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The response of root and microbial respiration to the experimental warming of a boreal black spruce forest

Jason G. Vogel, Dustin Bronson, Stith T. Gower, and Edward A.G. Schuur

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Abstract: We investigated the effects of a 5 °C soil + air experimental heating on root and microbial respiration in a boreal black spruce (*Picea mariana* (Mill.) B.S.P.) forest in northern Manitoba, Canada, that was warmed between 2004 and 2007. In 2007, the $^{14}C/^{12}C$ signatures of soil CO_2 efflux and root and soil microbial respiration were used in a two-pool mixing model to estimate their proportional contributions to soil CO_2 efflux and to examine how each changed in response to the warming treatments. In laboratory incubations, we examined whether warming had altered microbial respiration rates or microbial temperature sensitivity. The $^{14}C/^{12}C$ signature of soil CO_2 efflux and microbial respiration in the heating treatments were both significantly ($p < 0.05$) enriched relative to the control treatment, suggesting that C deposited nearer the atmospheric bomb peak in 1963 contributed more to microbial respiration in heated than control treatments. Soil CO_2 efflux was significantly greater in the heated than control treatments, suggesting the acclimation to temperature of either root or microbial respiration was not occurring in 2007. Microbial respiration in laboratory incubations was similar in heated and control soils. This study shows that microbial respiration rates still responded to temperature even after 4 years of warming, highlighting that ecosystem warming can cause a prolonged release of soil organic matter from these soils.

Key words: boreal, soil carbon, root respiration, warming, black spruce.

Résumé : Nous avons étudié les effets d'un réchauffement expérimental de 5 °C de la température de l'air et du sol sur la respiration racinaire et microbienne dans une forêt boréale d'épinette noire (*Picea mariana* (Mill.) Britton, Sterns, Poggenb.) du nord du Manitoba, au Canada, de 2004 à 2007. En 2007, la signature isotopique ($^{14}C/^{12}C$) du CO_2 émanant du sol et provenant de la respiration racinaire et microbienne du sol a été utilisée dans un modèle de mélange de deux fluides pour estimer leur contribution proportionnelle au CO_2 émanant du sol et la façon dont chacun a varié en réaction au réchauffement de la température. En utilisant des incubations en laboratoire, nous avons étudié si le réchauffement a modifié le taux de respiration microbienne ou la sensibilité microbienne à la température. La signature isotopique ($^{14}C/^{12}C$) du CO_2 émanant du sol et de la respiration microbienne dans les traitements ayant fait l'objet d'un réchauffement étaient toutes deux significativement ($p < 0,05$) enrichies comparativement au traitement témoin, ce qui indique que le C déposé plus près du point culminant des essais nucléaires atmosphériques en 1963 a davantage contribué à la respiration microbienne dans le traitement ayant fait l'objet d'un réchauffement que dans le traitement témoin. Le CO_2 émanant du sol était significativement plus important à la suite du réchauffement que dans le traitement témoin, ce qui indique que les racines ou la respiration microbienne ne s'étaient pas acclimatées à la température en 2007. Dans les incubations en laboratoire la respiration microbienne était similaire dans le sol ayant subi un réchauffement et dans le sol témoin. Cette étude démontre que le taux de respiration microbienne réagit encore à la température même après quatre années de réchauffement, faisant ressortir le fait que le réchauffement de l'écosystème peut engendrer une perte prolongée de matière organique dans ces sols. [Traduit par la Rédaction]

Mots-clés : boréal, carbone du sol, respiration racinaire, réchauffement, épinette noire.

Introduction

During the next century, air temperatures in some regions of the boreal forest may warm by as much as 8 °C (Intergovernmental Panel on Climate Change 2007). Warmer air temperatures will likely coincide with warmer soil temperatures, creating the possibility that the soil organic matter (SOM) pools of the region will be decomposed and lost to the atmosphere as a result of increased heterotrophic respiration. One means to document an increase in heterotrophic respiration could be by monitoring soil CO_2 efflux, which is the combined respiration of soil heterotrophs and roots. Using this approach, Bond-Lamberty and Thomson (2010) reviewed the literature and found that historic soil warming in temperate and tropical regions coincided with regional increases in soil CO_2 efflux; however, for the boreal forest and arctic regions, the temperature effect on soil CO_2 efflux was slightly negative. This result was surprising because measurements of root and microbial respiration have indicated that within a growing season, both components of soil CO_2 efflux are positively correlated with temperature in boreal ecosystems (Bond-Lamberty et al. 2004; Vogel et al. 2005). In contrast, Vogel et al. (2008) reported that across a climate gradient where temperature and precipitation were correlated, warmer and wetter conditions corresponded to significantly lower annual estimates of root respiration and soil CO_2 efflux.

In controlled warming experiments, the soil CO_2 efflux rate at a given temperature has often become less responsive to tempera-

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J.G. Vogel* and E.A.G. Schuur. Department of Biology, Bartram-Carr Hall, P.O. Box 118525, Gainesville, FL 32611, USA.
D. Bronson† and S.T. Gower. Forest and Wildlife Ecology, University of Wisconsin-Madison, 1630 Linden Drive, Madison, WI 53706, USA.
Corresponding author: Jason G. Vogel (e-mail: jason_vogel@tamu.edu).
*Present address: Texas A&M University, Department of Ecosystem Science and Management, College Station, TX 77845, USA.
†Present address: Wisconsin Department of Natural Resources, Bureau of Science Services, 107 Sutliff Ave., Rhinelander, WI 54501, USA.

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ture when the average temperature has been increased (Luo et al. 2001; Rustad et al. 2001; Melillo et al. 2002). Often described as soil CO_2 efflux acclimation to temperature (Kirschbaum 2004), this shift in temperature response could reflect either changes in the physiological function of roots and microbes or a reduction in the amount of C available to microbes. For plants, the respiration rate of individual roots could acclimate (Tjoelker et al. 1999; Atkin et al. 2000), or whole-plant C allocation could shift away from root processes (Bronson et al. 2008; Burton et al. 2008). The latter may occur as nutrients become more available during the enhanced decomposition of SOM (Melillo et al. 2002; Litton et al. 2007). For soil microbes, it is unclear if acclimation is related to changes in microbial physiological function or community structure (Bradford et al. 2008) or if it is simply an overall depletion of SOM that results in the decrease in microbial respiration (Kirschbaum 2004). In addition, a reduction in belowground C allocation would reduce C supply to the mycorrhizosphere.

We partitioned root and microbial respiration in soil CO_2 efflux for an experimentally heated black spruce (*Picea mariana* (Mill.) B.S.P.) forest, where both air and soil temperatures were maintained at 5 °C above ambient temperature during the growing season for four previous growing seasons (Bronson et al. 2008). In the first 2 years of the heating, soil CO_2 efflux in air + soil warming treatments decreased to levels below the control plots, which corresponded to a significant decrease in fine root biomass (Bronson et al. 2008). This result led us to hypothesize that in the 4th year of warming, a change in soil CO_2 efflux temperature response would occur because of a decrease in root respiration. We used an isotope partitioning technique to separate the components of soil CO_2 efflux (Czimczik et al. 2006; Hahn et al. 2006; Schuur and Trumbore 2006). With this technique, the unique radiocarbon signatures of root and microbial respiration are used in a two-pool mixing model to estimate the relative proportion of each in soil CO_2 efflux. Radiocarbon signatures differ for the two sources of soil CO_2 efflux because the ^{14}C in plant respiration more closely reflects the current atmosphere, whereas microbial respiration reflects a mixture of organic material, including some enriched in the radiocarbon that was incorporated into plant tissue closer to the peak of the atomic and hydrogen bomb atmospheric testing in 1963 (Schuur and Trumbore 2006). As a result, microbial respiration is often enriched in radiocarbon relative to plant respiration (Trumbore 2000). In addition to our primary hypotheses that warming had caused a proportional decrease in root respiration, we hypothesized that microbial respiration had decreased as a result of a decrease in the labile SOM available for decomposition.

Methods

Site description and experimental design

The study site is located 20 km south of Thompson, Manitoba, Canada (55°53′N, 98°20′W). The warming experiment was established in a 4 ha black spruce plantation planted in 1993 with trees placed at 2 m × 2 m spacing along a gently sloping hillside. The soils are classified as Typic Cryorthents, having a montmorillonite clay mineral soil and an organic layer 2–6 cm thick on the soil surface. The warming experiment was established in 2003; warming began in May of 2004 and continued through September of 2007. In 2003, the trees had an average diameter at breast height (1.37 m) of 5.62 ± 0.96 cm and an average height of 4.75 ± 0.39 m (Bronson et al. 2008).

The experimental design was a randomized complete block comprised of four replicate blocks. In each block, there was a heated plot and a control plot (15 m × 15 m), with the heating treatment being in a greenhouse in which both soil and air were warmed to ~5 °C above ambient temperature. Soil warming was applied across the entire plot area, and the air warming was maintained within a 7.3 m diameter × 7.5 m tall greenhouse enclosure located in the center of the plot. The enclosures contained approximately nine black spruce trees. Enclosures were also located in the control plots to account for enclosure-specific artifacts, and in these, air temperature was maintained at the ambient temperature through a combination of active cooling and passive venting. Air circulation was nearly constant between the chambers and outside environment for both ambient and heated treatments. The soil was heated using heating cable installed inside water-filled polyethylene tubing that had been placed at a depth of approximately 20 cm. Rather than cutting down from above, the tubing was installed horizontally under the 15 m plot using a modified diamond-bit drill, with horizontal holes placed at 30 cm intervals. From 2004 to 2007, soil warming was maintained continuously as soon as the control plot soil temperature exceeded 0 °C. To avoid thermal shock, the soil was heated gradually in the spring and allowed to cool slowly in the fall. Warming was not maintained during the winter.

From 2005 to 2007, soil temperature at a 10 cm depth for the heated treatments was maintained at 5 ± 1 °C above the control soil temperature for 100%, 97%, and 94% of the warming period, while air temperature was held above control air temperature for 96%, 83%, and 50% of the warming period (Bronson and Gower 2010). In 2007, new sections of chamber were added to accommodate tree growth, and the increased enclosure volume reduced the ability of the aging air heaters to maintain the target temperature. If a deviation of ±1.5 °C was the threshold, air temperature was within range for 77% of the treatment time period (Bronson and Gower 2010).

In situ radiocarbon measurements

On 31 July to 1 August, 2007, and 7 to 8 September, 2007, the $^{14}C/^{12}C$ and $^{13}C/^{12}C$ ratios of soil CO_2 efflux were measured in each heated and control enclosure using a customized version of the modified dynamic flow system (Norman et al. 1997; Bronson et al. 2008). In each enclosure, a polyvinyl chloride (PVC) collar (33 cm diameter × 5 cm tall) was used to seat an acrylic chamber (33 cm inside diameter × 11.5 cm high). The fit between the chamber and collar was snug and reasonably airtight because of an inside squared edge on the collar. The CO_2 concentration inside the chamber was monitored using the LI-6200 portable CO_2 infrared gas analyzer system (IRGA) (LI-COR Inc., Lincoln, Nebraska, USA). The bottom edge of the PVC collar was beveled to minimize soil compaction as it was placed in the ground. Four collars were placed in the soil in 2003; for the $^{14}CO_2$ measurements, one collar per treatment was selected for measurement. In addition to the $^{14}CO_2$ measurements, soil CO_2 efflux was measured within 4 days of the radiocarbon measurements, and these were used to assess treatment differences in efflux. These dates were 27–28 July and 11–12 September, 2007; four repeated measurements were made on each of the four collars within an experimental plot.

When making in situ radiocarbon measurements of soil CO_2 efflux, there are two objectives: (*i*) maintain a relatively normal CO_2 efflux rate from the soil surface and (*ii*) minimize drawing background air into the chamber (Gaudinski et al. 2003). To achieve these objectives, we passed the airstream from the IRGA through a soda lime scrub that removed all CO_2. The airstream then returned CO_2-free air to the chamber. The rate at which air flowed through the scrub was adjusted so that the chamber CO_2 concentrations remained slightly above the concentrations outside the chamber (~380–460 ppm). In effect, CO_2 was scrubbed from the system at a rate similar to the rate of CO_2 efflux from the soil. The flow rate was generally adjusted to be near 2.5 $L \cdot min^{-1}$. Scrubbing was maintained for 20 min, which resulted in 3.5 chamber volumes passing through the scrub. At the end of the scrub period, the airstream was diverted through a $MgClO_4$ moisture trap and then a molecular sieve trap (Alltech 13X) for 15 min for eventual isotopic analysis of $^{13}C/^{12}C$ and $^{14}C/^{12}C$ ratios.

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The delta notation was used for the stable isotopic signature of CO_2:

$$\delta^{13}C\ (‰) = \left[\left(^{13}C/^{12}C\right)_{sample}/\left(^{13}C/^{12}C\right)_{PDB} - 1\right] \times 1000$$

where the isotopic ratio of the sample ($(^{13}C/^{12}C)_{sample}$) is divided by the isotopic ratio of a common standard (Pee Dee Belemnite (PDB)) ($(^{13}C/^{12}C)_{PDB}$). Similarly, radiocarbon estimates of each sample were normalized by a standard, oxalic acid (OX1), that is radiocarbon age-corrected to the year 1950 (Stuiver and Polach 1977):

$$\Delta^{14}C\ (‰) = \left[\left(\left(^{14}C/^{12}C\right)_{sample}/\left(0.95 \times \left(^{14}C/^{12}C\right)_{OX1}\right)e^{(year-1950/8267)}\right) - 1\right] \times 1000$$

where year is the year in which the sample was collected.

The molecular sieves trapped all CO_2 that passed through them, resulting in between 1.5 and 2.5 mg CO_2-C captured in total for the soil CO_2 efflux measurements. Soil air is a mixture of atmospheric air and respired CO_2 (Schuur and Trumbore 2006), so a correction is applied to the $\Delta^{14}CO_2$ using the $\delta^{13}C$ of the soil CO_2 efflux, the atmospheric $\delta^{13}C$ value ($\delta^{13}C_{atm}$ = –8.2‰), and the actual $\delta^{13}C$ value of root and microbial respiration. The $\delta^{13}C$ of root and microbial respiration was assumed to be the average of root and microbial respiration for a given treatment (–24.9‰ to –26.7‰).

The fraction (F) of atmospheric air in the sample was estimated as

$$F = \left(\delta^{13}C_{measured} - \delta^{13}C_{expected}\right)/\left(\delta^{13}C_{atm} - \delta^{13}C_{expected}\right)$$

The actual $\Delta^{14}C$ of soil CO_2 efflux was then estimated using the mass balance equation:

$$\Delta^{14}C\ (‰) = \Delta^{14}C_{measured} - \left(\Delta^{14}C_{air} - \left(F \times \Delta^{14}C_{air}\right)\right)/(1 - F)$$

Removing the effect of background air increased the soil CO_2 efflux $\Delta^{14}C$ value by an average of 10.0‰.

In the laboratory, the molecular sieve traps were heated to 625 °C while connected to a vacuum line. This caused the CO_2 trapped in the sieve to evacuate into the vacuum line, where it was then cryogenically purified and subsampled for $\delta^{13}C$ analysis (Bauer et al. 1992). The remaining CO_2 was reduced to a graphite coating on a cobalt catalyst using a mixture of zinc powder and titanium hydride as reductants (Vogel 1992). Graphite samples were sent to the Lawrence Livermore AMS facility for radiocarbon analysis. The subsamples of purified CO_2 were analyzed for $\delta^{13}C$ at the University of Florida using a Delta Plus XL Continuous Flow isotope ratio mass spectrometer attached to a GasBench II (ThermoFinnigan, San Jose, California, USA).

Incubations for heterotrophic and root isotopic signatures

To estimate the radiocarbon signature of microbial respiration, we performed laboratory incubations on soil collected within the control and warming experiment at the same time as the field measurements. Three soil cores were collected from each of the heated and control plots from the surface to a depth of 20 cm using a 3 cm diameter corer. The cores were split into 0–10 cm and 10–20 cm fractions, with 0–10 cm horizon including the thin organic layer. The edges of the cores were inspected for live roots, which were gently removed so that the overall soil structure was maintained. The cores were also wrapped in foil to maintain soil structure and minimize the effect of disturbance on the $\Delta^{14}C$ signature of CO_2 (Schuur and Trumbore 2006). Before the radiocarbon measurement, the three cores were placed in a 490 mL glass canning jar and allowed to incubate for 72 h at room temperature (~20–25 °C). This step was performed to allow any very fine roots that might have been missed during the initial inspection to respire most of their stored C (Ruess et al. 2003). After 72 h, the jars were capped with lids containing two stopcock access ports. The access ports were connected via a closed loop to a brushless pump and a container of fine molecular sieve soda lime for the purpose of removing the CO_2 inside the jars before the incubation occurred. The CO_2 was scrubbed out by passing an airstream through the soda lime for 5 min at maximum flow (3.5 L·min^{-1}), resulting in approximately 35 jar volumes passing through the soda lime (Lavoie et al. 2011). The stopcocks were then closed, and the jar was made airtight. The jar was left capped and sealed for 12 h for the July sampling, after which time the CO_2 that accumulated in the jar was circulated through the molecular sieve traps. Prior to trapping, we estimated an incubation time (~12 h) that would yield ~0.5–1.0 mg of CO_2-C for the 0–10 cm depth interval, which is the optimal amount for analysis. When the traps were analyzed, however, only about half this amount was actually found in each trap. This occurred because these soils had lower C concentrations than expected for black spruce forests. Thus, two samples were combined within a treatment for subsequent radiocarbon analysis. For the September sampling, the amount of incubation time was doubled to 24 h, which doubled the amount of trapped C (~1.0–2.0 mg of CO_2-C) and allowed for individual analysis of each trap. Microbial respiration rates from the 10–20 cm depth were ~1/10 that of the 0–10 cm layer, and although the samples were subsequently incubated in the laboratory, the in situ rates were too low for radiocarbon analysis. The 0–10 cm molecular sieve traps were then analyzed as described earlier.

We measured the radiocarbon value of CO_2 emitted from root respiration by conducting short incubations of live roots that were less than 3 mm in diameter. No effort was made to separate the black spruce and understory roots as both contribute to the soil radiocarbon efflux. A 5 cm diameter corer was used to collect three 10 cm deep soil cores per plot. Intact roots were gently removed from the soil, rinsed with distilled water to remove soil particles, and then immediately placed in 490 mL glass canning jars. Each jar contained approximately 5–10 g of freshly cut roots. Jars were sealed, and the air in the jar was passed through a soda lime scrub for the equivalent of ~35 jar volumes to remove atmospheric CO_2. The roots were then allowed to incubate at room temperature (~20 °C) in the dark for 4 h. At the end of that period, accumulated CO_2 was collected for isotopic analysis as described for the SOM incubation. Root respiration samples collected from two plots per treatment were analyzed in the July sampling.

After the radiocarbon measurement, the soils collected in September were split into two halves and analyzed for rates of microbial respiration at 5 °C and 25 °C for both the 0–10 cm and 10–20 cm depths. The incubations were conducted at field volumetric moisture content (0–10 cm depth, 0.34–0.54 cm^3·cm^{-3}; 10–20 cm, 0.48–0.59 cm^3·cm^{-3}), and the rate of microbial respiration was measured immediately and then every 7–10 days. Microbial respiration rate was estimated by collecting an air sample after the jar's internal air was scrubbed free of atmospheric CO_2 and then again after 6 h (25 °C) and 36 h (5 °C). Air samples were analyzed on a LI-6262 (LI-COR Inc.) attached to a gas chromatograph injection loop. The 25 °C experiment was maintained for 60 days, but the 5 °C experiment ended after 30 days because of a malfunction in the climate-control system of the incubator. At the end of the incubation, the soils were dried at 60 °C for 72 h, ground with a mortar and pestle, and analyzed on a Costech ECS 4010 elemental analyzer (Costech Analytical Technologies, Inc., Valencia, California, USA) for both C and N.

Partitioning fluxes using $\Delta^{14}C$

The proportion of root and microbial respiration in soil CO_2 efflux was estimated using a two-pool mixing model. With this model, the values of root and microbial respiration are treated as end members, and the proportion of each needed to yield the $\Delta^{14}C$

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of soil CO_2 efflux is solved for analytically. Error terms were estimated following the methods outlined by Phillips and Gregg (2001). Briefly, this method uses the sample size, mean, and standard deviation of each member's $\Delta^{14}C$ signature to estimate a pooled variance. The microbial respiration values collected in September were used as the microbial respiration end member for both time periods, because the longer incubation time resulted in the optimal amount of C collected in each trap; as a result, the error terms reflected the variance of the four replicates rather than that of a composite.

Statistical analysis

The mixed model procedure in SAS 9.1 was used to compare the warming and control treatment for the isotopic ratios of soil CO_2 efflux, root and microbial respiration, and soil characteristics. For the estimates of soil CO_2 efflux and root and microbial respiration rates (both in situ and laboratory), the analysis included time to examine for treatment and temporal changes in soil CO_2 efflux. For the laboratory measurements, a spatial covariance structure was applied because the sample periods were unevenly spaced through time (Littel et al. 1997).

To estimate in situ temperature response functions and confidence intervals for root and microbial respiration, a Monte Carlo approach was used in which a random sampling was done within the range of temperature, soil CO_2 efflux, and root and microbial proportions estimated for the two different time points. A normally distributed data set of proportional root and microbial respiration values was generated using the SAS proc RAN command, with the random values restricted to the range of the 95% confidence intervals estimated by the isotope mixing model. These proportional estimates were multiplied by a random sample drawn from the normal distribution of soil CO_2 efflux and temperature measurements. This step was repeated 5000 times, creating a combined data set of 15 000 temperature and root and microbial respiration estimates. Root and microbial respiration covariance with temperature across treatments was then examined using the generated data set. The PROC NLIN procedure in SAS was used to construct exponential response equations, with the Gauss–Newton method used for parameter estimation. Confidence intervals for parameters were used to evaluate differences between the root and microbial response functions.

Results

Soil bulk density, C and N concentrations, and the C:N ratio were similar in the heated and control treatments, with statistical comparisons suggesting no significant differences in these soil attributes (Table 1). The largest percent difference for any soil attribute was for C concentration in the surface soil (0–10 cm), which was 32% less in the heated treatment than in the control (paired t test, $p = 0.17$). Given the variance in the soil measurements, a C concentration decrease of 65% would have been required to detect a significant difference between the heated and control treatments. During the active warming in the growing season, the average soil temperature in 2007 was 8.6 °C and 7.4 °C in the control treatments and 13.2 °C and 12.6 °C in the heated treatment at 10 cm and 25 cm depth, respectively.

The $\delta^{13}C$ signature of root and microbial respiration did not differ between heated and control treatments or between the two sampling dates (Table 2). Root and microbial respiration did not differ from one another in their $\delta^{13}C$ signature ($p = 0.23$). The $\delta^{13}C$ signature of soil CO_2 efflux was significantly more enriched than root or microbial respiration ($p < 0.001$), but it did not differ between treatments. Between sampling dates, soil CO_2 efflux did become significantly more enriched in $\delta^{13}C$ ($p < 0.001$).

The $\Delta^{14}C$ signature of soil CO_2 efflux was greater in the heated than control treatments ($p = 0.045$) but did not change significantly between time points. The soil CO_2 efflux $\Delta^{14}C$ signature was also significantly greater in heated than control ($p = 0.033$) treatments before the $\delta^{13}CO_2$ correction was applied. Microbial respiration was significantly more enriched in ^{14}C in the heated than control treatments (Tables 2, 3), and the microbial respiration sampled in September was significantly more enriched in ^{14}C compared with the July–August sampling (Table 3). Root respiration from the heated treatments tended to be more enriched in ^{14}C, but the difference between treatments was not significant ($p = 0.09$; Table 3).

The mean $\Delta^{14}C$ signature of soil CO_2 efflux fell between the average $\Delta^{14}C$ signatures of root and microbial respiration (September sampling) (Table 2), allowing for an analytical solution to the proportional contribution of roots and microbes to soil CO_2 efflux. The proportional root respiration estimate ranged from 68% ± 40% for the control treatments to 51% ± 14% for the heated treatments based on the unique $\Delta^{14}C$ values for soil CO_2 efflux and microbial respiration (Fig. 1). The 95% confidence intervals overlapped for the different treatments and between measurement periods, indicating no significant difference in treatments.

Soil CO_2 efflux during the two measurement periods nearest the partitioning were significantly greater in the heated than the control plots ($p = 0.03$) and decreased significantly from July to September ($p = 0.002$; Fig. 2A). Soil CO_2 efflux correlated with soil temperature at 10 cm across treatments ($Y = 0.70 \cdot \exp(0.057 \cdot \text{soil temperature})$, $r^2 = 0.54$, $p < 0.001$; Fig. 2B). The root and microbial temperature response functions generated from the Monte Carlo sampling were significant ($p < 0.001$), and temperature explained relatively low amounts of variance for roots ($r^2 = 0.07$) and microbes ($r^2 = 0.14$). The derived equations for roots and microbes had nonoverlapping parameters for the exponent, suggesting unique temperature response relationships for both components of soil CO_2 efflux across treatments (Table 4). Root respiration had a lower exponent (equivalent to a temperature sensitivity (Q_{10}) of 1.86, 95% confidence interval = 1.74–1.92) than microbial respiration (equivalent to a Q_{10} of 1.99, 95% confidence interval = 1.96–2.08).

In the laboratory incubations at a common temperature, the heated and control treatments had microbial respiration values that were similar for both the 0–10 cm and 10–20 cm depth intervals (Figs. 3A, 3B). The microbial respiration of the warmer laboratory temperature treatment (25 °C) was significantly ($p < 0.001$) greater than for the cooler temperature treatment (5 °C) for both depth intervals, and microbial respiration for the 10–20 cm depth interval was 8–15 times less than respiration for the 0–10 cm interval. This latter result likely reflected the lower C concentrations in the deeper soils (Table 1). For the 0–10 cm interval, the change in respiration rate over time and time × treatment interaction was not significant; however, during the course of the incubation, there was a slight upward trend in microbial respiration for the 10–20 cm interval and significant time effect for both temperatures (Fig. 3B). At the 25 °C incubation temperature, the soils from the heated and control plots lost a similar amount of cumulative C for both depth intervals. For the first 24 days of the incubation, the Q_{10} was not significantly different for the 0–10 cm depth interval (heated, 2.10 ± 0.51, vs. control, 1.81 ± 0.33) or for the 10–20 cm depth interval (heated, 1.22 ± 0.5, vs. control, 1.41 ± 0.22).

Discussion

During the first 2 years of this warming experiment, soil CO_2 efflux in the plots receiving the air + soil heating treatments acclimated to levels below the controls with a ~5 °C warming, which coincided with a significant 20%–30% reduction in fine root biomass (Bronson et al. 2008). We had hypothesized that a downward shift in soil CO_2 efflux for 2007, the 4th year of warming, would correspond to a proportional decrease in the respiration attributed to root processes. However, at the time of our measurements, soil CO_2 efflux was greater in the heated treatments than in the control treatments, and the temperature response was

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Table 1. Mean (±SE) soil characteristics of heated and control treatments at two different depth intervals.

	Depth (cm)	Bulk density (g·cm^{-3})	% C	% N	C:N
Control	0–10	0.89±0.14	8.2±2.1	0.36±0.11	21±1.7
	10–20	1.67±0.09	0.67±0.11	0.11±0.01	6.1±0.9
Heated	0–10	0.96±0.04	5.6±0.90	0.27±0.04	20±2.0
	10–20	1.58±0.10	0.74±0.16	0.10±0.01	7.1±1.4

Table 2. The average isotopic signature ($\delta^{13}C$, $\Delta^{14}C$ in ‰) of root and microbial respiration, and soil CO_2 efflux collected from control and heated treatments (mean ± SE; n = 4 unless otherwise noted).

	Root respiration		Microbial respiration		Soil CO_2 efflux	
	$\delta^{13}C$	$\Delta^{14}C$	$\delta^{13}C$	$\Delta^{14}C$	$\delta^{13}C$	$\Delta^{14}C$
31 July to 1 August						
Control	–26.1±0.3	55±2.4[a]	–26.1±0.4	73±12.1[b]	–22.3±0.1	66±9.3
Heated	–25.7±0.6	60±1.3	–26.4±0.7	83±7.0	–20.5±0.6	86±6.5
7–8 September						
Control	–24.9±0.5	57±2.3	–25.7±0.3	83±1.8	–17.8±1.2	68±6.5
Heated	–25.6±0.6	63±0.2	–26.4±0.2	111±2.5	–17.4±0.5	84±4.9

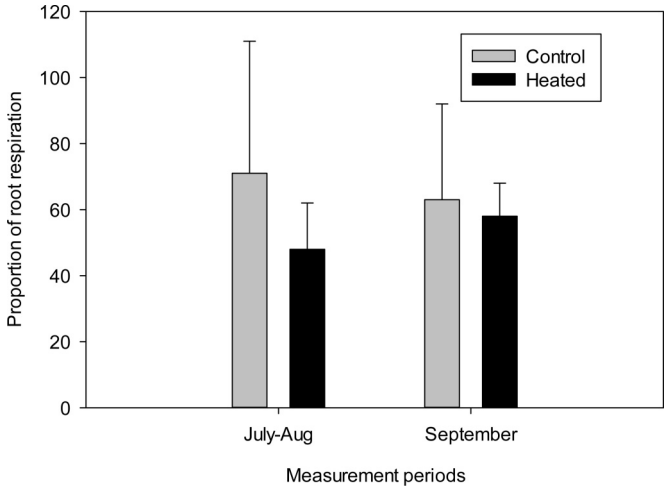
[a]July root respiration is an average of two replicates.
[b]July microbial respiration is an average of two composite samples from two different replicates.

Table 3. ANOVA results for the $\Delta^{14}C$ values of respiration components for the two treatments (heated and control) and time points (31 July to 1 August and 7–8 September).

	Root respiration		Microbial respiration		Soil CO_2 efflux	
	F	p value	F	p value	F	p value
Treatment	4.9	0.09	8.3	0.02	4.1	0.05
Time	1.5	0.29	7.8	0.02	1.5	0.89
Treatment × time	0.0	0.95	2.0	0.20	0.2	0.93

Note: Degrees of freedom: for root respiration, df = 4; for microbial respiration, df = 6; and for soil CO_2 efflux, df = 8.

Fig. 1. The estimated proportion of root respiration (mean ± SE) in soil CO_2 efflux calculated from a two-pool mixing model. The September estimates of microbial respiration are used for the partitioning in both months.



significant across treatments (Fig. 2A). In effect, there was no evidence of overall reduction in soil CO_2 efflux. This might be because the air heating treatment had begun to decline in efficacy in 2007 (Bronson and Gower 2010), and in this context, it is worth noting that the soil warming part of the experiment alone in 2004 and 2005 caused an increase in soil CO_2 efflux (Bronson et al.

Fig. 2. (A) Soil CO_2 efflux estimates that were within 4 days of the radiocarbon measurements, and (B) the relationship between soil CO_2 efflux and soil temperature at 10 cm depth across treatments. Each data point represents a treatment plot: solid symbols are heated treatment soils and shaded symbols are the control soils.

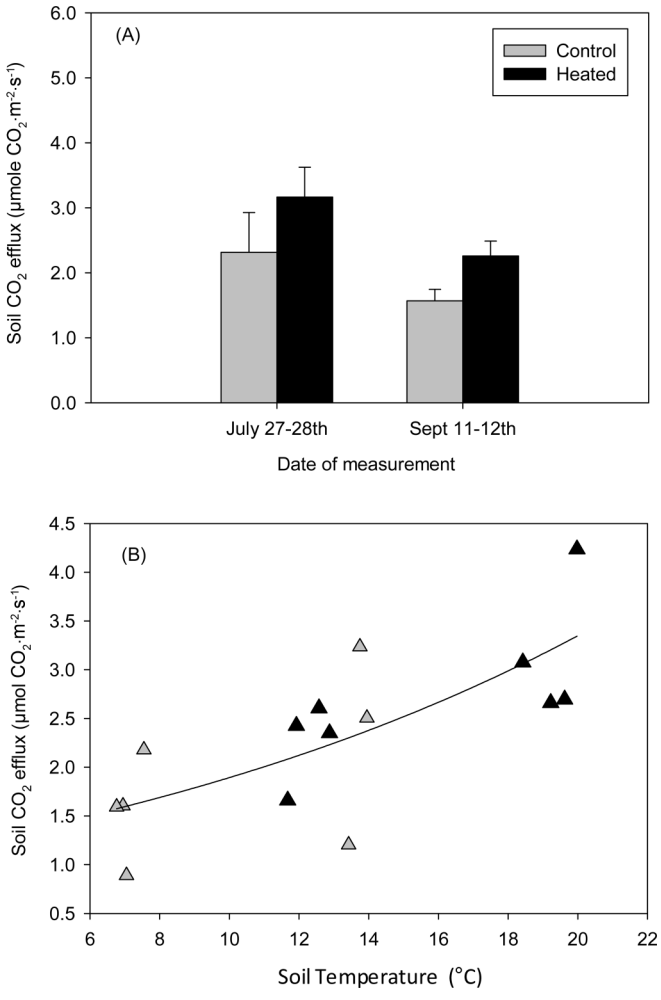


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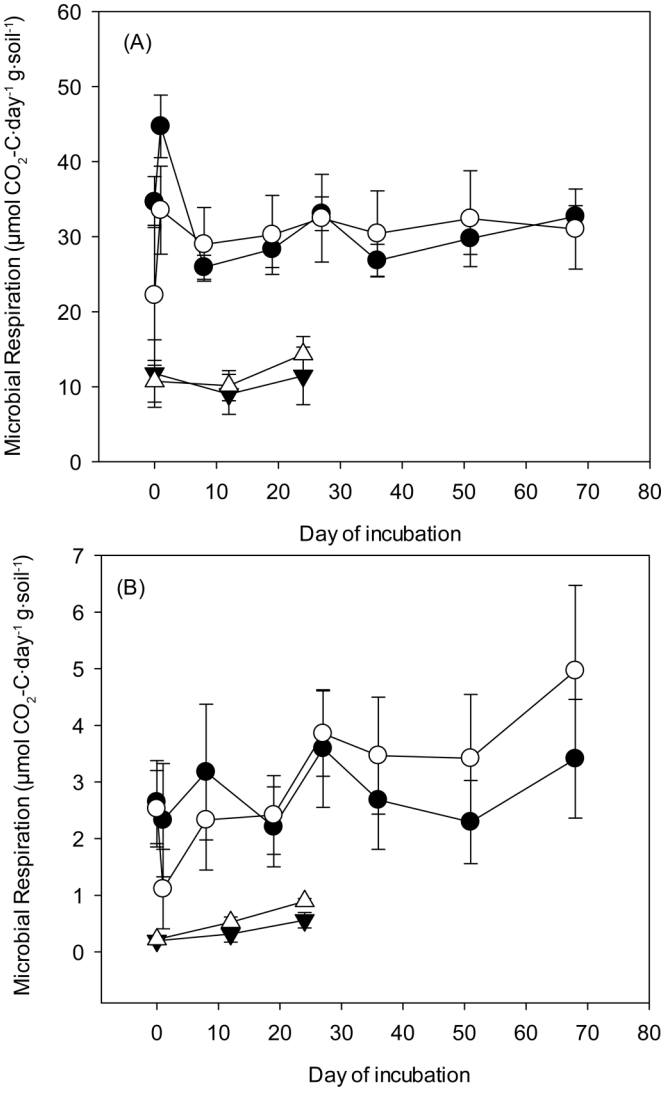
Table 4. Temperature response models ($Y = a \cdot \exp(b \cdot \text{soil temperature})$) for root and microbial respiration across the control and heated treatments generated from random sampling from distributions of soil temperature, root and microbial proportions, and soil CO_2 efflux.

Parameter	Estimate	SE	95% Confidence interval
Root respiration			
a	0.451	0.0103	0.430–0.471
b	0.062	0.0015	0.058–0.064
Microbial respiration			
a	0.430	0.0095	0.412–0.450
b	0.069	0.0014	0.068–0.072

Note: Soil temperature was measured at a depth of 10 cm.

Fig. 3. Change in microbial respiration over the course of laboratory incubations at 5 °C (triangles) and 25 °C (circles) for (A) the 0–10 cm soils and (B) the 10–20 cm soils. Open symbols are heated treatment soils and solid symbols are the control soils.



2008). Air + soil warming may be critical to creating an acclimation-type response in the components of soil CO_2 efflux in these ecosystems or may reflect a decrease in net C gain for black spruce (Way and Sage 2008).

An important unknown for boreal soils is how the temperature sensitivity of microbial respiration is affected by past temperatures. Our results suggest that past warming had no discernible effect on microbial temperature sensitivity, and as a result, more C was lost from heated than control plots over the duration of the in situ warming experiment. Using the in situ microbial respiration model (Table 4) and the continuous soil temperature measurements for each treatment (D. Bronson, unpublished), we estimate that microbial respiration was 218 g $C \cdot m^{-2}$ in heated treatments and 150 g $C \cdot m^{-2}$ in the control plots in the summer of 2007, or 31% greater in the heated than control plots. In addition, the temperature sensitivity of microbial respiration from root exclusions near this experiment suggested that there should have been 27% more microbial respiration from the heated treatments than from the control (Bond-Lamberty et al. 2011). Although these estimates are uncertain because the potential effects of moisture are not included, the values provide context to the overall effect of soil C loss, as the difference between heated and control treatments (68 g $C \cdot m^{-2} \cdot year^{-1}$) was potentially larger than the aboveground wood biomass increment (24–92 g $C \cdot m^{-2} \cdot year^{-1}$) in mature black spruce forests (Vogel et al. 2008; Hermle et al. 2010).

Whether the potential increases in microbial respiration translated to an actual decrease in SOM would depend on how C inputs to the soil via litter, roots, and mycorrhizal fungi and outputs from SOM as dissolved organic C (DOC) may have changed in response to the treatment. Aboveground growth, in the form of shoot elongation, had only started to increase in the 3rd year of warming (Bronson et al. 2009), and belowground biomass was decreased by warming in the first 2 years of the study (Bronson et al. 2008), suggesting a moderate, if any, increase in C inputs. Conversely, even with additional losses from DOC, it is likely that a 27%–32% loss from microbial respiration would have been difficult to directly measure as a SOM change given that the variation in SOM estimates suggest that a 65% decrease would be needed to directly estimate a significant (alpha = 0.05) change in SOM. A larger number of soil samples, or samples collected in each year of warming, would have increased the power to detect changes in SOM.

We found no evidence that microbial respiration was more limited by C availability in the heated plots or that microbial temperature sensitivity had shifted with past warming. Although the 5 °C incubation may have been too short to detect differences in microbial respiration caused by past heating (Karhu et al. 2010), differential carbon limitation between the heated and control treatments also did not seem to occur during the 68-day incubation at 25 °C. These results highlight that SOM in boreal soils is sensitive to soil warming for relatively long periods of time (Karhu et al. 2010; Lavoie et al. 2011), which is likely a reflection of the high SOM concentrations and low degree of microbial processing of SOM in cold soils (Streit et al. 2014). Given the decreases in SOM observed along temperature gradients (Kane and Vogel 2009), it is reasonable to assume that with prolonged warming, the microbial respiration that is dependent on labile pools of SOM will become C-limited (Bradford et al. 2008); however, we have yet to see evidence of this effect after 4 years of warming. Important and unresolved questions are how long can these and other boreal soils support elevated rates of microbial respiration under warmer temperatures and how might reduced soil moisture interact with temperature to affect soil respiration (Allison and Treseder 2008; Schindlbacher et al. 2012).

The more that microbial respiration is enriched in ^{14}C relative to the current atmosphere, the greater is the fraction of microbial respiration derived from fractions of SOM that were deposited near the 1963 peak for atmospheric radiocarbon (Trumbore 2000). Our observation of microbial respiration being enriched in ^{14}C in the warming experiment suggests that in the heated treatment, microbes were using more C that was deposited near this peak. Others have observed a shift in microbial use from more recently deposited to older C with warming soil temperatures (Lavoie et al. 2011; Streit et al. 2014). Notably, a change in $\Delta^{14}CO_2$ requires little

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change in the $\Delta^{14}C$ signature of the substrates that microbes are using because ^{14}C is a very small fraction (<0.01%) of the total composition of carbon isotopes. Waldrop et al. (2012) found a significant shift towards more enriched $\Delta^{14}CO_2$ between 48 h and one month of a laboratory incubation. This temporal sensitivity is also potentially one reason why the microbial respiration collected in September had become more enriched in ^{14}C relative to the July–August sampling, as the continued microbial activity over the growing season would be similar to a prolonged incubation. The change in microbial respiration $\Delta^{14}CO_2$ between July–August and September also highlights that the $\Delta^{14}CO_2$ signature of the microbial respiration end members will likely change over the course of an incubation and that this has implications for partitioning with radiocarbon (Waldrop et al. 2012). For example, if a researcher wishes to group microbial rhizosphere respiration with the microbial respiration derived from older SOM, then the collection should be timed very close to the extraction of the root biomass. However, if a researcher wishes to group microbial rhizosphere respiration with root respiration, then waiting longer after root removal will effectively accomplish this goal. In the latter situation, a researcher is likely approaching estimates of root respiration that are similar in the proportional contribution to those observed by root exclusions (Schuur and Trumbore 2006).

Because of the priming effect, or the potential effect of recently deposited organic matter on the decomposition of other organic matter (Gaumont-Guay et al. 2008), we attempted to group the microbial respiration from the rhizosphere into the heterotrophic proportion by collecting CO_2 soon after root removal (72 h). However, this also increases the probability that the ^{14}C signature of microbial respiration and of soil CO_2 efflux will be indistinguishable, as was observed for the July–August sampling after the 12 h collection (Table 2). We found that a 24 h collection in September resulted in the ^{14}C signatures of microbial respiration being more clearly separated from soil CO_2 efflux. In effect, a 14% increase in the incubation time resulted in better separation among end members, which likely reflects the rapid decay of root exudates (Hicks Pries et al. 2013). In applying the September microbial respiration to the July–August mixing model, there is likely some error that is associated with differing levels of rhizodeposition of organic matter. For example, Hicks Pries et al. (2013) used a 120 h preincubation period and found only a 0.01‰ difference for $\Delta^{14}C$ in heterotrophic respiration across different months and two different years for surface tundra soils. It is likely that as the preincubation period lengthens and the decomposition of rhizodeposits continues, the $\Delta^{14}C$ signature of microbial respiration will become more stable because it will more closely resemble the bulk SOM. It is also likely, however, that any priming effect will be missed with a longer preincubation period.

Soil CO_2 from deep within the soil profile can also add to the $\Delta^{14}C$ signature of surface soil CO_2 efflux (Schuur and Trumbore 2006), but in this study, we were unable to constrain this contribution because of low C yields on the microbial respiration from deep soils. Because the soil profile was heated from both below (20 cm depth) and above in this experiment, it is likely that deep soil CO_2 efflux was greater in the heated plots. Below 10 cm in the mineral soil of boreal forests, $\Delta^{14}CO_2$ indicates that the sources of CO_2 are predominately from before the bomb testing (Winston et al. 1997; Dioumaeva et al. 2003; Czimczik et al. 2006), suggesting that the more enriched $\Delta^{14}CO_2$ soil efflux estimates from the heated plots were diluted by greater amounts of deep, depleted $\Delta^{14}CO_2$ than in the control plots (Table 1). This might explain why the difference in $\Delta^{14}CO_2$ for microbial respiration was greater than the difference for soil CO_2 efflux (Table 2). Although the addition of deeper soil microbial respiration could also contribute to the change in $^{13}CO_2$ between sampling dates (Table 2), in this case, it is likely due to the lower overall soil CO_2 efflux in September, which allows for a greater exchange of atmospheric air with the soil profile's atmosphere (Schuur and Trumbore 2006).

Conclusions

Our results support the hypothesis that with warming, SOM will be lost from these soils as microbes do not appear to acclimate to warming or become C-limited, at least within the time frames of either the in situ warming or the laboratory experiment. This suggests that the greater soil CO_2 efflux in the heated plots relative to control plots could have been supported by microbial respiration and that the previous observations of lower soil CO_2 efflux in the air + soil heated treatments were most likely from the co-occurring reduction in fine root biomass (Bronson et al. 2008). An important remaining question is whether the past reduction in root biomass and, potentially, root turnover was responsible for the radiocarbon signature of microbial respiration that was nearer the bomb peak or whether a greater fraction of "older" C was being accessed under warm temperatures.

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