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Thawing permafrost increases old soil and autotrophic respiration in tundra: Partitioning ecosystem respiration using $\delta^{13}C$ and $\Delta^{14}C$

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Abstract

Ecosystem respiration (R_{eco}) is one of the largest terrestrial carbon (C) fluxes. The effect of climate change on R_{eco} depends on the responses of its autotrophic and heterotrophic components. How autotrophic and heterotrophic respiration sources respond to climate change is especially important in ecosystems underlain by permafrost. Permafrost ecosystems contain vast stores of soil C (1672 Pg) and are located in northern latitudes where climate change is accelerated. Warming will cause a positive feedback to climate change if heterotrophic respiration increases without corresponding increases in primary production. We quantified the response of autotrophic and heterotrophic respiration to permafrost thaw across the 2008 and 2009 growing seasons. We partitioned R_{eco} using $\Delta^{14}C$ and $\delta^{13}C$ into four sources–two autotrophic (above – and belowground plant structures) and two heterotrophic (young and old soil). We sampled the $\Delta^{14}C$ and $\delta^{13}C$ of sources using incubations and the $\Delta^{14}C$ and $\delta^{13}C$ of R_{eco} using field measurements. We then used a Bayesian mixing model to solve for the most likely contributions of each source to R_{eco}. Autotrophic respiration ranged from 40 to 70% of R_{eco} and was greatest at the height of the growing season. Old soil heterotrophic respiration ranged from 6 to 18% of R_{eco} and was greatest where permafrost thaw was deepest. Overall, growing season fluxes of autotrophic and old soil heterotrophic respiration increased as permafrost thaw deepened. Areas with greater thaw also had the greatest primary production. Warming in permafrost ecosystems therefore leads to increased plant and old soil respiration that is initially compensated by increased net primary productivity. However, barring large shifts in plant community composition, future increases in old soil respiration will likely outpace productivity, resulting in a positive feedback to climate change.

Keywords: autotrophic respiration, ecosystem respiration, heterotrophic respiration, partitioning, permafrost thaw, radiocarbon, seasonality, $\delta^{13}C$

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Introduction

Ecosystem respiration (R_{eco}) is the largest carbon (C) flux from the terrestrial biosphere to the atmosphere (Raich & Schlesinger, 1992). Thus, understanding the response of R_{eco} to climatic changes is critically important to making predictions about the C cycle on local, regional, and global scales. However, measured responses of R_{eco} to temperature increases have been highly variable (Davidson & Janssens, 2006) because R_{eco} is a combination of respiration by autotrophs and heterotrophs, which often respond differently to changes in climate (Borken *et al.*, 2006; Muhr & Borken, 2009; Gomez-Casanovas *et al.*, 2012). The relative responses of autotrophic (R_a) and heterotrophic respiration (R_h) to climatic changes affect the C balance of ecosystems: on short timescales, R_a is generally balanced by current production but R_h does not have to be. Their relative responses are particularly important in permafrost ecosystems, which have historically been C sinks (Hicks Pries *et al.*, 2012) and have the potential to cause a large positive feedback to climate change as they thaw (Schuur *et al.*, 2008).

Soils in the permafrost zone store 1672 Pg C, over twice as much C as the atmosphere currently holds (Schuur *et al.*, 2008), because frozen soil has protected organic C from decomposition. These permafrost soils are found mainly in high latitudes where up to 7 °C temperature increases are predicted over the next century (IPCC, 2007). In Alaska, some permafrost is already thawing downward at a rate of 0.1–0.9 m yr^{-1} (Osterkamp & Romanovsky, 1999; Osterkamp, 2007). As permafrost thaws, soil organic C is exposed to microbial degradation, increasing R_h (Goulden *et al.*, 1998; Schuur *et al.*, 2009). However, warmer, thawed soils can also increase biomass C storage and R_a by increasing nutrient availability and causing plant communities to shift to larger growth forms (e.g. shrubs; Schuur *et al.*, 2007).

With CO_2 flux measurements, researchers have shown permafrost thaw increases R_{eco} in tundra (Vogel

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et al., 2009) and peatlands (Dorrepaal et al., 2009). However, measuring fluxes alone does not reveal whether the increase is being driven by R_a or R_h. Increases in R_{eco} as a result of thaw may indicate different outcomes of an ecosystem's C cycle depending on which respiration source drives the change. For example, if R_h of old soil C is driving the increase, the system is losing C that had been stored for hundreds to thousands of years to the atmosphere, likely resulting in a positive feedback to climate change. If R_a is driving the increase, the system is either turning over newly photosynthesized C faster, a neutral or constrained positive feedback to climate change, or is fixing more C, a negative feedback to climate change. To help predict the strength of the permafrost thaw feedback, autotrophic and heterotrophic contributions to R_{eco} must be known.

Partitioning R_{eco} into its sources is necessary for a mechanistic understanding of how respiration responds to climate change, and many partitioning methods have been developed. Natural abundance $\delta^{13}C$ or $\Delta^{14}C$ is used for partitioning R_{eco} when their values differ among respiration sources. Isotope partitioning is less destructive than methods like trenching or girdling, which can change environmental conditions (e.g. Luan et al., 2011; Subke et al., 2011), and may cause less sampling artifacts than methods like excising roots from soil to measure fluxes separately (Kuzyakov, 2006; Yi et al., 2007). Many studies have used either $\delta^{13}C$ or $\Delta^{14}C$ to estimate source contributions to soil or ecosystem respiration (Ehleringer et al., 2000; Gaudinski et al., 2000; Trumbore, 2000; Ngao et al., 2005; Schuur et al., 2009). In permafrost ecosystems, deep soil contributions to R_{eco} have been estimated using $\delta^{13}C$ (Dorrepaal et al., 2009) and $\Delta^{14}C$ (Schuur et al., 2009). However, source contributions can be determined with increased accuracy using both carbon isotopes simultaneously (Phillips & Gregg, 2003).

Combining C isotopes is powerful because $\delta^{13}C$ and $\Delta^{14}C$ separate sources based on different principles–^{13}C via biological fractionation and water relations (Bowling et al., 2002) and ^{14}C via age (Trumbore, 2000). $\delta^{13}C$ differs among sources because many enzymatic processes, like C fixation by Rubisco, discriminate against the heavy isotope. $\delta^{13}C$ also varies among autotrophs due to different photosynthetic strategies, water relations, and CO_2 concentrations because the less C limited plants are, the more they discriminate against ^{13}C (Dawson et al., 2002). $\Delta^{14}C$ acts as a timestamp once isotopic fractionation effects have been corrected for because ^{14}C undergoes radioactive decay. Further separating sources based on age is the 1963 ^{14}C bomb peak, caused by atmospheric nuclear weapons testing, which causes C fixed in the past 50 years to have enriched $\Delta^{14}C$ (Levin & Hesshaimer, 2000). Despite the potential of dual isotope partitioning, both C isotopes have rarely been used together in natural abundance studies (Mayorga et al., 2005; Billett et al., 2007; Hardie et al., 2009).

The main objective of this study was to quantify the response of plant and microbial respiration to permafrost thaw and seasonality by utilizing $\delta^{13}C$ and $\Delta^{14}C$ to partition R_{eco} into four sources: aboveground plant structures (AG), belowground plant structures (BG), young soil (YS; 0–15 cm), and old soil (OS; 15–80 cm). Specific objectives were to: (1) Determine spatial and temporal variability in source and R_{eco} $\delta^{13}C$ and $\Delta^{14}C$, (2) Determine whether permafrost thaw increases the contribution of R_h from old soil C, (3) Determine how the contributions of R_a and its components, AG and BG plant respiration, change seasonally (May through September) and with thaw. This mechanistic approach to understanding how the components of R_{eco} respond to seasonality and permafrost thaw will increase our understanding of permafrost ecosystems' responses to climate change. The resulting R_{eco} partitioning estimates can be used to parameterize C cycling models, increasing our capacity to predict the future state of the Earth system.

Materials and methods

Site description

Our site is tundra located near Eight Mile Lake (EML; 63°52′ 42″ N, 149°15′12″ W) in Healy, Alaska. The vegetation is moist acidic tussock tundra underlain by soils that have permafrost within a meter of the surface (Gelisols). The soils consist of about 0.5 m of organic soil on top of mineral soil that is a mixture of loess deposits and glacial till (Vogel et al., 2009). The water table is usually 15–25 cm below the soil surface (Trucco et al., 2012), and so methane production out of the ecosystem is negligible (E.A.G. Schuur and C. Trucco, unpublished data). Permafrost temperatures in this region are around −1 °C, and therefore the permafrost is susceptible to thaw (Osterkamp & Romanovsky, 1999). Within the study site, some areas have undergone active layer thickening and thermokarst formation due to permafrost thaw (Vogel et al., 2009). Thaw has been documented for the past two decades at this site but likely began earlier (Osterkamp et al., 2009). This site has had ongoing monitoring of soil temperature, active layer depth, water table depth, and CO_2 fluxes since 2004 (Schuur et al., 2009; Vogel et al., 2009; Trucco et al., 2012).

Ecosystem respiration

To measure the $\delta^{13}C$ and $\Delta^{14}C$ of R_{eco}, we installed 12 permanent PVC collars (25.4 cm diameter × 10 cm deep) 8 cm into the soil across 1 km of the study site. For sampling R_{eco}, 10 L dark chambers (13 cm high) were fit onto the collars over the soil and encompassing the aboveground plant biomass. Ecosystem respiration $\delta^{13}C$ and $\Delta^{14}C$ was sampled over a week in June, July, and August 2008 and May, July, and

September 2009. Sampling occurred from 06.00 to 11.00 hours to limit diurnal variation and ensure calm conditions. After each set of R_{eco} samples was taken, thaw depth was measured twice adjacent to and once within each collar using a metal probe (2 mm in diameter) pushed into the ground until it hit resistance. Active layer depth (AL), the thaw depth at the end of the growing season and a measure of the permafrost thaw extent, was measured in September and used to stratify collars into three categories for the partitioning model.

We used Keeling plots to measure the $\delta^{13}C$ of R_{eco} wherein we took air samples from the chamber every 2–3 min while pCO_2 increased for a total of seven samples (Keeling, 1958). The air samples were collected into exetainers (septa-topped vials; Labco Limited, Lampeter, UK) in line with the chamber, a pump, and an infrared gas analyzer (IRGA; LI-820, LI-COR, Lincoln, Nebraska, USA). We recorded the pCO_2 from the IRGA when each exetainer was removed from the line. The exetainers were sent back to the University of Florida to be run using a GasBench II coupled with a Finnigan Delta Plus XL stable isotope ratio mass spectrometer (precision ± 0.2‰, n = 215). Their holding time was no longer than 10 days, and the majority of the samples were run within 7 days (Mortazavi & Chanton, 2004; Midwood *et al*., 2006). Standard samples of similar pCO_2 and a $\delta^{13}C$ of −10.46‰ (Oztech Trading Corporation, Safford, AZ, USA) were sent with each batch of exetainers to correct for changes in $\delta^{13}C$ due to travel and storage. The $\delta^{13}C$ and $1/pCO_2$ of each collar's air samples were fit with a linear regression to find the intercept, which was the $\delta^{13}C$ of R_{eco}.

For measuring $\Delta^{14}C$, we first removed as much atmospheric CO_2 from the chamber as possible by pumping chamber air through soda lime for 45 min while maintaining ambient pCO_2 (Schuur & Trumbore, 2006). After scrubbing, we pumped chamber air through a zeolite molecular sieve (Alltech 13X; Alltech Associates, Deerfield, IL, USA) trap that quantitatively adsorbs CO_2 for 15 min (Hardie *et al*., 2005). By maintaining pCO_2 around ambient levels, we removed CO_2 from the chamber at roughly the same rate it was fluxing out of the ecosystem and avoided an unnatural CO_2 concentration gradient. Sampling only occurred under calm wind conditions to minimize atmospheric CO_2 entering chambers through the soil. The molecular sieve traps were baked at 625 °C to desorb CO_2 (Bauer *et al*., 1992), which was purified using liquid N_2 on a vacuum line and reduced to graphite by Fe reduction in H_2 (Vogel *et al*., 1987). The graphite was sent to the UC Irvine W.M. Keck carbon cycle accelerator mass spectrometry (AMS) Laboratory for $\Delta^{14}C$ analysis (precision ± 2.3‰, n = 102). $\Delta^{14}C$ data are reported at the same $\delta^{13}C$ value to correct for mass-dependent fractionation effects. $\Delta^{14}C$ data were corrected for the atmospheric CO_2 remaining in the chambers using $\delta^{13}C$ data from each chamber in a 2-pool (atmospheric and R_{eco}) mixing model as in Schuur & Trumbore (2006).

Autotrophic respiration

Short-term incubations were used to measure the $\delta^{13}C$ and $\Delta^{14}C$ of AG and BG R_a. Autotrophic respiration $\delta^{13}C$ was measured from nine randomly chosen sites in June and August 2008 and in May, July, and August 2009. Aboveground and BG R_a $\Delta^{14}C$ was measured in July 2008 and in May and July 2009 from three sites. To measure the $\delta^{13}C$ and $\Delta^{14}C$ of R_a, we collected plants from a randomly placed 20 cm^2 quadrat; we clipped all the aboveground material (including lichens and mosses) to the soil surface, and collected all live roots and rhizomes (>2 mm in diameter) from the thawed soil. Aboveground samples were immediately placed into foil-covered mason jars (0.24 L) whereas belowground samples were rinsed twice in water to remove soil particles and shaken dry before being put into foil-covered mason jars. We incubated plants as soon as possible after clipping (within 5 min for AG and 30 min for BG), as the $\delta^{13}C$ of excised root respiration can change slightly after 40 min (Midwood *et al*., 2006). Air from the sealed mason jars was then pumped through soda lime for 5 min at 1 L min^{-1} to remove CO_2 from the headspace before starting a 5–10 min or 4 h incubation (until pCO_2 reached the range needed for $\delta^{13}C$ and $\Delta^{14}C$ analyses, respectively). At the end of the incubation, headspace air was pumped into a Helium-flushed exetainer for $\delta^{13}C$ analysis or a molecular sieve trap for $\Delta^{14}C$ analysis.

Heterotrophic respiration

To measure the $\delta^{13}C$ and $\Delta^{14}C$ of R_h, nine surface soil cores (0–25 cm) were randomly sampled in May, July, and August 2009, and 12 deep soil cores (25+ cm) were sampled in May 2009. We sampled surface soil more often to test if respired $\delta^{13}C$ and $\Delta^{14}C$ change throughout the growing season due to rhizosphere processes. We manually cored surface soils using a serrated knife to the depth of thaw or 25 cm, whichever came first, and sectioned them into 0–5 cm, 5–15 cm, and 15–25 cm depths. For deeper soils (25+ cm), we used a Tanaka TIA-340 permafrost drill with carbide bits to drill through frozen soil down to about 80 cm, below which gravel impeded coring. Surface soil incubations were started the day of sampling, whereas deep soils were kept frozen until February 2010 when they were thawed, cut into 10 cm sections, and incubated. Roots (>1 mm in diameter) were removed from all soil sections before soils were put into mason jars (0.95 L) for incubations. Care was taken to minimize disturbance to the soil structure, which was a minor problem for mineral soils as they had very few roots. Surface soils sat at room temperature for 5 days before $\delta^{13}C$ and $\Delta^{14}C$ sampling to ensure the majority of labile C from small roots and root exudates decomposed and would not affect the R_h isotope ratio. As in previous studies (e.g. Schuur & Trumbore, 2006), we assumed that the CO_2 respired from soils after 5 days was dominated by the heterotrophic flux and therefore included fast-cycling rhizosphere C in the autotrophic flux. Soils were incubated at field moisture under aerobic conditions. Deep soils sat at room temperature for 10 days to allow microbial populations to stabilize after thaw. During the 2 days preceding isotopic sample collection, three short-term (3 h) incubations were performed to measure rates of R_h flux. Samples were run on an IRGA (LI-820) connected to an injection loop to measure pCO_2. The $\delta^{13}C$ and $\Delta^{14}C$ sample collection was performed as for R_a except that incubation times were based on the time it took for 1.5 mg C to accumulate in the headspace, which ranged from 12 to 72 h.

Heterotrophic respiration was split into two sources, YS (0–15 cm) and OS (15–80 cm), based on soil $\Delta^{14}C$ values; YS included the top of the soil profile that contained post 'bomb peak' $\Delta^{14}C$ (Hicks Pries *et al.*, 2012). To calculate $\delta^{13}C$ and $\Delta^{14}C$ of YS and OS, we weighted the isotopic signatures of each incubated depth by its CO_2 flux per g C, corrected for each section's average bulk density, %carbon, and average monthly field temperature using the Q_{10} (Table S1; Schuur & Trumbore, 2006). We calculated this weighted average for each replicate core separately and then averaged all cores to obtain monthly mean $\delta^{13}C$ and $\Delta^{14}C$ for YS and OS. Before averaging, $\delta^{13}C$ values were corrected for the incubation temperature shift as in Dorrepaal *et al.* (2009). This correction was needed because $\delta^{13}C$ is depleted by 0.12–0.35‰ for each 1 °C temperature rise (Andrews *et al.*, 2000; Biasi *et al.*, 2005) and our soils were incubated at temperatures warmer than field conditions. The same $\Delta^{14}C$ values from the 2009 cores were used to calculate 2008 R_h end members. The effect of radioactive decay (<1‰ yr^{-1}) and the addition of new OC with slightly depleted values (due to the Suess effect and biosphere mixing) on R_h $\Delta^{14}C$ are likely below the 2–3‰ precision error of the AMS (Schuur *et al.*, 2009).

A subset of 12 soil samples from various soil cores and depths were used to calculate Q_{10} and the $\delta^{13}C$ temperature correction. We removed roots, homogenized, and split each sample into two jars–one was incubated for 10 days at 2.5 °C and the other at 12.5 °C. The average CO_2 flux from three short-term incubations was used to calculate each sample's Q_{10}. For $\delta^{13}C$, 12 mL samples from the headspace were injected into vacuumed exetainers after a 6 h incubation preceded by scrubbing CO_2 from the headspace. The average Q_{10} of these soils was 2.5 and the average $\delta^{13}C$ shift was −0.157‰ per 1 °C. To calculate the adjusted $\delta^{13}C$, we used the following equation:

$$^{13}C_{adj} = {}^{13}C_{inc} + 0.157 * (T_{inc} - T_{field})$$

where the $^{13}C_{inc}$ is the $\delta^{13}C$ from incubating a soil depth section, T_{inc} is the temperature of the incubation, and T_{field} is the *in situ* field temperature of that soil depth from soil temperature sensors (Table S1; see Trucco *et al.*, 2012 for sensor details).

Data analysis and partitioning model

Ecosystem respiration was partitioned into AG, BG, YS, and OS using SIAR (stable isotope analysis in R; Parnell *et al.*, 2010). For partitioning, the 12 R_{eco} collars were stratified into three categories based AL depth because SIAR gives more robust estimates when fitting parameters to a group of R_{eco} values than to a single R_{eco} value (Inger *et al.*, 2010). Six collars were classified as shallow AL (46–55 cm), three as intermediate AL (62–69 cm), and three as deep AL (80–103 cm). Active layer depth is a good indicator of permafrost thaw extent; ALs are deeper where the soil surface has subsided due to ground ice thaw (Osterkamp *et al.*, 2009). Partitioning was performed separately for each AL category and each month sampled. The SIAR method uses Markov chain Monte Carlo to find possible solutions to this set of three equations:

$$^{13}C_{Ecosystem} = f_{AG} * (^{13}C_{AG}) + f_{BG} * (^{13}C_{BG}) + f_{YS} * (^{13}C_{YS}) + f_{OS} * (^{13}C_{OS})$$

$$^{14}C_{Ecosystem} = f_{AG} * (^{14}C_{AG}) + f_{BG} * (^{14}C_{BG}) + f_{YS} * (^{14}C_{YS}) + f_{OS} * (^{14}C_{OS})$$

$$1 = f_{AG} + f_{BG} + f_{YS} + f_{OS}$$

where the unknowns are f, each source's proportional contribution to R_{eco}, and the $\delta^{13}C$ and $\Delta^{14}C$ of each source and R_{eco} have known distributions. The input data for this model include the mean and standard deviation of all source isotopic values (Tables 2 and 3) and the individual isotopic values of R_{eco} collars measured for each AL category (Table S2). For July 2008 partitioning, R_a $\delta^{13}C$ was an average of June and August 2008 values because July R_a $\delta^{13}C$ was not sampled. The results of the model are probability density distributions of each source's f. While SIAR uses a Bayesian framework, we used uninformative priors because previous partitioning results are limited.

For statistical analyses of source isotopes, we used one- and two-way analyses of variance (ANOVA's) in JMP (SAS, Cary, NC, USA) with source type (R_a only) and month as main effects, and core as a random effect (YS and OS only). For R_{eco} isotopes and thaw depth, repeated measures ANOVA's were used with AL category and month as main effects and collar as a random effect. Analyses were done for 2008 and 2009 separately. To analyze how isotopes varied in the soil profile, we used a one-way ANOVA with depth as the main effect and soil core as a random effect for deep soil cores and a two-way ANOVA with month as an additional main effect for surface soil cores. One-way ANOVA's were used to compare source contributions among months or AL categories. We investigated the relationships between mean thaw depth of the AL categories and the mean source contributions (f) using linear regressions with category as a random effect in R (R Development Core Team, 2012). Source contributions were logit transformed before analyses. All residuals were checked for normality and homogeneity of variances to ensure the assumptions of ANOVA and regressions were met.

Respiration fluxes

Ecosystem respiration fluxes were sampled with static and auto chambers throughout the 2008 and 2009 growing seasons from plots adjacent to the isotopic sampling collars. The respiration measurements were part of a C balance study and are described in Vogel *et al.* (2009) and Trucco *et al.* (2012). We used the same data as presented in Trucco *et al.* (2012), except that we averaged the respiration plots into the three AL depth categories instead of by site, to pair with our partitioning estimates (Table S3). To estimate growing season respiration from each source, we combined 2008 and 2009 source estimates so we had the sources' proportional contributions for each month of the growing season (May through September; we averaged 2008 and 2009 July values) and multiplied the proportions by their corresponding mean flux (for each month and AL category). We then summed the flux of each source across all

growing season months by AL category. This first approximation of a growing season flux includes some uncertainties because our isotopic sampling reflected only the big changes over the growing season. However, sampling respiration at low frequencies has been shown to accurately capture seasonal variation (Savage & Davidson 2003). Logistical constraints prevented frequent isotopic sampling, so we did not focus on shorter timescales.

Results

Ecosystem respiration

Ecosystem respiration collars were stratified into three categories based on their AL depth. Among these AL categories, thaw depths differed significantly throughout the growing season in 2008 and 2009 (Table 1; repeated measures ANOVAs, $P < 0.005$). Thaw depths significantly increased throughout each growing season (Table 1; repeated measures ANOVAs, $P < 0.0001$) and differences among categories became larger throughout the growing season in both years (category x month interaction, $P < 0.028$).

In both 2008 and 2009, R_{eco} $\delta^{13}C$ did not differ among AL categories (Table 1; repeated measures ANOVA, $P > 0.60$), but did differ among months ($P < 0.056$). There were significant AL category differences in R_{eco} $\Delta^{14}C$. Shallow AL R_{eco} was significantly more enriched in $\Delta^{14}C$ than deep AL R_{eco} in 2009 (Fig. 1, repeated measures ANOVA, $P = 0.035$), but the effect was marginally significant in 2008 ($P = 0.082$). Early growing season R_{eco} was generally more enriched in $\Delta^{14}C$ than mid and late season R_{eco}, a difference which was significant in 2008 (Fig. 1, repeated measures ANOVA, $P = 0.0007$) and marginally significant in 2009 ($P = 0.064$). Across all categories and years, there is a negative linear relationship between thaw depth and R_{eco} $\Delta^{14}C$ ($R^2 = 0.37$, $P < 0.0001$), but no relationship between thaw depth and R_{eco} $\delta^{13}C$ ($R^2 = 0.01$, $P = 0.44$).

Source respiration

Aboveground R_a was generally 2‰ more enriched in $\delta^{13}C$ than BG R_a (Table 2; two-way ANOVAs, $P < 0.0001$). Early growing season R_a $\delta^{13}C$ was more enriched than later growing season R_a in both 2008 and 2009 (Table 2; two-way ANOVAs, $P < 0.0001$). In 2009, AG R_a was 2–3‰ more enriched in $\delta^{13}C$ in July than in May or September (Table 2; two-way ANOVA, type x month interaction, $P = 0.043$). Autotrophic respiration $\Delta^{14}C$ ranged from 44.2 to 49.7‰, but there were no significant differences among AG and BG structures or month sampled (Table 2; two-way ANOVAs, $P > 0.4$).

Heterotrophic respiration $\delta^{13}C$ became more enriched with depth, $\Delta^{14}C$ became more depleted with soil depth, and variability of both $\delta^{13}C$ and $\Delta^{14}C$ increased with depth (Fig. 2). Statistical analyses of R_h were split into surface cores (0–25 cm) and deep cores (25+ cm) due to different sampling frequencies (see methods). Both $\delta^{13}C$ and $\Delta^{14}C$ differed significantly with depth in surface (Fig. 2; two-way ANOVA, $P < 0.021$) and deep cores (Fig. 2; one-way ANOVA, $P < 0.0073$). Correcting R_h $\delta^{13}C$ from each depth section for *in situ* soil temperatures caused the top 15 cm of soil to be more $\delta^{13}C$ depleted in July than in other months due to warmer soil temperatures (Fig. 2b). On average, the R_h $\delta^{13}C$ temperature correction caused 2‰ and 3.5‰ enrichments relative to measured values of YS and OS, respectively.

Heterotrophic respiration sources, YS and OS (calculated from averaged core sections; see methods), differed greatly in their $\Delta^{14}C$ and $\delta^{13}C$ (Table 3). Young soil was more depleted in $\delta^{13}C$ (−25.7 to −24.4‰) but more enriched in $\Delta^{14}C$ (76‰) than OS, which had $\delta^{13}C$ around −22.8‰ and $\Delta^{14}C$ around −30‰. Young soil $\delta^{13}C$ differed by month in 2009 but not 2008 (Table 3; one-way ANOVAs, $P > 0.0001$ and $P = 0.41$, respectively). Young soil $\Delta^{14}C$ did not differ by month in either 2008 or 2009 (Table 3; one-way ANOVAs, $P > 0.99$). The $\Delta^{14}C$ and $\delta^{13}C$

Table 1 Mean (±SE) thaw depth and $\delta^{13}C$ of ecosystem respiration within the active layer categories throughout the growing season in 2008 and 2009. Significance was tested separately for each year with two-way ANOVA's ($\alpha = 0.05$). Categories that do not share a capital letter, months that do not share a lowercase letter, and individual means that do not share a number are significantly different. $\delta^{13}C$ was significantly different among months, but not among active layer categories

	Thaw (cm)			$\delta^{13}C$ (‰)		
2008	*June*^{a}	*July*^{b}	*August*^{c}	*June*^{a}	*July*^{b}	*August*^{ab}
Shallow^{A}	24.4 ± 1.2^{1}	36.1 ± 1.1^{2,3}	49.5 ± 1.1^{4}	−24.4 ± 0.8	−22.8 ± 0.3	−23.3 ± 0.3
Intermediate^{B}	29.2 ± 1.2^{1,2}	46.0 ± 3.9^{3,4}	67.8 ± 2.8^{5}	−23.8 ± 0.4	−22.6 ± 0.4	−23.3 ± 0.5
Deep^{C}	43.5 ± 2.4^{2,3,4}	68.0 ± 6.0^{5}	86.0 ± 7.6^{6}	−23.9 ± 0.5	−23.1 ± 0.4	−23.1 ± 0.2
2009	*May*^{a}	*July*^{b}	*September*^{c}	*May*^{ab}	*July*^{a}	*September*^{b}
Shallow^{A}	9.82 ± .36^{1}	32.9 ± .71^{2}	50.8 ± 1.4^{3}	−23.1 ± 0.6	−22.6 ± 0.2	−23.4 ± 0.2
Intermediate^{AB}	10.7 ± .60^{1}	41.9 ± 2.1^{2,3}	65.1 ± 2.0^{4}	−22.6 ± 0.6	−22.3 ± 0.2	−24.8 ± 1.6
Deep^{B}	17.9 ± 5.7^{1}	56.9 ± 4.8^{3,4}	88.3 ± 7.2^{5}	−23.8 ± 0.2	−22.8 ± 1.0	−24.0 ± 0.5

of the other R_h source, OS, did not differ by month in either year (Table 3; one-way ANOVAs, $P > 0.69$).

Partitioning ecosystem respiration

The greatest contributions to R_{eco} came from AG R_a, whose contributions ranged from 16 to 48% in 2008 and 26 to 43% in 2009 (Fig. 3). Belowground R_a contributions ranged from 17 to 34% in 2008 and 15 to 32% in 2009 (Fig. 3). Results reported in this section are the means of the posterior probability density distributions for each source's proportional contribution to R_{eco}. Combining mean contributions across years and AL categories, AG R_a had the greatest contributions at the height of the growing season, July and August, whereas BG R_a did not significantly differ throughout the growing season (Fig. 4; two-way ANOVA, month x type interaction, $P < 0.0001$). We did not use July 2008 partitioning results in this analysis because of the uncertainty associated with estimating the July 2008 AG and BG source isotopes. The greatest proportional contributions of AG R_a at the height of the growing season corresponded to the lowest proportional contributions of BG R_a while AG and BG R_a were similar during months of seasonal transition, May and September. Combining all AL categories, months, and years, mean total R_a (AG plus BG) contributions to R_{eco} ranged from 40 to 70% and increased with increasing thaw depth (Fig. 5a; regression, $n = 18$, $R^2 = 0.25$, $P = 0.039$). One-way ANOVA's were performed to tease apart the effects of AL category and month, as both contribute to increasing thaw depths. Autotrophic respiration did not differ significantly among AL categories (one-way ANOVA, $P = 0.37$), but did vary significantly among months (one-way ANOVA, $P = 0.0047$) with the greatest contributions occurring in July and August, implying the relationship between R_a and thaw depth was driven by seasonal differences.

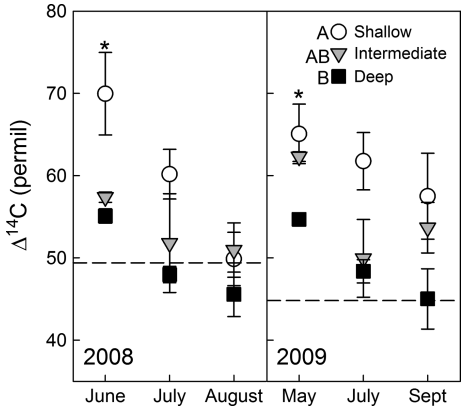

Fig. 1 Mean R_{eco} $\Delta^{14}C$ for all active layer (AL) categories in all months sampled (error bars = SE). In both years, early growing season R_{eco} was more enriched in $\Delta^{14}C$ than later growing season R_{eco} (indicated by asterisks, $\alpha = 0.06$). Letters not shared indicate significant differences among AL categories, which were only marginally different in 2008 ($\alpha = 0.08$). The dashed line is the $\Delta^{14}C$ of the atmosphere in each year.

For heterotrophic contributions to R_{eco}, YS had greater contributions than OS (Fig. 3). Young soil contributions ranged from 20 to 53% in 2008 and 20 to 41% in 2009. Young soil generally contributed more to R_{eco} where the AL was shallow. Old soil contributions ranged from 6 to 17% in 2008 and 8 to 18% in 2009. The greatest contributions from OS occurred in areas with intermediate and deep AL's. Combining all AL categories, months, and years (except May 2009 outliers), OS contributions increased with increasing thaw depth (Fig. 5b; regression, $n = 16$, $R^2 = 0.52$, $P = 0.025$). Old soil respiration did not differ significantly among months (one-way ANOVA, $P = 0.43$), but did differ significantly among AL categories (one-way ANOVA, $P = 0.0022$) with shallow AL having the smallest OS contributions. These results imply that the relationship between OS and thaw depth was driven by AL category and not seasonality. The relative contributions of autotrophic and heterotrophic respiration to R_{eco} can be compared using a ratio. Combining all AL categories, months, and years, R_a:R_h increased with increasing thaw depth (Fig 5c; regression, $n = 18$, $R^2 = 0.23$, $P = 0.047$).

We performed sensitivity analyses to test how model estimates responded to uncertainty in source isotopic

Table 2 Mean (±SE) $\delta^{13}C$ and $\Delta^{14}C$ of aboveground and belowground plant respiration during the 2008 and 2009 growing season. Significance was tested separately for each year with two-way ANOVA's. Asterisks represent a significant difference between aboveground and belowground R_a $\delta^{13}C$ only ($\alpha = 0.05$). Months that do not share a lowercase letter and individual means that do not share a number are significantly different in $\delta^{13}C$. There were no $\Delta^{14}C$ significant differences. NS stands for not sampled

	$\delta^{13}C$ (‰)	$\Delta^{14}C$ (‰)	$\delta^{13}C$ (‰)	$\Delta^{14}C$ (‰)	$\delta^{13}C$ (‰)	$\Delta^{14}C$ (‰)
2008	*June*[a]		*July*		*August*[b]	
Aboveground*	−20.5 ± 0.3	NS	NS	44.2 ± 1.7	−22.7 ± 0.3	NS
Belowground	−23.1 ± 0.6	NS	NS	48.5 ± 1.8	−25.8 ± 0.3	NS
2009	*May*[a]		*July*[a]		*September*[b]	
Aboveground*	−22.2 ± 0.3[1]	48.1 ± 2.0	−20.3 ± 0.4[2]	48.8 ± 2.8	−23.2 ± 0.5[1,3]	NS
Belowground	−24.4 ± 0.5[3,4]	49.1 ± 0.4	−24.5 ± 0.2[3,4]	49.7 ± 2.4	−25.8 ± 0.4[4]	NS

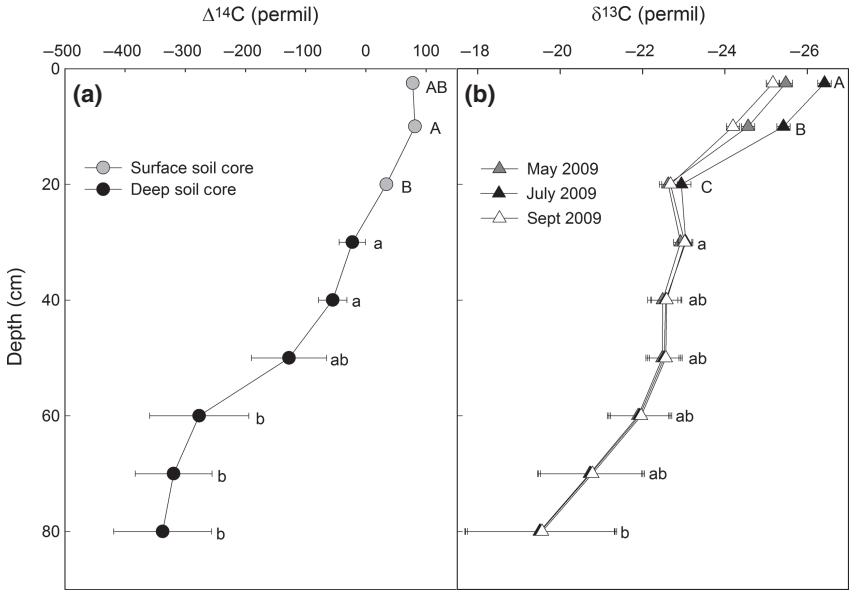

Fig. 2 Mean $\Delta^{14}C$ and $\delta^{13}C$ (error bars = SE) of R_h by depth. Statistical analyses were split into surface soil cores (capital letters, sampled in May, July, and September 2009) and deep soil cores (lowercase letters, sampled in May 2009 only). Depths that do not share a letter are significantly different ($\alpha = 0.05$). For $\delta^{13}C$, laboratory soil respiration values had to be corrected for *in situ* soil temperatures for each month we partitioned (see methods). The 2009 corrected data are shown here.

Table 3 Mean (±SE) $\delta^{13}C$ and $\Delta^{14}C$ of young and old soil respiration. Significance was tested separately for each year and each source with one-way ANOVA's. The only significant difference was for young soil $\delta^{13}C$ in 2009 ($\alpha = 0.05$, indicated with letters not shared)

	$\delta^{13}C$ (‰)	$\Delta^{14}C$ (‰)	$\delta^{13}C$ (‰)	$\Delta^{14}C$ (‰)	$\delta^{13}C$ (‰)	$\Delta^{14}C$ (‰)
2008	*June*		*July*		*August*	
Young Soil	−24.7 ± 0.2	76.4 ± 4.2	−25.0 ± 0.2	76.4 ± 4.2	−25.0 ± 0.2	76.4 ± 4.2
Old Soil	−22.7 ± 0.3	−30.5 ± 38	−22.8 ± 0.2	−26.8 ± 35	−23.0 ± 0.2	−26.3 ± 35
2009	*May*		*July*		*September*	
Young Soil	-24.6 ± 0.2^{ab}	76.4 ± 4.0	-25.7 ± 0.2^{a}	76.5 ± 4.1	-24.4 ± 0.2^{b}	76.5 ± 4.2
Old Soil	−22.6 ± 0.3	−30.7 ± 37	−22.9 ± 0.3	−26.3 ± 34	−22.8 ± 0.3	−30.3 ± 37

values (Table S4). Changing OS $\Delta^{14}C$ by one standard deviation in either direction shifted OS's mean contributions up to five percentage points and all other source contributions less than three percentage points. Using uncorrected $\delta^{13}C$ soil values (see methods) resulted in 3–10 percentage point changes in contributions. Shifting the soil $\delta^{13}C$ by 1‰ resulted in zero to six percentage point changes. Relationships between source contributions and month or thaw depth only changed slightly as a result of these changes and maintained their significance. Our results are therefore qualitatively robust to source isotope uncertainty.

Respiration fluxes

Areas with deep AL's generally had greater growing season and monthly respiration rates than areas with shallow AL's (Table S3). Growing season C flux from AG and BG R_a and OS R_h were greatest in the deep AL category (Fig. 6). This larger growing season C flux from R_a and OS reflect both the larger contributions of R_a and OS to R_{eco} and the greater R_{eco} flux rates where the AL was deepest.

Discussion

Both autotrophic and old soil heterotrophic respiration increased with permafrost thaw. We were able to measure the crucial loss of old soil C because we used a dual isotope approach and explicitly measured the isotopic value of all sources. This method allowed us to more accurately partition R_{eco} into more sources than past studies. We even detected seasonal differences in aboveground and belowground R_a contributions to R_{eco}. Taken together, the thaw-induced increases in R_a and old soil R_h can have different C balance outcomes depending on the response of primary production. If the magnitude of C input (net primary productivity)

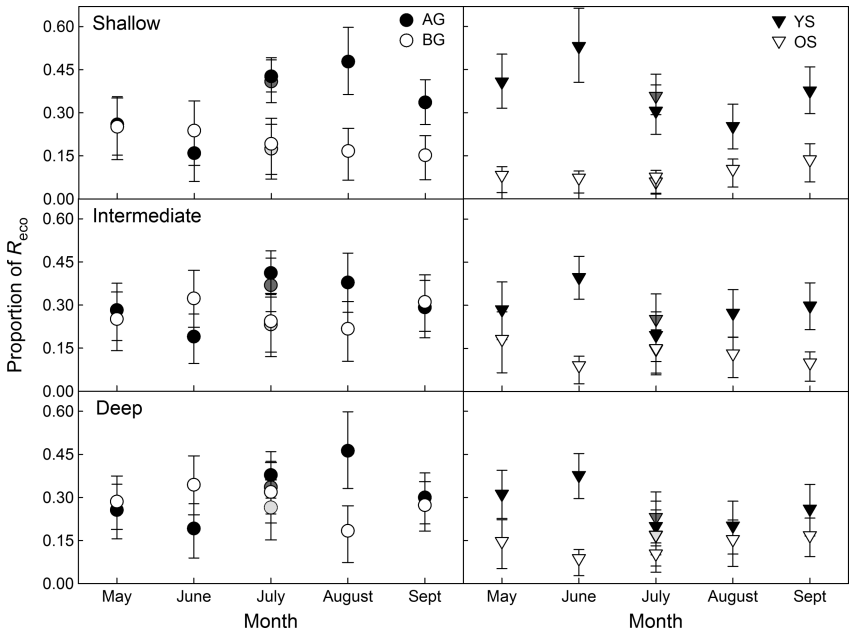

Fig. 3 Percent contributions of sources to R_{eco} by month for each active layer category. Autotrophic sources are on the left (AG is for aboveground and BG is for belowground plant structures) and heterotrophic sources are on the right (YS is for young soil and OS is for old soil). These contributions are the mean *f* estimates from SIAR, and the error bars are the 25 and 75 percentiles of the estimates. Both 2008 (June, July, and August) and 2009 (May, July, and September) data are shown. Please note that July 2008 data (gray symbols) were partitioned with estimated AG and BG source $\delta^{13}C$ (see methods).

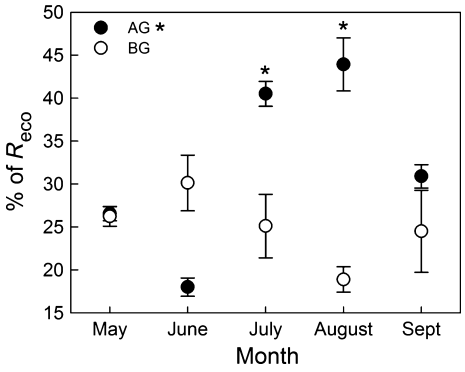

Fig. 4 SIAR estimates for contributions of aboveground R_{auto} (AG, black symbols) and belowground R_{auto} (BG, open symbols) averaged over active layer category and year for each month sampled. The asterisks show significant differences ($\alpha = 0.05$). AG contributions were greater than BG contributions, and AG contributions were greatest in July and August. BG contributions did not vary by month.

response to permafrost thaw is not greater than the C output (respiration) response, thaw will have caused the system to become a C source.

Variability of autotrophic source isotopes

Our first objective was to sample source isotopes temporally and spatially, which will elucidate how to best sample source $\delta^{13}C$ and $\Delta^{14}C$ in future partitioning studies. Previous studies have made untested assumptions, such as assuming R_a has the same $\Delta^{14}C$ as the atmosphere (e.g. Subke *et al.*, 2011) or assuming R_h has the same isotopic values as solid organic C (e.g. Dutta *et al.*, 2006). Isotopic variation in R_a was driven by plant structure and temporal changes in soil moisture. Respired $\delta^{13}C$ from BG plant structures was consistently 2‰ depleted compared with respiration from AG plant structures as observed in previous studies (e.g. Badeck *et al.*, 2005; Klumpp *et al.*, 2005). Belowground respiration is likely depleted relative to AG respiration because root metabolism is fueled by depleted substrates, like lipids, whereas leaf metabolism is fueled by enriched substrates, like sugars (Bowling *et al.*, 2008). In contrast, AG and BG respiration $\Delta^{14}C$ did not differ indicating that they were respiring substrates of similar ages. In 2009, both AG and BG respiration were about 4‰ more enriched in $\Delta^{14}C$ relative to the atmosphere (≈1 year older), indicating plants were using a mixture of stored carbohydrates and recently fixed photosynthates for respiration (Czimczik *et al.*, 2006; Schuur & Trumbore, 2006). As R_a $\Delta^{14}C$ enrichment has been previously seen in other perennial plants (Czimczik *et al.*, 2006; Schuur & Trumbore, 2006), only annual plants should be assumed to respire CO_2 with the same $\Delta^{14}C$ as the atmosphere.

Autotrophic respiration $\delta^{13}C$ varied temporally across the growing season in both years, but R_a $\Delta^{14}C$

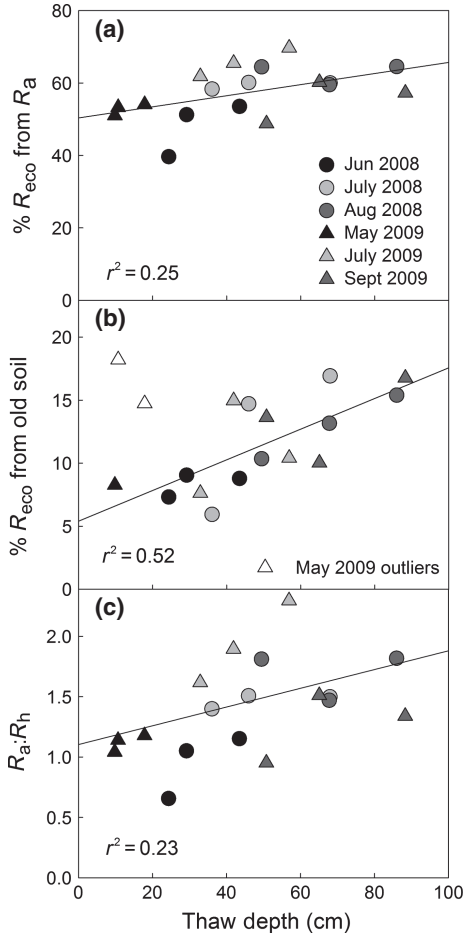

Fig. 5 Autotrophic respiration contributions to R_{eco} (a), old soil respiration contributions to R_{eco} (b), and the autotrophic to heterotrophic respiration ratio (c) with thaw depth for all active layer (AL) categories and months. Note that for (a) and (b), the contributions are the means of the probability density distributions that were a result of running the SIAR model for each AL category. Ratios greater than one indicate autotrophic respiration dominates. The r^2 values are from linear regressions (α = 0.05). For (b), the regression was performed without the May 2009 outliers (see text for details).

did not. Temporal variation in $\delta^{13}C$ was likely due to plant/water relations wherein C_3 plants become more $\delta^{13}C$ enriched under dry conditions due to limitations on stomatal conductance (McDowell *et al*., 2004; Bowling *et al*., 2008). In July 2009, when the study site experienced below-average rainfall, the $\delta^{13}C$ of AG R_a was 2–3‰ enriched compared with the relatively wetter months of May and September. In August 2008, after a wet July, both AG and BG R_a were 2‰ depleted compared with June, when rainfall was normal. Unlike $\delta^{13}C$, R_a $\Delta^{14}C$ did not vary temporally, indicating atmospheric ^{14}C was well mixed and that plants used a similar amount of stored C during the growing season, reinforcing results in forests (Cisneros-Dozal *et al*., 2006; Czimczik *et al*., 2006). Due to temporal variability, when partitioning R_a $\delta^{13}C$ should be measured at the same time as R_{eco} $\delta^{13}C$ and potential differences in isotopic values before and after precipitation events should be considered. In contrast, sampling R_a $\Delta^{14}C$ once a growing season is sufficient.

Variability of heterotrophic source isotopes

Carbon isotopes of R_h mainly varied with depth in the soil profile. In general, $\Delta^{14}C$ became depleted with depth, so the deeper the soil, the older the organic C respired. The exception to the depletion trend was in the top 15 cm of soil where $\Delta^{14}C$ became more enriched than the atmospheric due to respiration of 'bomb' peak C as previously measured in black spruce forests (Schuur & Trumbore, 2006). While R_h $\Delta^{14}C$ became depleted with depth, R_h $\delta^{13}C$ became enriched up to 6‰ as seen in numerous other studies (Ehleringer *et al*., 2000; Högberg *et al*., 2005; Bostrom *et al*., 2007). This enrichment may be due, in part, to the Suess effect in which fossil fuel burning has caused the atmosphere to become depleted in $\delta^{13}C$. This effect causes surface soils (formed in the past 150 years) to be about 1.5‰ more depleted than deeper soils (Ehleringer *et al*., 2000; Högberg *et al*., 2005). The rest of the 6‰ enrichment may be explained by increasing proportions of microbial-derived enriched C with depth relative to depleted plant-derived C (Ehleringer *et al*., 2000; Högberg *et al*., 2005; Bostrom *et al*., 2007). Both $\delta^{13}C$ and $\Delta^{14}C$ of R_h were more variable in deep soils than in surface soils. Respired CO_2 from the top 5 cm was all less than 20 years old whereas respired CO_2 from soil 80 cm deep ranged from 1500 to 7000 years old. This variability could be a result of cryoturbation (i.e. soil mixing caused by freeze/thaw cycles; Hicks Pries *et al*., 2012) or variable C accumulation rates. Respired CO_2 from most depth sections was considerably younger and more $\delta^{13}C$ enriched than bulk organic C (Hicks Pries *et al*., 2012) indicating isotopic values of bulk soil organic C should not be used to partition R_{eco}. Overall, heterotrophic respiration $\delta^{13}C$ and $\Delta^{14}C$ did vary spatially, but did not show consistent temporal variation, supporting previous results from a pine forest (Carbone *et al*., 2011).

Partitioning ecosystem respiration

Ecosystem respiration $\Delta^{14}C$ became more depleted throughout the growing season and in areas with deeper active layers. Ecosystem respiration $\Delta^{14}C$ was also more enriched than the atmosphere and more enriched than R_a across all categories and times with few exceptions,

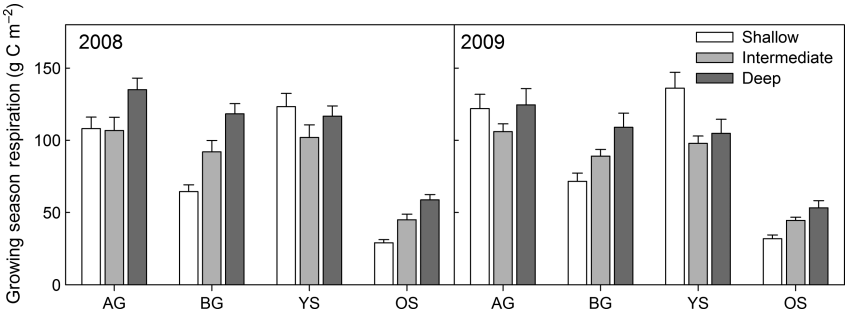

Fig. 6 Estimates of growing season fluxes from each respiration source [aboveground plant structures (AG), belowground plant structures (BG), young soil (YS), and old soil (OS)] for active layer (AL) categories in 2008 and 2009. The error bars are the spatial error of the respiration fluxes. To obtain the growing season flux, the mean estimates of source proportional contributions from SIAR for each month and AL category were multiplied by the fluxes for each month and AL category in each year. The results were then summed over May, June, July, August, and September.

whose $\Delta^{14}C$ were equal to the atmosphere. The depletion in R_{eco} $\Delta^{14}C$ could therefore be due to: (1) increasing contributions of R_a, which had $\Delta^{14}C$ only slightly more enriched than the atmosphere, (2) increasing contributions of OS respiration, which had negative $\Delta^{14}C$ (e.g. Schuur *et al.*, 2009), (3) decreasing contributions of YS respiration, which had enriched $\Delta^{14}C$ (e.g. Borken *et al.*, 2006), or (4) a combination of the above. Areas with deeper active layers at our study site also have been shown to have greater R_{eco} C losses (Vogel *et al.*, 2009). Only partitioning can decipher the mechanism behind the depleting R_{eco} $\Delta^{14}C$ and increasing R_{eco} losses.

Partitioning revealed that the seasonal and thaw-induced decreases in R_{eco} $\Delta^{14}C$ were driven by increases in both R_a and heterotrophic respiration of old soil C. Autotrophic contributions to R_{eco} increased throughout the growing season peaking in August, likely a result of increased plant biomass, increased net primary production, and warmer soils. We were able to detect seasonal changes in R_a by sampling several times throughout the growing season. Our results differed from previous studies, which found R_a contributions peaked at the start of the growing season (Chiti *et al.*, 2011) or did not change during the growing season (Nowinski *et al.*, 2010). Outside the growing season, R_a contributions have predictably decreased when plants are dormant (Ruehr & Buchmann, 2010; Subke *et al.*, 2011).

Across all AL depths and times, R_a contributions ranged from 40 to 70% of R_{eco}. Similar wide ranges of R_a contributions, 41–54% in a peatland (Hardie *et al.*, 2009) and 40–80% in tundra (Nowinski *et al.*, 2010), were found in previous R_{eco} partitioning studies. In terms of total growing season respiration flux, autotrophs respired 58% and 28% more C in areas with deep permafrost thaw than where thaw was shallow in 2008 and 2009, respectively. Similarly, permafrost thaw induced by a 1 °C warming doubled autotrophic contributions to R_{eco} in a Swedish permafrost peatland (Dorrepaal *et al.*, 2009). In contrast, autotrophic contributions to R_{eco} decreased with snow fence-induced permafrost thaw in Toolik, AK; although the snow fence shortened the growing season, likely eliciting the negative plant response (Nowinski *et al.*, 2010). Plants have generally responded to warming by increasing primary production (Rustad *et al.*, 2001). At our site, plant biomass is 35% greater where permafrost thaw is extensive relative to where thaw is minimal (Schuur *et al.*, 2007). Nearby in Healy, AK, warming soil by 2.3 °C caused a 20% increase in aboveground productivity (Natali *et al.*, 2012). Ecosystem respiration is positively correlated with aboveground net primary productivity at our study site (Vogel *et al.*, 2009). The maintenance of higher productivity where active layers are deepest may therefore necessitate higher rates of R_a.

Autotrophic respiration is made up of respiration from AG and BG plant structures, and respiration from AG plant structures drove the R_a increases discussed above. Aboveground R_a increased as the unfrozen layer deepened throughout the growing season and among AL categories, but BG R_a did not vary temporally as in a previous study (Cisneros-Dozal *et al.*, 2006). Relative contributions of AG and BG R_a shifted seasonally indicating changes in plant C allocation. At the shoulders of the growing season, respiration contributions from AG and BG plant structures were similar, whereas at the height of the growing season (July and August), AG R_a contributions were roughly twice that of BG. In absolute magnitude, however, BG R_a remained steady indicating root and rhizome respiration does not increase even when plants reallocate C from and to stored reserves at the beginning and end of the growing season. The difference between AG and BG R_a contributions in July and August was therefore driven by an increase in AG R_a, which temporally corresponds with

the greatest rates of primary production at and near our study site (Vogel *et al.*, 2009; Natali *et al.*, 2011).

The observed depletion in R_{eco} $\Delta^{14}C$ with deepening thaw (seasonally and across AL categories) was not only caused by increased R_a but also by increased OS R_h, which has a depleted $\Delta^{14}C$ value around $-30‰$ (a mean calendar age of ~250 years, but likely a mix of younger and much older C). With deepening thaw, more soil C is available to decomposition. In September, there is 21.1 kg more C m^{-2} available to above-freezing respiration where thaw is deep than where thaw is shallow, a 90% increase in thawed soil C (soil data from Hicks Pries *et al.*, 2012). However, thawed soil below 50 cm is still very cold with temperatures less than 1 °C and is often saturated. Thaw may therefore increase OS R_h in other ways, by causing soils to be warmer farther up in the soil profile or via priming as plant productivity increases and roots grow deeper. Our estimates for the contributions of OS to R_{eco} across the study site (6–18%) fall within the ranges of previous peatland studies (Dorrepaal *et al.*, 2009; Hardie *et al.*, 2009; Schuur *et al.*, 2009). The increase in OS respiration is particularly of note as this soil C has been stored away from the atmosphere for hundreds of years, making its release a potentially huge positive feedback to climate change (Schuur & Abbott, 2011): as permafrost thaws, more old C is respired, which increases atmospheric CO_2 causing more warming, which in turn thaws additional permafrost. This potential for a positive feedback is illustrated by the 67–103% increase in growing season old C flux where the permafrost thaw is deep relative to where thaw is shallow, similar to the 78% increase measured previously at our study site (Schuur *et al.*, 2009). The increase in OS flux with deepening thaw was 25–50% less than the R_a growing season increase (21–30 g C m^{-2} vs. 40–81 g C m^{-2}), demonstrating OS losses can be obscured by changes in plant respiration, except when revealed by isotopes.

Although OS R_h generally increased as thaw deepened throughout the growing season, there were two exceptions. In May 2009, the highest OS contributions reported in this study occurred in places with deep active layers. These high contributions early in the growing season are indicative of a burst of CO_2 from deeper in the soil profile during thaw (Lee *et al.*, 2010). Due to the downward movement of soil freeze in the autumn, microbial respiration continues to occur at above-freezing temperatures while the distance between the frozen surface soil and the permafrost closes. Decomposition can also occur within unfrozen soil micro sites during winter. When the surface soil thaws in the spring, some of the old C decomposed during the autumn and winter is released.

Challenges of the isotopic partitioning approach

The isotope partitioning method assumes isolated incubations of soil, roots, and aboveground plant structures minimally affect the $\delta^{13}C$ or $\Delta^{14}C$ of respiration. For $\Delta^{14}C$, the assumption is supported by several studies (Dioumaeva *et al.*, 2002; Czimczik & Trumbore, 2007), but the assumption does not hold for $\delta^{13}C$. Plant respiration $\delta^{13}C$ can change ($<1‰$) in the hours after plants are excised, but the effect is small when measurements are made directly after excision (Midwood *et al.*, 2006) as we did in this study. Soil respiration $\delta^{13}C$ from incubations have also been shown to change over times from a few hours (Millard *et al.*, 2008) to months (Blagodatskaya *et al.*, 2011) to years (Follett *et al.*, 2007), resulting in uncertainty about YS and OS $\delta^{13}C$. One mechanism for the hourly $\delta^{13}C$ shift is the respiration and loss of labile root exudates from the soil. For example, the 0.8–2‰ change in soil respiration from Millard *et al.* (2008) was a shift away from the depleted root respiration value. In this study, we waited for 5 and 10 days before measuring the respired $\delta^{13}C$ from surface and deep soil cores, respectively. The 5 day wait was based on a field study wherein soil respiration decreased by 50% in the 5 days after trees were girdled (Högberg *et al.*, 2001). By waiting, we excluded root exudates from our YS source and included them in the BG source. Root exudates turnover quickly and are tied to the autotrophic response, so including them in the BG source is appropriate for this study, which was principally designed to measure how OS respiration responded to permafrost thaw. During incubations, soil respiration $\delta^{13}C$ also shifts due to changes in microbial substrate preference from labile to more recalcitrant C as the labile C pool is exhausted. Our incubation length was specifically planned to measure $\delta^{13}C$ while the labile C pool was respired. Incubation studies demonstrate that the labile C pool takes at least 5–20 days to be exhausted in tundra soils, the longer time applying to deeper soils (Lee *et al.*, 2012; Lavoie *et al.*, 2011). Lastly, soil oxygen gradients in incubations may differ from *in situ* conditions, which may also affect isotopic values.

We tested the sensitivity of our results to a 1‰ shift in soil $\delta^{13}C$ based off the change measured during soil incubations in Follett *et al.* (2007). A 1‰ enrichment caused the AG proportion to increase and the YS and OS proportions to decrease, and vice versa for a 1‰ depletion. The average absolute change in all sources was only 2.7% of R_{eco}. All AL categories responded similarly, so a shift in soil $\delta^{13}C$ would not change the relationships between thaw depth and source contributions. Our results are robust to soil respiration uncertainty. However, mechanisms behind the $\delta^{13}C$ shift

during short incubations and how the shift affects partitioning results warrant further study as the isotope partitioning method becomes more widely used.

Implications for net ecosystem carbon balance

Flux measurements indicate that areas with deeper permafrost thaw at our study site had the greatest gross primary production and were likely a net C sink in 2008 and 2009 (Trucco *et al*., 2012). Therefore, the increased growing season losses from R_a and old soil R_h with increasing permafrost thaw are currently more than compensated for by increased net primary production. However, the loss of old soil C is concerning in the long term because sustained losses of old soil C are likely given the climate change trajectory. Because the size of the soil C pool (55–70 kg m^{-2}; Hicks Pries *et al*., 2012) is much larger than the tundra plant C pool (0.35 kg m^{-2}; Shaver & Chapin, 1991), future losses of old soil C will likely outpace increases in plant production, barring a major shift in plant community from tundra to boreal forest (e.g. Callaghan *et al*., 2004). Even then, a mature boreal spruce forest stores about 6 kg C m^{-2} in plant biomass (Gower *et al*., 2001; Goulden *et al*., 2011), only enough to compensate for a 10% loss of soil C. The questions that remain are what proportion of permafrost soil C is vulnerable to permafrost thaw and to what extent primary production increases can compensate soil C losses.

Acknowledgements

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Supporting Information

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

Table S1. The measured $\delta^{13}C$, $\Delta^{14}C$, flux rates, and estimated carbon pools and soil temperatures of individual soil sections used to calculate the depth-integrated $\delta^{13}C$ and $\Delta^{14}C$ of young soil and old soil.

Table S2. $\delta^{13}C$ and $\Delta^{14}C$ of each R_{eco} collar for all active layer (AL) categories and months.

Table S3. Mean (±SE) R_{eco} flux during the growing season for all AL categories in 2008 and 2009.

Table S4. Sensitivity analysis using July 2009 data to quantify how changing the mean values of source isotopes affect model results.