}\text{C}$; +S soils never fell below $0.21\text{ }^{\circ}\text{C}$. Variability in soil moisture among treatments was likely influenced by FTCs. The Ambient treatment experienced two FTCs toward the end of winter as snowpack diminished (Fig. 1b). Soil moisture in –S treatment was more variable due to this treatment experiencing four FTCs throughout winter season. In the +S treatment, soils experienced no FTCs and moisture remained relatively constant at $0.30\text{ m}^3\text{ H}_2\text{O m}^{-3}$.

3.2. C substrate availability and chemistry

Our snow manipulations influenced soil DOC concentrations over the course of the experiment. DOC concentrations were lower in Ambient and –S than +S soils prior to spring–thaw (RM-ANOVA: time \times treatment, $F_{(10, 19)} = 3.80$, $P < 0.01$; Supplementary Fig. 1).

However, DOC concentrations were similar among treatments at the final sampling date (April 3rd, Julian day = 94). DOC concentrations across the time series for each treatment ranged as follows: ambient = $0.09\text{--}0.19\text{ g C kg}^{-1}$ soil, –S = $0.11\text{--}0.16\text{ g C kg}^{-1}$ soil, and +S = $0.09\text{--}0.36\text{ g C kg}^{-1}$ soil. The snow manipulations did not influence DON (RM-ANOVA: treatment, $F_{(2, 4)} = 0.05$, $P > 0.20$) or inorganic N concentrations (RM-ANOVA: treatment, $F_{(2, 4)} = 2.95$, $P > 0.21$). Dissolved organic N ranged from 1.1 to 7.3 mg kg^{-1} soil, N-NH_4^+ concentrations ranged from 6.0 to 19 mg kg^{-1} soil, and NO_3^- concentrations ranged from 3.8 to 7.3 mg kg^{-1} soil.

3.3. Respiration sensitivity to temperature

Respiration was most sensitive to fluctuations in temperature in soils that never experienced sub-zero temperatures. Specifically,

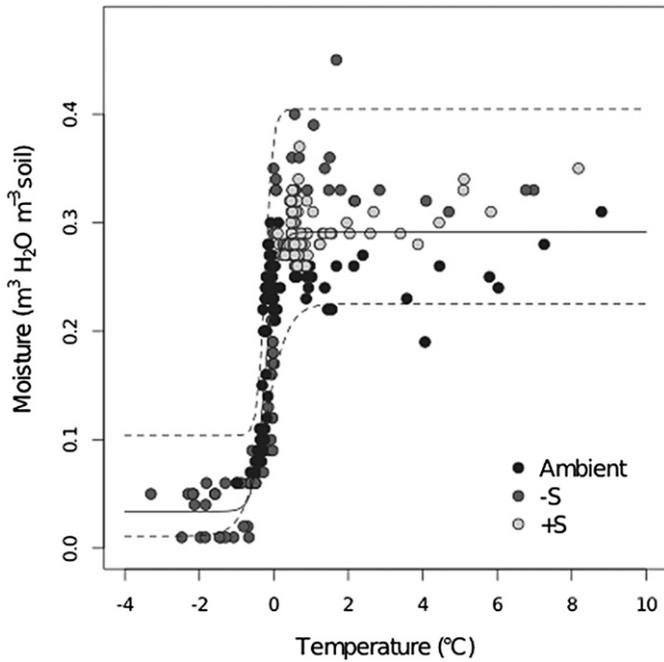


Fig. 2. Logistic relationship describing the phase transition of soil H₂O between a solid (ice) and liquid as a function of soil temperature in the three snow treatments. All points are daily means from sensor data generated every 10 s and averaged to create a 30 min value. The solid line represents the predicted relationship and the dashed lines are the 95% confidence intervals.

respiration sensitivity to temperature was seven-times higher under the +S than Ambient treatment, and +S was marginally higher than the -S treatment ($P = 0.07$, Figs. 3a and 1d). In contrast, respiration sensitivity was depressed in the +S treatment when there was an increase in temperature the previous day ($\text{temp}(t - 1)$) (Fig. 3b). During both time-steps, t and $t - 1$, temperature sensitivities in the -S and Ambient treatment were not significantly different than zero (i.e., the 95% confidence intervals overlapped zero).

We created causal networks to visualize the complex influence of temperature on respiration responses in our experiment (Fig. 4). For example, direct temperature effects on respiration were only apparent for +S, and indirect temperature effects on respiration were mediated through moisture for the -S and Ambient treatments. The standardized regression coefficients for $\text{temp}(t)$ and $\text{temp}(t - 1)$ paths were 0.50 and -0.42 respectively. Fits for the time-series were higher for the +S (adjusted $R^2 > 0.68$) than Ambient (adjusted $R^2 < 0.40$) and -S treatments (adjusted $R^2 < 0.21$), further suggesting that temperature was a major factor influencing CO₂ under the snow addition.

3.4. Respiration sensitivity to moisture

Respiration was most sensitive to fluctuations in moisture in soils that occasionally lacked a snowpack and underwent FTCs. For example, respiration was sensitive to fluctuations in moisture that occurred during the previous day ($\text{moisture}(t - 1)$) in the Ambient and -S treatments (Figs. 3d and 1d). In the -S treatment,

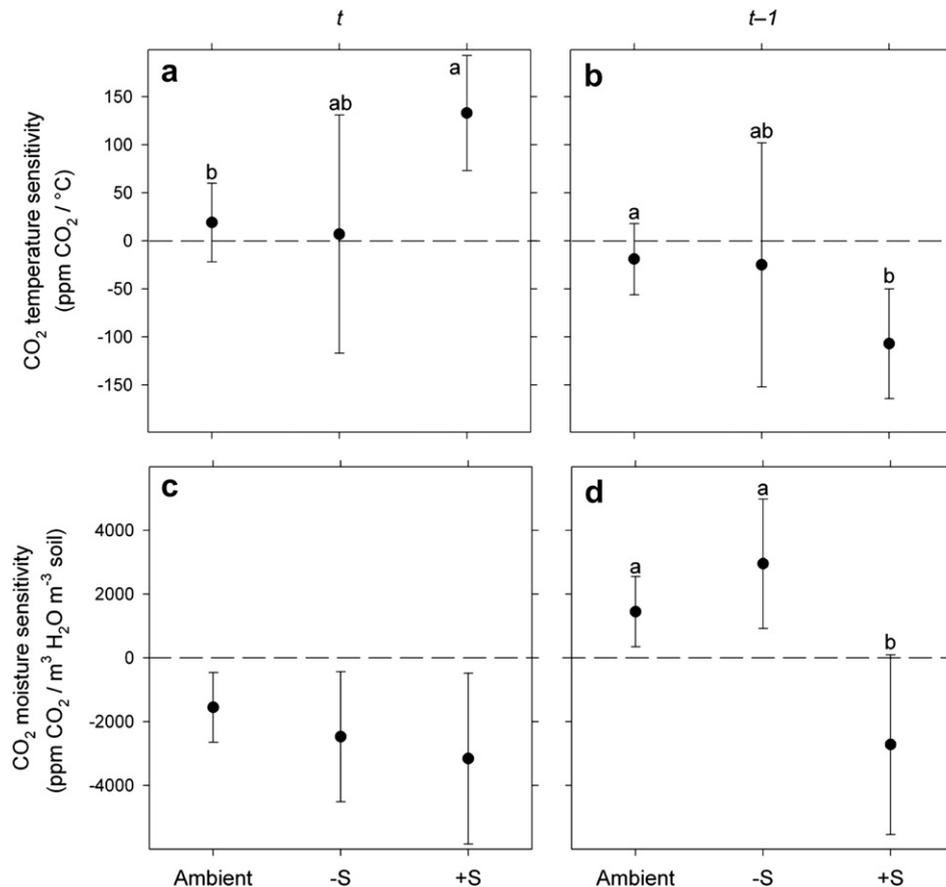


Fig. 3. Sensitivities of soil respiration to fluctuations in temperature (a, b) and moisture (c, d) in the snow treatments. All sensitivities are parameter estimates from time-series regression modeling and are defined as the change in CO₂ per unit change of an environmental variable (i.e., soil moisture or soil temperature). Values are expressed on two different time-steps: t (current day) and $t - 1$ (the day before the current day) and are shown with accompanying 95% confidence intervals.

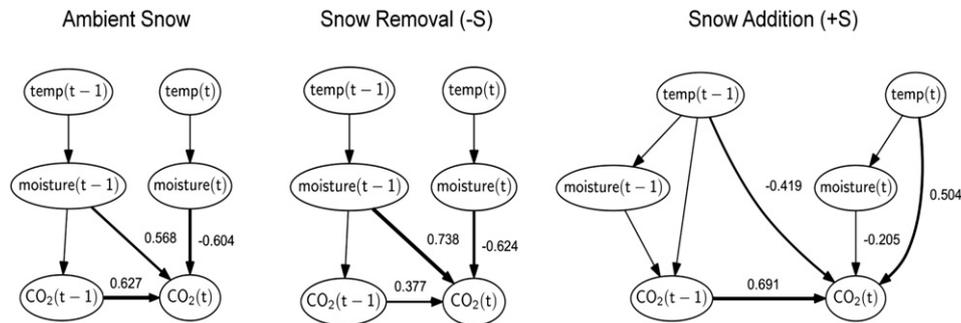


Fig. 4. Causal networks representing the relative importance of moisture and temperature on respiration dynamics in three snow treatments. Values are standardized regression coefficients generated from time-series regression modeling. These coefficients identify the relative effect of each path on CO₂(t) within a treatment. All significant paths directly influencing CO₂(t) are labeled with a coefficient. The coefficients can be interpreted as the change in the variable at the head of the arrow (in units of standard deviation (STDV)) if the variable at the tail of the arrow was changed by one STDV and all other variables were held constant. For example, in the ambient snow treatment, if moisture($t-1$) were increased by one STDV and all other variables remained constant, CO₂(t) would increase by 0.568 STDV. Similarly, if moisture(t) was increased by one STDV (and all other variables were held constant), CO₂(t) would decrease by 0.604 STDV.

respiration sensitivity contributed to pulses of CO₂ that were 5- to 19-times higher than respiration prior to a FTC. This trend was less apparent in the Ambient treatment, which only experienced two FTCs. Last, soil respiration did not respond to moisture fluctuations in the +S treatment, which never experienced freezing conditions. These effects are highlighted in the causal networks where the soil moisture of the previous day (moisture($t-1$)) directly influenced respiration and generated some of the highest standardized regression coefficient within the Ambient and -S networks (Fig. 4). Alternatively, increases in moisture during any given day (moisture(t)) depressed near-surface CO₂ concentrations in all three treatments and was the only variable that directly influenced respiration in every treatment (Figs. 3c and 4).

3.5. Wintertime bacterial and fungal community structure

Our snow manipulations had a strong effect on the structure and dynamics of soil microbial communities during the winter season. The composition of fungal communities, as determined by T-RFLP, significantly differed among the snow treatments (PERMANOVA: $F_{(2, 52)} = 1.51$, $P < 0.001$, Supplementary Fig. 2). Likewise, bacterial composition was affected by snow treatments throughout the four-month experiment (treatment \times time, PERMANOVA: $F_{(16, 47)} = 1.21$, $P = 0.024$). Further, the relative contribution of fungi and bacteria to the soil microbial community, measured as F:B ratios, was significantly affected by the snow treatments during winter (treatment \times time, RM-ANOVA: $F_{(16, 48)} = 5.70$, $P < 0.0001$; Supplementary Fig. 3). This interaction was driven mostly by a spike in F:B ratios (mean \pm SEM = 1.6 ± 0.20) in the -S treatment on Feb 15th (Julian day = 46) as soils began to freeze.

3.6. Bacterial community structure during spring–thaw

Our results suggest that by spring, soil microbial communities had mostly recovered from the effects of an altered snowpack. Based on T-RFLP, there were no effects of snow manipulations on bacterial OTU richness (ANOVA: $F_{(2, 8)} = 0.12$, $P = 0.88$) or fungal OTU richness (ANOVA: $F_{(2, 8)} = 0.72$, $P = 0.52$), nor were there any effects of snow on the composition of bacteria (PERMANOVA: $F_{(2, 8)} = 1.18$, $P = 0.30$) or fungi (PERMANOVA: $F_{(2, 8)} = 1.01$, $P = 0.43$). The inability to detect snow effects could have been due in part to the low resolution of the fingerprinting approach. Therefore, we also tested for the effects of the snow manipulation on bacterial communities using bar-coded pyrosequencing of the 16S rRNA gene. There was a small, but significant (3%) decrease in average

OTU richness when comparing soils from the Ambient to the +S treatment, but a more substantial increase (17%) in average OTU richness when comparing -S to +S soils (ANOVA: $F_{(2, 6)} = 7.8$, $P = 0.04$). Chao-1 OTU richness estimates were as follows (mean \pm SEM): ambient = 2703 ± 78 , -S = 3068 ± 87 , and +S = 2621 ± 77 . However, there were no significant effects of the snow treatments on bacterial community composition when sequences were grouped at the OTU level (i.e., 97% similarity) (PERMANOVA: $F_{(2, 8)} = 1.13$, $P = 0.18$) or phylum level (PERMANOVA: $F_{(2, 8)} = 1.30$, $P = 0.67$). Nevertheless, patterns from our hierarchical clustering indicated a snow signal that mirrored the patterns observed for respiration sensitivity. For example, bacterial communities from the Ambient and -S treatments clustered together with only a 7% difference between their communities (Fig. 5), and these treatments were the only ones that demonstrated respiration sensitivity to increases in moisture occurring the previous day (moisture($t-1$), Fig. 3d). Further, communities from the +S treatment were at least 16% different in composition compared to the other two communities (Fig. 5), and this was the only treatment where respiration sensitivity to temperature was significantly greater than zero (Fig. 3a, b).

4. Discussion

Mid-latitude ecosystems experience tremendous variation in the timing and amount of snowfall. Despite this, details describing how snow influences soil respiration and microbial communities are generally lacking for mid-latitude terrestrial ecosystems. We addressed this knowledge gap by exposing soils and microorganisms to snowpack manipulations during winter in a seasonally snow-covered deciduous forest. Overall, we found that soil respiration was sensitive to soil temperature above freezing and sensitive to soil moisture fluctuations that were induced by the absence of snow. The snow manipulations also altered microbial community structure, which may have contributed, in part, to the observed winter CO₂ dynamics. Although our treatments affected the richness of spring–thaw soil bacteria, other aspects of microbial community structure were not influenced by our manipulations, which suggests that soil microbial communities may be resilient to wintertime snow variability.

4.1. Respiration sensitivity to temperature

Soil respiration was sensitive to fluctuations in temperature, but only under the continual insulating effects of snow. Winter

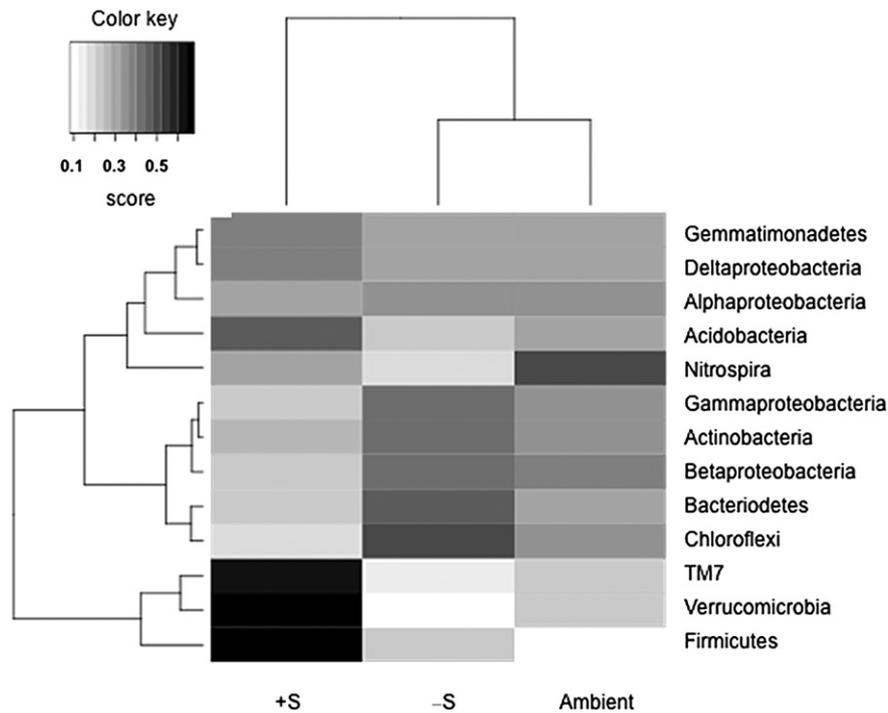


Fig. 5. Heatmap showing the trends in relative recovery for thirteen of the most highly represented bacterial taxa (i.e., nine bacterial phyla and four proteobacterial classes) from the treatments during spring–thaw. The hierarchical clusterings of the snow treatments and bacterial taxa, based on multivariate similarities, are included. Colors in the heatmap represent a standardized score for the relative recovery for each bacterial taxon. The scores or colors for each phylum equal one and are calculated as the relative recovery in a given treatment divided by the sum of the relative recoveries in all three snow treatments. Thus, a higher recovery is represented by darker colors and a lower recovery is represented by lighter colors. Recovery is based on the OTUs from 16S rRNA pyrosequencing data.

respiration was seven-times as responsive to increases in temperature during a given day under the snow addition than the ambient treatments. Temperature sensitivity improved the model fit for CO₂ dynamics in the snow addition treatment, suggesting that microbial metabolic activity responded linearly and predictably to increases in the observed temperature range. However, under sub-zero conditions, respiration slowed considerably in the snow removal and ambient treatments, with no evidence for the enhanced sensitivity of respiration to temperatures as reported in northern-latitudes (Mikan et al., 2002; Karhu et al., 2010; Tilston et al., 2010). This discrepancy between northern and mid-latitude systems may reflect the distribution of traits that allow microorganisms to adapt to different environmental pressures (e.g., Wallenstein and Hall, 2011). For example, there is growing evidence that communities in colder environments are able to capitalize on increases in temperature under sub-zero conditions (Mikan et al., 2002; Karhu et al., 2010; Tilston et al., 2010). Further, bacteria in colder environments maintain metabolic processes under freezing and sub-zero conditions by modifying the fluidity of cell membranes, accumulating polyols and antifreeze proteins, and generating cold-shock proteins and cold-active enzymes (Walker et al., 2006; Hall et al., 2010). It is possible that the transient snow levels and shorter winter season in mid-latitude systems may not alter temperatures long enough to generate a quantifiable respiration response under sub-zero conditions.

4.2. Respiration sensitivity to moisture

We found that respiration was enhanced by fluctuations in moisture, but only in treatments where snow was absent. The ambient and snow removal treatments experienced several FTCs and respiration in these treatments was stimulated by moisture. However, respiration was sensitive to increases in moisture that

occurred one day after thawing, indicating there was a temporal lag, possibly associated with soil microbial recovery from freezing and osmotic stresses that are commonly associated with FTCs (Clein and Schimel, 1995; Schimel et al., 2007). For microorganisms to remain active as soils freeze, they must physiologically acclimate to declines in C substrates, O₂, and free water by synthesizing antifreeze proteins (Mihoub et al., 2003), altering membranes to maintain fluidity of metabolites (Methe et al., 2005), and/or producing solutes to control internal water potential (Mindock et al., 2001). Alternatively, as soils thaw, microorganisms must rapidly reverse these processes to prevent cell rupture and other effects (Schimel et al., 2007). Together, these physiological acclimations require time and energy and may be the basis for our measured delay in respiration sensitivity to moisture fluctuations. In addition, soil thawing induced by our snow manipulations most likely contributed to pulses of CO₂. The CO₂ comprising the pulses most likely originated from an increase in the metabolic activity of microorganisms in surface soils and not from a build-up of CO₂ in a deeper, unfrozen subsurface layer since we found no evidence for the accumulation of CO₂ at deeper depths (15 cm) prior to thaw events (Supplementary Fig. 4). Last, respiration was depressed by increases in moisture that occurred during that day, but this negative sensitivity of respiration to moisture was most likely a physiochemical response (i.e., diffusion of O₂ and/or CO₂) and not one driven by biology since it occurred in all treatments regardless of soil moisture variability.

We identified a strong, non-linear relationship between temperature and moisture that helped clarify how these two variables interact and potentially co-regulated winter respiration. When temperatures were greater than 1 °C or less than –1 °C, soil moisture was relatively constant. In contrast, when temperatures ranged from –0.5 to 0.5 °C soil moisture fluctuated dramatically owing to the phase transition of H₂O between ice and free water.

This threshold relationship between temperature and moisture had a strong influence on respiration sensitivity. For example, when soils were thawing or freezing, respiration was insensitive to minor fluctuations in temperature. But once moisture became available, respiration responded more to fluctuations in temperature and did not respond to minor variations in soil moisture. Therefore, snow-driven differences in moisture and temperature appear to co-regulate the sensitivity of winter soil respiration. This type of co-regulation has been observed in other systems, too. For example, as snowpack levels increased in a subalpine meadow the factor controlling respiration shifted from soil moisture and FTCs to soil temperature (Liptzin et al., 2009), and freezing conditions decoupled the temperature dependence of respiration in a tundra soil (Mikan et al., 2002).

Although our respiration and DOC measurements were conducted on different timescales, it is well established that carbon substrate availability stimulates winter respiration rates. Interactions between DOC and respiration may help explain why respiration sensitivity to temperature was dampened by increases in temperature from the previous day under the snow addition treatment. For example, additions of glucose increased winter respiration by 52–160% in seasonally snow-covered ecosystem (Brooks et al., 2004; Liptzin et al., 2009). If microorganisms preferentially depleted the availability of simple, low-molecular weight C substrates in conjunction with the increase in temperature, respiration may actually be depressed the next day due to the lower availability of these labile substrates relative to more recalcitrant organic compounds.

4.3. Wintertime bacterial and fungal community structure

We detected differences in bacterial and fungal communities between the snowpack treatments that may have contributed in part to observed respiration sensitivity. Based on our fingerprinting results, both bacterial and fungal communities were altered by the snow treatments during the winter season. For example, bacterial communities in our mid-latitude system were susceptible to snowfall changes, the same as microbial communities in high-latitude or high-altitude ecosystems (Buckeridge and Grogan, 2008; Zinger et al., 2009; Lipson et al., 2009). The potential for fungal communities to influence respiration may be greater in mid-latitude than northern systems. In northern soils and under laboratory incubations, fungi are generally susceptible to freezing soil conditions, leading to pronounced differences in fungal communities and lower biomass (Yergeau and Kowalchuk, 2008). In contrast, as soils began to freeze, we measured a spike in F:B ratios. One explanation for this pattern is that fungi were more tolerant of freezing than bacteria. Fungi may contend with mid-latitude winter conditions by using hyphae to spatially exploit resources and water from deeper soils below the frozen surface. For example, under snow removal and ambient conditions soils only froze to a relatively shallow depth (approximately 5–7 cm), while northern latitude soils may freeze to much deeper depths (Hofle et al., 1998); these differences may be a key factor influencing fungal tolerance to winter conditions in different biogeographic locations.

4.4. Microbial community structure during spring–thaw

Recent studies have shown that the composition and function of soil microbial communities can be extremely dynamic during spring–thaw conditions (Mackelprang et al., 2011). However, it is unknown whether or not spring–thaw microbial responses are influenced by variation in antecedent snowfall patterns. Results obtained from our pyrosequencing of 16S rRNA genes suggest that snow-induced differences in bacterial richness may persist

into the growing season. Richness, or the number of OTUs, was 17% higher in the snow removal than snow addition treatment, suggesting that soil freezing may potentially create new niches for certain microbial taxa. More extreme conditions, such as FTCs, may act as physiological stresses or disturbances that prevent specific taxa from becoming dominant and thus promoting the coexistence of additional, cold-adapted taxa. By most other metrics, however, our results suggest that the snow manipulations did not produce lasting effects on microbial community structure. Growing evidence suggests that microbial seed banks in the soil may be important for buffering the composition and function of microbial communities in response to environmental change (Lennon and Jones, 2011). Therefore, as soil conditions transitioned from winter to spring and became more conducive for bacteria growth (i.e., warmer while remaining moist), dormant bacteria may be recruited from the seed bank and ameliorate the snow effects that we observed earlier in the winter season.

Although the effects of our manipulation appeared to diminish over time, we observed a qualitative signal of the snow treatments on springtime bacterial communities that mirrored patterns of respiration sensitivity. The enhanced sensitivity to moisture under the ambient and snow removal treatments coincided with an increase in the relative abundance of Actinobacteria, Bacteroidetes, β -Proteobacteria, Chloroflexi, and γ -Proteobacteria following spring–thaw. These phyla and proteobacterial classes comprised 29% of the overall community, experienced multiple FTC, and thus may have been more adapted to the osmotic and desiccation stresses that are typically associated with sub-zero conditions. Other researchers have found similar taxonomic responses in northern systems. For example, in a subalpine forest, *Janthinobacterium*, a genus of β -Proteobacteria, increased in abundance when soils dropped below 0 °C (Monson et al., 2006). In another study, Actinobacteria increased, γ -Proteobacteria remained constant, but β -Proteobacteria decreased in abundance following multiple FTCs in tundra soils (Mannisto and Haggblom, 2006). Alternatively, the enhanced sensitivity to temperature under snow addition coincided with an increase in the relative abundance of Firmicutes, Verrucomicrobia, and TM7. These phyla, however, only comprised a minor fraction (<1%) of the overall community. Therefore, FTCs may act more strongly to structure communities than optimal wetter and warmer winter conditions. As such, links between winter respiration and microorganisms may be more apparent in soils that experience the selective pressures of FTCs.

5. Conclusion

Future climate scenarios forecast more variable snowfall and frequent FTCs for many terrestrial ecosystems (IPCC, 2007; Henry, 2008; Kreyling et al., 2008). Given these projections, it is essential to better understand regulations on wintertime respiration by moisture and temperature, especially as moisture variability is enhanced by freezing and thawing conditions. Similar to results from high-latitude and high-altitude ecosystems, our mid-latitude findings suggest that moisture and temperature co-regulate respiration by switching in relative importance as a function of snowpack depth. However, unlike northern systems, we found no evidence for enhanced sensitivity of respiration to sub-zero temperatures, with respiration only being sensitive to temperature fluctuations above freezing. Our snow manipulations altered bacterial community composition, but the seasonal transition from winter to spring seemed to “reset” microbial community structure, suggesting that soil microbial communities are likely to be adapted to annual fluctuations in snowpack.

Acknowledgments

We thank the KBS LTER field technicians for monitoring climate over the last several decades and B. Lehmkuhl for assistance in the field and lab. In addition, we thank S. Placella, M. Muscarella, M. Larsen, K. Miller, and two anonymous reviewers for valuable comments on an early version of this manuscript. We acknowledge the support from the USDA National Institute of Food and Agriculture through a National Research Initiative Grants and Rackham Research Endowment, the National Science Foundation, and the Michigan Agricultural Experiment Station.

Appendix A. Supplementary material

Supplementary material associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.soilbio.2012.07.022>.

References

- Aanderud, Z.T., Schoolmaster, D.R., Lennon, J.T., 2011. Plants mediate the sensitivity of soil respiration to rainfall variability. *Ecosystems* 14, 156–167.
- Aanderud, Z.T., Lennon, J.T., 2011. Validation of heavy-water stable isotope probing for the characterization of rapidly responding soil bacteria. *Applied and Environmental Microbiology* 77, 4589–4596.
- Anderson, M.J., 2001. A new method for a non-parametric multivariate analysis of variance. *Austral Ecology* 26, 32–46.
- Avis, P.G., Dickie, I.A., Mueller, G.M., 2006. A 'dirty' business: testing the limitations of terminal restriction fragment length polymorphism (TRFLP) analysis of soil fungi. *Molecular Ecology* 15, 873–882.
- Borken, W., Matzner, E., 2008. Focus issue: soil processes under extreme meteorological conditions preface. *Journal of Plant Nutrition and Soil Science-Zeitschrift Fur Pflanzenernahrung Und Bodenkunde* 171, 665.
- Brooks, P.D., Schmidt, S.K., Williams, M.W., 1997. Winter production of CO₂ and N₂O from Alpine tundra: environmental controls and relationship to inter-system C and N fluxes. *Oecologia* 110, 403–413.
- Brooks, P.D., McKnight, D., Elder, K., 2004. Carbon limitation of soil respiration under winter snowpacks: potential feedbacks between growing season and winter carbon fluxes. *Global Change Biology* 11, 231–238.
- Buckeridge, K.M., Grogan, P., 2008. Deepened snow alters soil microbial nutrient limitations in arctic birch hummock tundra. *Applied Soil Ecology* 39, 210–222.
- Carbone, M.S., Winston, G.C., Trumbore, S.E., 2008. Soil respiration in perennial grass and shrub ecosystems: linking environmental controls with plant and microbial sources on seasonal and diel timescales. *Journal of Geophysical Research-Biogeosciences* 113.
- Chao, A., 1984. Nonparametric-estimation of the number of classes in a population. *Scandinavian Journal of Statistics* 11, 265–270.
- Clein, J.S., Schimel, J.P., 1995. Microbial activity of tundra and taiga soils at subzero temperatures. *Soil Biology & Biochemistry* 27, 1231–1234.
- Daly, E., Oishi, A.C., Porporato, A., Katul, G.G., 2008. A stochastic model for daily subsurface CO₂ concentration and related soil respiration. *Advances in Water Resources* 31, 987–994.
- DeGryze, S., Six, J., Paustian, K., Morris, S.J., Paul, E.A., Merckx, R., 2004. Soil organic carbon pool changes following land-use conversions. *Global Change Biology* 10, 1120–1132.
- DeSantis, T.Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E.L., Keller, K., Huber, T., Dalevi, D., Hu, P., Andersen, G.L., 2006. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Applied and Environmental Microbiology* 72, 5069–5072.
- Drott, S.H., Sparrman, T., Nilsson, M.B., Schleucher, J., Oquist, M.G., 2010. Both catabolic and anabolic heterotrophic microbial activity proceed in frozen soils. *Proceedings of the National Academy of Sciences of the United States of America* 107, 21046–21051.
- Elder, J.K., Southern, E.M., 1983. Measurement of DNA length by gel electrophoresis II: comparison of methods for relating mobility to fragment length. *Analytical Biochemistry* 128, 227–231.
- Fierer, N., Jackson, J.A., Vilgalys, R., Jackson, R.B., 2005. Assessment of soil microbial community structure by use of taxon-specific quantitative PCR assays. *Applied and Environmental Microbiology* 71, 4117–4120.
- Fierer, N., Carney, K.M., Horner-Devine, M.C., Megonigal, J.P., 2009. The biogeography of ammonia-oxidizing bacterial communities in soil. *Microbial Ecology* 58, 435–445.
- Groffman, P.M., Driscoll, C.T., Fahey, T.J., Hardy, J.P., Fitzhugh, R.D., Tierney, G.L., 2001. Effects of mild winter freezing on soil nitrogen and carbon dynamics in a northern hardwood forest. *Biogeochemistry* 56, 191–213.
- Hall, E.K., Singer, G.A., Kainz, M.J., Lennon, J.T., 2010. Evidence for a temperature acclimation mechanism in bacteria: an empirical test of a membrane-mediated trade-off. *Functional Ecology* 24, 898–908.
- Hamady, M., Walker, J.J., Harris, J.K., Gold, N.J., Knight, R., 2008. Error-correcting barcoded primers for pyrosequencing hundreds of samples in multiplex. *Nature Methods* 5, 235–237.
- Henry, H.A.L., 2008. Climate change and soil freezing dynamics: historical trends and projected changes. *Climatic Change* 87, 421–434.
- Hofle, C., Ping, C.L., Kimble, J.M., 1998. Properties of permafrost soils on the northern Seward Peninsula, Northwest Alaska. *Soil Science Society of America Journal* 62, 1629–1639.
- Huse, S.M., Huber, J.A., Morrison, H.G., Sogin, M.L., Mark Welch, D., 2007. Accuracy and quality of massively parallel DNA pyrosequencing. *Genome Biology* 8, 9.
- IPCC, 2007. *Climate Change 2007: the Scientific Basis*. Cambridge Press, Cambridge, UK.
- Karhu, K., Fritze, H., Hamalainen, K., Vanhala, P., Jungner, H., Oinonen, M., Sonninen, E., Tuomi, M., Spetz, P., Kitunen, V., Liski, J., 2010. Temperature sensitivity of soil carbon fractions in boreal forest soil. *Ecology* 91, 370–376.
- Keselman, K.J., Algina, J., Kowalchuk, R.K., Wolfinger, R.D., 1999. The analysis of repeated measurements: a comparison of mixed-model satterthwaite F tests and a nonpooled adjusted degrees of freedom multivariate test. *Communications in Statistics-Theory and Methods* 28, 2967–2999.
- Kreyling, J., Beierkuhnlein, C., Pritsch, K., Schloter, M., Jentsch, A., 2008. Recurrent soil freeze-thaw cycles enhance grassland productivity. *New Phytologist* 177.
- Lauber, C.L., Knight, R., Hamady, M., Fierer, N., 2009. Soil pH as a predictor of soil bacterial community structure at the continental scale: a pyrosequencing-based assessment. *Applied and Environmental Microbiology* 75, 5111–5120. 938–945.
- Lennon, J.T., Martiny, J.B.H., 2008. Rapid evolution buffers ecosystem impacts of viruses in a microbial food web. *Ecology Letters* 11, 1178–1188.
- Lennon, J.T., Jones, S.E., 2011. Microbial seed banks: the ecological and evolutionary implications of dormancy. *Nature Reviews Microbiology* 9, 119–130.
- Lipson, D.A., Schmidt, S.K., 2004. Seasonal changes in an alpine soil bacterial community in the Colorado Rocky Mountains. *Applied and Environmental Microbiology* 70, 2867–2879.
- Lipson, D.A., Monson, R.K., Schmidt, S.K., Weintraub, M.N., 2009. The trade-off between growth rate and yield in microbial communities and the consequences for under-snow soil respiration in a high elevation coniferous forest. *Biogeochemistry* 95, 23–35.
- Liptzin, D., Williams, M.W., Helmig, D., Seok, B., Filippa, G., Chowanski, K., Hueber, J., 2009. Process-level controls on CO₂ fluxes from a seasonally snow-covered subalpine meadow soil, Niwot Ridge, Colorado. *Biogeochemistry* 95, 151–166.
- Ljung, G.M., Box, G.E.P., 1978. On a measure of lack of fit in time series models. *Biometrika* 65, 297–303.
- Mackelprang, R., Waldrop, M.P., DeAngelis, K.M., Maude, D.M., Chavarria, K.L., Blazewicz, S.J., Rubin, E.M., Jansson, J.K., 2011. Metagenomic analysis of a permafrost microbial community reveals rapid response to thaw. *Nature* 480, 368–371.
- Mannisto, M.K., Haggblom, M.M., 2006. Characterization of psychrotolerant heterotrophic bacteria from Finnish Lapland. *Systematic and Applied Microbiology* 29, 229–243.
- Mariko, S., Bekku, Y., Koizumi, H., 1994. Efflux of carbon dioxide from snow-covered forest floors. *Ecological Research* 9, 343–350.
- McMahon, S.K., Wallenstein, M.D., Schimel, J.P., 2011. A cross-seasonal comparison of active and total bacterial community composition in Arctic tundra soil using bromodeoxyuridine labeling. *Soil Biology & Biochemistry* 43, 287–295.
- Methe, B.A., Nelson, K.E., Deming, J.W., Momen, B., Melamud, E., Zhang, X.J., Mout, J., Madupu, R., Nelson, W.C., Dodson, R.J., Brinkac, L.M., Daugherty, S.C., Durkin, A.S., DeBoy, R.T., Kolonay, J.F., Sullivan, S.A., Zhou, L.W., Daviden, T.M., Wu, M., Huston, A.L., Lewis, M., Weaver, B., Weidman, J.F., Khouri, H., Utterback, T.R., Feldblyum, T.V., Fraser, C.M., 2005. The psychrophilic lifestyle as revealed by the genome sequence of *Colwellia psychrerythraea* 34H through genomic and proteomic analyses. *Proceedings of the National Academy of Sciences of the United States of America* 102, 10913–10918.
- Mihoub, F., Mistou, M.Y., Guillot, A., Leveau, J.Y., Boubetra, A., Billaux, F., 2003. Cold adaptation of *Escherichia coli*: microbiological and proteomic approaches. *International Journal of Food Microbiology* 89, 171–184.
- Mikan, C.J., Schimel, J.P., Doyle, A.P., 2002. Temperature controls of microbial respiration in arctic tundra soils above and below freezing. *Soil Biology & Biochemistry* 34, 1785–1795.
- Mindock, C.A., Petrova, M.A., Hollingsworth, R.I., 2001. Re-evaluation of osmotic effects as a general adaptive strategy for bacteria in sub-freezing conditions. *Biophysical Chemistry* 89, 13–24.
- Monson, R.K., Turnipseed, A.A., Sparks, J.P., Harley, P.C., Scott-Denton, L.E., Sparks, K., Huxman, T.E., 2002. Carbon sequestration in a high-elevation, subalpine forest. *Global Change Biology* 8, 459–478.
- Monson, R.K., Lipson, D.L., Burns, S.P., Turnipseed, A.A., Delany, A.C., Williams, M.W., Schmidt, S.K., 2006. Winter forest soil respiration controlled by climate and microbial community composition. *Nature* 439, 711–714.
- Panikov, N.S., Flanagan, P.W., Oechel, W.C., Mastepanov, M.A., Christensen, T.R., 2006. Microbial activity in soils frozen to below-39 degrees C. *Soil Biology & Biochemistry* 38, 785–794.
- R Development Core Team, 2008. *R: a Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria, ISBN 3-900051-07-0. URL: [http://www\(R-project.org\)](http://www(R-project.org)).
- Raich, J.W., Potter, C.S., 1995. Global patterns of carbon-dioxide emissions from soils. *Global Biogeochemical Cycles* 9, 23–36.

- Rey, A., Pegoraro, E., Tedeschi, V., De Parri, I., Jarvis, P.G., Valentini, R., 2002. Annual variation in soil respiration and its components in a coppice oak forest in Central Italy. *Global Change Biology* 8, 851–866.
- Riveros-Iregui, D.A., Emanuel, R.E., Muth, D.J., McGlynn, B.L., Epstein, H.E., Welsch, D.L., Pacific, V.J., Wraith, J.M., 2007. Diurnal hysteresis between soil CO₂ and soil temperature is controlled by soil water content. *Geophysical Research Letters* 34.
- Schimel, J., Balsler, T.C., Wallenstein, M., 2007. Microbial stress-response physiology and its implications for ecosystem function. *Ecology* 88, 1386–1394.
- Schimel, J.P., Bilbrough, C., Welker, J.A., 2004. Increased snow depth affects microbial activity and nitrogen mineralization in two Arctic tundra communities. *Soil Biology & Biochemistry* 36, 217–227.
- Sharma, S., Szele, Z., Schilling, R., Munch, J.C., Schloter, M., 2006. Influence of freeze-thaw stress on the structure and function of microbial communities and denitrifying populations in soil. *Applied and Environmental Microbiology* 72, 2148–2154.
- Sommerfeld, R.A., Mosier, A.R., Musselman, R.C., 1993. CO₂, CH₄ and N₂O flux through a Wyoming snowpack and implications for global budgets. *Nature* 361, 140–142.
- Tang, J.W., Baldocchi, D.D., Qi, Y., Xu, L.K., 2003. Assessing soil CO₂ efflux using continuous measurements of CO₂ profiles in soils with small solid-state sensors. *Agricultural and Forest Meteorology* 118, 207–220.
- Tilston, E.L., Sparrman, T., Oquist, M.G., 2010. Unfrozen water content moderates temperature dependence of sub-zero microbial respiration. *Soil Biology & Biochemistry* 42, 1396–1407.
- Walker, V.K., Palmer, G.R., Voordouw, G., 2006. Freeze-thaw tolerance and clues to the winter survival of a soil community. *Applied and Environmental Microbiology* 72, 1784–1792.
- Wallenstein, M.D., Hall, E., 2011. A trait-based framework for predicting when and where microbial adaptation to climate change will affect ecosystem functioning. *Biogeochemistry* 109, 35–47.
- Winston, G.C., Sundquist, E.T., Stephens, B.B., Trumbore, S.E., 1997. Winter CO₂ fluxes in a boreal forest. *Journal of Geophysical Research-Atmospheres* 102, 28795–28804.
- Yergeau, E., Kowalchuk, G.A., 2008. Responses of Antarctic soil microbial communities and associated functions to temperature and freeze-thaw cycle frequency. *Environmental Microbiology* 10, 2223–2235.
- Zinger, L., Shahnavaz, B., Baptist, F., Geremia, R.A., Choler, P., 2009. Microbial diversity in alpine tundra soils correlates with snow cover dynamics. *The ISME Journal* 3, 850–859.