

Winter forest soil respiration controlled by climate and microbial community composition

Russell K. Monson^{1,2*}, David L. Lipson^{4*}, Sean P. Burns^{1,5}, Andrew A. Turnipseed⁵, Anthony C. Delany⁵, Mark W. Williams³ & Steven K. Schmidt¹

Most terrestrial carbon sequestration at mid-latitudes in the Northern Hemisphere occurs in seasonal, montane forest ecosystems¹. Winter respiratory carbon dioxide losses from these ecosystems are high, and over half of the carbon assimilated by photosynthesis in the summer can be lost the following winter^{2,3}. The amount of winter carbon dioxide loss is potentially susceptible to changes in the depth of the snowpack; a shallower snowpack has less insulation potential, causing colder soil temperatures and potentially lower soil respiration rates. Recent climate analyses have shown widespread declines in the winter snowpack of mountain ecosystems in the western USA and Europe that are coupled to positive temperature anomalies⁴⁻⁶. Here we study the effect of changes in snow cover on soil carbon cycling within the context of natural climate variation. We use a six-year record of net ecosystem carbon dioxide exchange in a subalpine forest to show that years with a reduced winter snowpack are accompanied by significantly lower rates of soil respiration. Furthermore, we show that the cause of the high sensitivity of soil respiration rate to changes in snow depth is a unique soil microbial community that exhibits exponential growth and high rates of substrate utilization at the cold temperatures that exist beneath the snow. Our observations suggest that a warmer climate may change soil carbon sequestration rates in forest ecosystems owing to changes in the depth of the insulating snow cover.

The recent global proliferation of tower-measurement networks has made it possible to analyse details of the coupling between climate dynamics and the carbon (C) cycle⁷. Most past studies have focused on ecosystem-atmosphere CO₂ exchange during the growing season because the instantaneous flux rates are so much higher than during colder periods. In seasonal forests, however, small but

continuous rates of ecosystem respiration during the winter can, in some cases, completely determine annual rates of C sequestration^{2,3}. Studies using artificial snow removal have demonstrated a critical role for thermal insulation in determining winter biogeochemical cycling⁸⁻¹⁰. In this study, we aimed to move beyond experimental manipulations of snow cover, which can cause artificial treatment effects, and examine the factors that control winter soil C cycling within the context of natural climate variation.

We used the eddy covariance approach between 1 November 1998 and 31 October 2004 to continuously measure net ecosystem CO₂ exchange (NEE) at the Niwot Ridge Ameriflux site in the Rocky Mountains. Interannual variation in late-winter (1 March–15 April) cumulative NEE was not correlated with variation in mean air temperature ($P < 0.05$, Fig. 1a), but was correlated with variation in mean soil temperature (Fig. 1b) which, in turn, was correlated with variation in the 1 April snow-water equivalent (SWE), a measure of the cumulative winter snow pack (Fig. 1c). The relationship between mean soil temperature and SWE is best explained by a second-order polynomial, reflecting an asymptote at high snow depths as soil temperature approached its natural limit of 0 °C. The mean 1 April SWE for the period 1982–2004 at the Niwot Ridge site is 36.7 ± 3.3 cm, which falls just above the threshold of 31.4 cm, below which decreases in SWE affect late-winter NEE; nine of the past 23 years (39%) have been characterized by 1 April SWE values that fall below this threshold.

We developed a first-order exponential coefficient (R_T) to describe the temperature sensitivity of respiration (analogous to the Q_{10} coefficient used in biochemical studies). The interannual temperature dependency described by R_T was 6.6 when determined across all six years (Fig. 1b). The interannual range in late-winter NEE

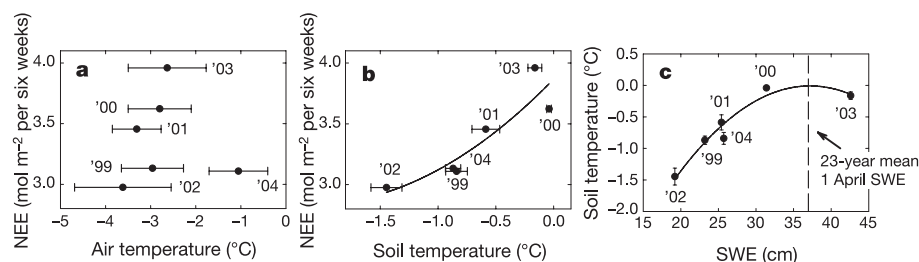


Figure 1 | Responses of NEE to air and soil temperature as influenced by SWE for the indicated years. a, The response of NEE to average daily air temperature. **b**, The response of NEE to average daily soil temperature ($y = a \exp^{bx}$, $a = 3.8244$, $b = 0.1963$, $R^2 = 0.81$, $P = 0.0152$). **c**, The

relationship between SWE (on 1 April) and average daily soil temperature ($y = ax^2 + bx + c$, $a = -0.0046$, $b = 0.339$, $c = -6.320$, $R^2 = 0.944$, $P = 0.0129$). Error bars are mean \pm s.e. Data are for 1 March to 15 April.

¹Department of Ecology and Evolutionary Biology, ²Center for Interdisciplinary Research in Environmental Science, ³Department of Geography and Institute of Arctic and Alpine Research, University of Colorado, Boulder, Colorado 80309, USA. ⁴Department of Biology, San Diego State University, San Diego, California 92182, USA. ⁵National Center for Atmospheric Research, PO Box 3000, Boulder, Colorado 80305, USA.

*These authors contributed equally to this work.

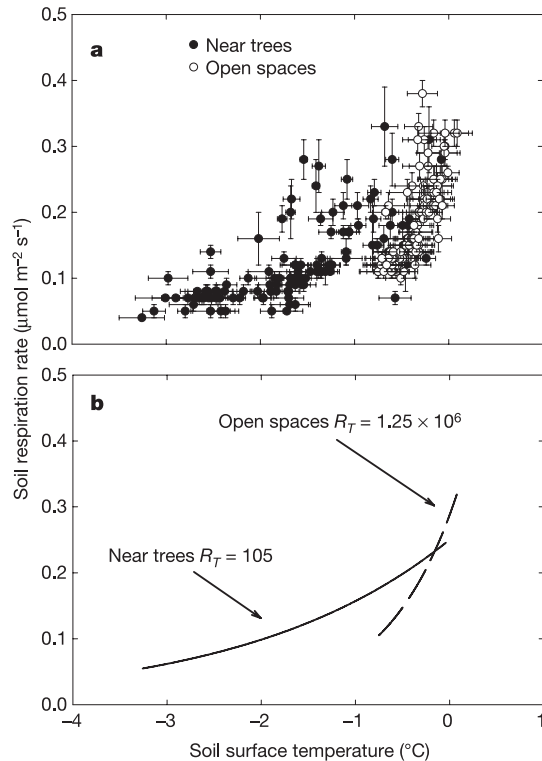


Figure 2 | The temperature sensitivity of soil respiration rate beneath the snow pack. **a**, The response of beneath-snow soil-respiration rate to soil temperature measured during the snow-covered period between 20 November 2003 and 22 March 2004 in open spaces between trees or in the spaces next to tree boles. Vertical and horizontal error bars represent the mean \pm s.e. of four chambers located in open spaces and three chambers located next to trees. **b**, The mean temperature response and estimates of R_T from a first-order exponential model applied to the data in **a**.

evaluated across all six years ($0.99 \text{ mol C m}^{-2}$) represents 21% of the mean cumulative annual NEE for this site ($-4.81 \pm 0.8 \text{ mol C m}^{-2}$). Thus, the natural variance in late-winter snow pack and its effect on soil temperature has the potential to cause large variance in the capacity for annual C sequestration.

One past study has shown that high SWE coupled with persistent snow pack late into the spring stimulates photosynthetic gross

ecosystem CO_2 exchange (GEE), resulting in higher ecosystem CO_2 uptake². The results depicted in Fig. 1 demonstrate that when SWE is high late into the winter, before GEE has been stimulated, ecosystem respiration (R_e) is stimulated, resulting in higher CO_2 loss. The relative importance of these two opposing effects, as well as environmental conditions during the middle part of the growing season, will ultimately determine the NEE in any single year. In the six-year observation period of the present study, it was clear that springtime dynamics in GEE caused the largest interannual swings in cumulative annual NEE (ref. 2); however, late-winter differences in R_e were of sufficient magnitude to completely rearrange the ranking of years with respect to annual NEE, demonstrating the importance of spring SWE on annual NEE.

We made nearly continuous observations of soil respiration rate and soil temperature using an automated system. The measured CO_2 concentration was used to calculate the through-snow soil respiration rate. When data were pooled for the entire season, the calculated R_T was 105 and 1.25×10^6 , depending on whether measurements were made next to the boles of trees or in the open spaces between trees, respectively; these values are several orders of magnitude higher than the range of Q_{10} values (2–6) typically recorded for snow-free forest soils (Fig. 2)^{11–13}. When the analysis was restricted to observations between 0 and -1°C to achieve a temperature-normalized set of observations, the R_T was calculated as 104 for respiration near the boles of trees and 6.62×10^5 for respiration in the open spaces between trees.

Past studies of soils under laboratory conditions have also reported high temperature sensitivities for respiration at temperatures below 0°C ; though still several orders of magnitude below the highest values reported here¹⁴. One hypothesis that was proposed for the high temperature sensitivity invokes a physical limitation to substrate diffusion as liquid water disappears below 0°C . The disappearance of water between 0 and -1°C exhibits exponential behaviour, similar to what we observed for the temperature dependence of respiration¹⁵, and substrate limitations to microbial growth and respiration have been demonstrated in frozen soils¹⁶.

Our observation of lower R_T values for the soil respiration rate near tree boles, compared to open spaces, might be explained by differences in substrate diffusion limitations; substrate concentrations may be higher near the trees due to higher rates of rhizodeposition and litter inputs, and the higher concentration gradient for these substrates might overcome slow diffusion rates at subzero temperatures and render the overall process less sensitive to temperature. Substrate diffusion limitations would impose a

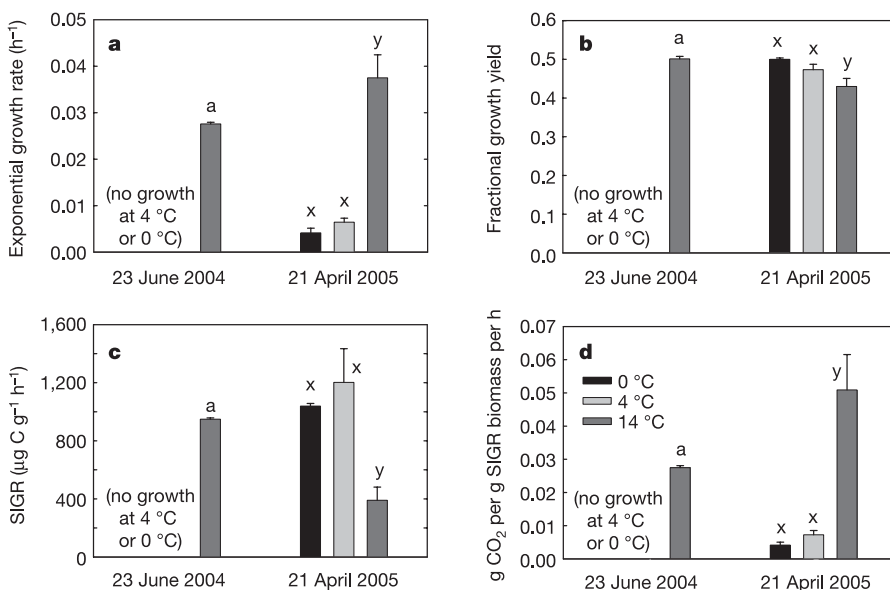


Figure 3 | Microbial growth kinetics in soils collected in summer and late winter, determined by SIGR experiments at temperatures ranging from 0 to 14°C . **a**, Exponential growth rate; **b**, growth yield; **c**, microbial biomass C capable of growth at the indicated temperature; **d**, rate of CO_2 production per unit of actively growing microbial biomass C. Different letters next to the bars of the same sample date indicate significant differences at $P < 0.05$ using Fisher's Protected Least Significant Difference Test. Vertical error bars represent s.e.

second-order constraint on respiratory metabolism and render first-order models inadequate. Whatever the limiting mechanism, the first-order exponential model driven by an R_T coefficient fits our observations well ($y = a \exp^{bx}$, $a = 0.287$, $b = 1.341$, $R^2 = 0.60$, $P < 0.0001$ for open spaces; $y = a \exp^{bx}$, $a = 0.250$, $b = 0.466$, $R^2 = 0.50$, $P < 0.0001$ for near trees). Thus, our conclusion that there is high R_T in the beneath-snow soil is supported by empirical regression.

We collected soils from beneath the spring snowpack and during the summer to determine whether the microbial communities differed in their seasonal composition, whether the microbial biomass was capable of increasing beneath the snow pack and whether microbial respiration responded to changes in substrate availability (Fig. 3). The summer microbial community was not capable of growth below 4 °C. However, microbes collected from under the snowpack could grow exponentially at 0 °C and their growth rates increased rapidly with increasing temperature. The growth yield (yield of biomass per unit of substrate assimilated) was highest at the lowest temperatures. These two parameters determine the rate of respiration per unit biomass and its response to temperature in a growing microbial population. The application of potassium glutamate substrate to soils collected from beneath the snowpack induced the largest substrate-induced growth respiration (SIGR) response at the lowest temperatures (Fig. 3c), and the rate of respiration per unit of SIGR biomass was especially sensitive to increases in temperature (Fig. 3d).

We constructed 16S ribosomal RNA clone libraries from the winter and summer soils after incubation in the glutamate SIGR experiments to identify which bacteria were responsible for the differential growth kinetics. The dominant bacteria in the winter library had sequences closely related to *Janthinobacterium* (38% of sequences) and, in summer, the sequences were closely related to *Burkholderia* (17% of the sequences). The overall DNA libraries were significantly different between the winter and summer soils by phylogenetic tail permutation (PTP) analysis ($P = 0.006$). These results show clearly that in addition to exhibiting different growth kinetic responses at low temperature, the soil microbial communities beneath the snowpack and during the summer are distinct in their taxonomic composition.

Long-term monitoring of mountain snow packs in the western USA and Europe have shown trends towards decreasing depth, with several mountain ranges experiencing 50–75% decreases, and these have been attributed to positive temperature anomalies^{4–6}. In other north-latitude ecosystems, climate interactions with local features of the landscape, including large lakes, are predicted to result in an increase in winter snow in the face of climate warming¹⁷. Trends in snow pack provide a critical, indirect coupling between climate and forest C cycles. Decreases in the winter snow pack will generally cause decreases in the loss of respired CO₂ from the soils of forest ecosystems, thus, enhancing the potential for soil C sequestration. The existence of a low-temperature adapted microbial flora living beneath the snow pack explains the connection between soil respiration rate and snow depth. These organisms function in a relatively narrow range of sub-zero soil temperatures, and are highly sensitive to warming or cooling outside this range. Our observations reveal that a warmer climate may change the beneath-snow soil temperature in forest ecosystems because of changes in the depth of the insulating snow cover, changing soil respiration rates and, concomitantly, soil C sequestration rates.

METHODS

Eddy covariance and beneath-snow respiration measurements. The Niwot Ridge AmeriFlux site is located at 3,050 m above sea level at 40° 1' 58" N; 105° 32' 47" W. Details of NEE and climate measurements can be found at http://urquell.colorado.edu/data_ameriflux/ (refs 13, 18, 19). The forest is dominated by lodgepole pine (*Pinus contorta*), subalpine fir (*Abies lasiocarpa*), and Engelmann spruce (*Picea engelmannii*). Soil temperatures were measured using copper-constantan thermocouples (1999–2001) or platinum resistance

thermometers (2001–2003) located within 50 m of the flux tower and in an area approximately 2 m from the nearest tree bole, and next to each of the soil chambers used for respiration measurements. Beneath-snow CO₂ concentrations were measured using a multi-inlet air-sampling system combined with a single LI-7000 IRGA gas analyser (LI-Cor Inc). Eleven chambers (2.5-litre volume) were placed on the ground before the first winter snow. An additional three inlets were placed above snow height to obtain the atmospheric CO₂ concentration. Air from the soil and air inlets was pumped (1.4 litres min⁻¹) to glass buffer volumes (2 litres) from which samples were drawn for analysis. Each buffer volume was sampled for 80 s every 30 min and only data from the final 20 s of the sampling time were used. Data on CO₂ concentration was continuously recorded at 1-s intervals during each sampling period. The IRGA was calibrated at 3–5-hour intervals. Calibration was obtained from three gases of a known CO₂ concentration (413, 504 and 749 p.p.m.v.) and calibrated accuracy to within ±2%. The maximum beneath-snow CO₂ concentration we observed was 3,295 p.p.m.v., which exceeded that for our highest calibration gas and introduced the possibility of error due to nonlinearity. We conducted analyses during the subsequent winter to determine the signal linearity across the range 0–3,000 p.p.m.v. using the original calibration gases plus a fourth mixture of 5,000 p.p.m.v. We calculated an upper-bounded, systematic error of ~5% at an observed value of 3,000 p.p.m.v. if the calibration was limited to the lower three gases only.

The flux of CO₂ through the snowpack was calculated using a steady-state diffusion model with driving variables being the difference in CO₂ concentration from soil to atmosphere, atmospheric pressure, air temperature, and snowpack depth, porosity and tortuosity^{20–24}. Snowpack physical characteristics were measured at 10-cm vertical intervals on a weekly basis after manually digging snow pits. SWE, snow temperature, grain type, size and snowpack stratigraphy were measured following previously published protocols and used to calculate snow density²⁵. Values of 1 April SWE were obtained from the US Natural Resources Conservation Service Snotel Database.

We explored the possibility that the CO₂ efflux was due to the release of ice-trapped air during soil warming. We assumed that the CO₂ efflux originates from the top 20 cm of the soil²⁶, that the maximum soil water content during the autumn is 30% (ref. 26), that the solubility of CO₂ in water is 0.35 g per 100 cm³, and we measured the pre-snow soil surface CO₂ partial pressure at 5.5×10^{-4} atm. We calculated that the CO₂ that could have been dissolved in autumnal soil water and released during the winter was 0.003 mol m⁻², which is less than 0.1% of the total observed CO₂ efflux (3.3 mol CO₂ m⁻²).

Values of R_T were calculated according to $R_T = (R_2/R_1)^{10/((T_2-T_1))}$, where R_2 and R_1 represent respiration rates measured at two different temperatures T_2 and T_1 , respectively. We note that R_T reflects responses to temperature, such as altered substrate supply and turnover of microbial populations, that are broader than the enzyme kinetics that drive traditional Q_{10} notation.

Microbial growth kinetics and substrate-use measurements. The growth kinetics of soil microbial communities were measured with the substrate-induced growth response method using potassium glutamate^{27,28}. Soils were incubated with sufficient substrate to induce growth, along with tracer levels of ¹⁴C-glutamate. Respired CO₂ was trapped in 1.0 M NaOH and counted by liquid scintillation. Exponential growth curves were fitted to respiration rate versus time²⁸. Experimental soils were frozen for later DNA extraction, polymerase chain reaction (PCR) amplification of the 16S rRNA gene, and clone library construction as described elsewhere²⁹. Sequences were either aligned manually or using the CLUSTAL algorithm within BioEdit. A maximum-likelihood tree was produced, and microbial communities were compared using the PTP test in PAUP. This method tests the null hypothesis that the samples are from the same community by comparing the original tree to a probability distribution made from 10,000 random permutations of the tree³⁰.

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