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Original Article

Carbon isotopic composition of forest soil respiration in the decade following bark beetle and stem girdling disturbances in the Rocky Mountains

Gregory E. Maurer[1,2], Allison M. Chan[1], Nicole A. Trahan[3], David J. P. Moore[3] & David R. Bowling[1]

[1]*Department of Biology, University of Utah, Salt Lake City, UT 84112, USA,* [2]*Department of Biology, University of New Mexico, Albuquerque, NM 87131, USA and* [3]*School of Natural Resources and the Environment, University of Arizona, 1064 E. Lowell St., Tucson, AZ 85721, USA*

ABSTRACT

Bark beetle outbreaks are widespread in western North American forests, reducing primary productivity and transpiration, leading to forest mortality across large areas and altering ecosystem carbon cycling. Here the carbon isotope composition ($\delta^{13}C$) of soil respiration (δ_J) was monitored in the decade after disturbance for forests affected naturally by mountain pine beetle infestation and artificially by stem girdling. The seasonal mean δ_J changed along both chronosequences. We found (a) enrichment of δ_J relative to controls (<1 ‰) in near-surface soils in the first 2 years after disturbance; (b) depletion (1‰ or no change) during years 3–7; and (c) a second period of enrichment (1–2‰) in years 8–10. Results were consistent with isotopic patterns associated with the gradual death and decomposition of rhizosphere organisms, fine roots, conifer needles and woody roots and debris over the course of a decade after mortality. Finally, δ_J was progressively more ^{13}C-depleted deeper in the soil than near the surface, while the bulk soil followed the well-established pattern of ^{13}C-enrichment at depth. Overall, differences in δ_J between mortality classes (<1‰) and soil depths (<3‰) were smaller than variability within a class or depth over a season (up to 6‰).

Key-words: *Abies lasiocarpa*; *Dendroctonus ponderosae*; *Picea engelmannii*; *Pinus contorta*; carbon cycle; chronosequence; decomposition.

INTRODUCTION

Bark beetle outbreaks have occurred in western North America during much of the Holocene (Baker & Veblen 1990; Brunelle *et al*. 2008; Morris & Brunelle 2012) and are part of a complex disturbance regime that shapes the structure and function of coniferous forests in the region (Veblen *et al*. 1994; Bradford *et al*. 2008). These disturbances influence the demography and biophysical environment of forests, and a variety of ecosystem processes such as photosynthesis, evapotranspiration and nutrient cycling [reviewed by Hicke *et al*. (2012)]. In the past two decades, attacks by bark beetles have reached epidemic proportions in many western forests and are contributing to broad declines in forest health (Breshears *et al*. 2005; Kurz *et al*. 2008; Raffa *et al*. 2008; van Mantgem *et al*. 2009; Logan *et al*. 2010). Mountain pine beetles (*Dendroctonus ponderosae*) have significantly impacted montane and subalpine conifer forests, which are responsible for the majority of ecosystem carbon exchange in the western USA (Houghton & Hackler 2000; Schimel *et al*. 2002). Over the long term, forest recovery is likely (Diskin *et al*. 2011), but little is known about how forest carbon cycle processes in the region will be altered by this disturbance over the next few decades.

Two mechanisms of damage to trees affect plant carbon and water relations during beetle infestation. Firstly, beetle larvae and pupae consume phloem and cambial tissue in the stems of infested trees, disrupting the supply of photosynthate to roots and the rhizosphere (Safranyik & Carroll 2006; Raffa *et al*. 2008). Secondly, fungi introduced to the tree by beetles grow into the xylem, blocking water transport to the leaves (Plaut *et al*. 2012; Hubbard *et al*. 2013). Photosynthetic CO_2 uptake [gross primary productivity (GPP)], respiration by roots and the rhizosphere and transpiration decline significantly as transport of water and carbohydrates diminishes (Amiro *et al*. 2010; Hubbard *et al*. 2013). Needles typically fall 2–6 years after the initial attack, and dead trees may fall anytime from years to decades later (Mitchell & Preisler 1998; Klutsch *et al*. 2009). Annually in the western USA the accumulation of beetle-killed organic matter is a carbon reservoir comparable to the carbon stocks in trees killed by fire (Hicke *et al*. 2013). When conditions allow beetle populations in a forest to enter an eruptive phase, severe tree mortality with large spatial extent can occur (Safranyik & Carroll 2006; Raffa *et al*. 2008), altering ecosystem properties at landscape to regional scales.

A number of studies have suggested that following beetle-induced mortality, decomposition of added dead biomass will lead to increases in ecosystem respiration (ER) and large-scale ecosystem carbon loss (Breshears & Allen 2002; Kurz *et al*. 2008; Edburg *et al*. 2011; Pfeifer *et al*. 2011). However, whether an ecosystem becomes a net carbon source is dependent on the balance between decomposition and recovery of GPP by surviving trees and vegetation regrowth. Ecosystem respiration is composed of aboveground plant respiration and soil

Correspondence: D. R. Bowling. e-mail: david.bowling@utah.edu

respiration, the latter an aggregate flux from plant roots, rhizosphere organisms and the primarily microbial heterotrophic community. The autotrophic component of ER, including respiration aboveground and from the roots and rhizosphere, is driven by recently assimilated carbon and thus declines sharply during beetle infestation as carbon gain and belowground carbon transport diminish (Högberg *et al.* 2001; Andersen *et al.* 2005; Amiro *et al.* 2010; Edburg *et al.* 2011). However, much of the soil respiration flux results from the turnover of organic matter by the heterotrophic community. Accumulation of dead plant tissue (woody tissue, leaves and roots) and dead rhizosphere organisms (mycorrhizal hyphae and other microbial biomass) following disturbance increases the substrate available to decomposers and is likely to enhance heterotrophic activity (Ekberg *et al.* 2007; Brown *et al.* 2010; Edburg *et al.* 2011). Importantly, studies thus far have not observed sustained increases in ER after beetle-induced mortality (Morehouse *et al.* 2008; Brown *et al.* 2010; Moore *et al.* 2013).

Stable isotopes may offer additional insight into carbon cycle processes following forest mortality. There are small but systematic differences in the carbon isotope composition ($\delta^{13}C$) of many plant and ecosystem carbon pools [reviewed by Bowling *et al.* (2008) and Brüggemann *et al.* (2011)], and these are likely to change following shifts in carbon cycling associated with bark beetle-induced mortality. Woody plant tissues are more enriched in ^{13}C than leaves, but in general, the CO_2 produced by respiring plant tissues exhibits the opposite pattern (root respiration is more depleted than leaf respiration; Ghashghaie & Badeck 2014). Belowground carbon pools, including mycorrhizal, soil and detrital pools, are further enriched in ^{13}C (Ehleringer *et al.* 2000; Boström *et al.* 2007, 2008; Werth & Kuzyakov 2010). Most of these differences in the $\delta^{13}C$ of ecosystem carbon pools are the result of post-photosynthetic fractionation during plant or microbial metabolism, with many of the mechanisms of fractionation unknown (Bowling *et al.* 2008; Brüggemann *et al.* 2011; Ghashghaie & Badeck 2014).

Stem girdling experiments also offer insight into carbon cycle processes following bark beetle infestation. When the phloem and cambium are intentionally removed from a section of tree bole, the connection between aboveground photosynthate and belowground tissues is severed. These manipulations have revealed declines in soil respiration from a few days to a few years after girdling, while observations of the $\delta^{13}C$ of soil respiration have allowed attribution of this change to the removal of the belowground labile carbon supply (Högberg *et al.* 2001; Bhupinderpal-Singh *et al.* 2003; Subke *et al.* 2004; Andersen *et al.* 2005; Scott-Denton *et al.* 2006). Presumably, terminating belowground carbon allocation by stem girdling or bark beetle damage is followed by the death and decay of root symbionts, other rhizosphere organisms and roots. Researchers have found evidence of this biomass decomposing and being released as ^{13}C-enriched soil respiration in the two years following girdling (Bhupinderpal-Singh *et al.* 2003; Ekberg *et al.* 2007). However, it is unclear whether similar isotopic changes can be observed over intermediate time scales (>2 years to decades), during which decomposition of newly dead leaves, roots and woody tissue and possibly other long-term shifts in forest carbon cycling are expected to occur.

This paper describes an investigation of forest carbon pool turnover in subalpine forests of the western USA using natural abundance stable carbon isotopes. This study follows earlier work in the same forests, where we demonstrated sustained decreases in GPP and respiration following forest mortality (Moore *et al.* 2013), and decreases in microbial biomass, soil organic carbon and altered nitrogen and phosphorus cycling (Trahan *et al.* 2015). In the present study, the $\delta^{13}C$ of soil respiration was monitored over two seasons across decadal-scale chronosequences (representing time since mortality) for forests affected naturally by mountain pine beetle and artificially by stem girdling. We predicted that (1) decomposition of mycorrhizal fungi would lead to enrichment in $\delta^{13}C$ of soil respiration within 1–2 years of mortality and (2) over a decadal post-disturbance period, the dominant carbon sources contributing to soil respiration would gradually change from more labile carbon (microbial biomass, needle tissue and fine roots), to more recalcitrant carbon pools (wood, coarse roots and soil carbon), leading to enrichment in $\delta^{13}C$ of soil respiration in later years.

MATERIALS AND METHODS

Study forests

Field research was conducted in 2011–2012 in two subalpine forests in the Rocky Mountains of Colorado, USA. The sites are described briefly here, with more detail and a map in Trahan *et al.* (2015). Both forests were dominated by lodgepole pine (*Pinus contorta*), with Engelmann spruce (*Picea engelmanii*) and subalpine fir (*Abies lasiocarpa*) as common sub-dominant species.

Beetle-induced mortality chronosequence

The Fraser Experimental Forest (FEF, http://www.fs.fed.us/rm/fraser) was severely impacted by mountain pine beetle beginning in 2002, with approximately 70% mortality of lodgepole pine in the watershed (Collins *et al.* 2011; Hubbard *et al.* 2013). The FEF study plots were located at 39°54′27″N, 105°51′10″W at 2910 m elevation. This forest has mean annual temperature of 0.5 °C and mean annual precipitation of 740 mm year^{-1}. Soils at FEF are well-drained Ustic Haplocryalfs (Collins *et al.* 2011).

At FEF, 18 circular plots (9–15 m diameter) with similar stand structure, topographic and parent substrate characteristics were selected along a disturbance chronosequence and divided into four groups, three based on the year in which a high degree of beetle-induced mortality occurred and one low mortality group that was designated as a control. In this analysis plots at FEF were grouped based on the 'years since disturbance', defined as the end of diameter growth assessed using dendrochronological analysis (Trahan *et al.* 2015). This experimental design has a varying number of plots in each years since disturbance class. Percent mortality of the plots was calculated using basal area of live and dead trees. Low mortality (Control) plots averaged 34% tree mortality ($n = 6$ plots), 4–5 year post-disturbance plots averaged 71% ($n = 5$), 5–6 year plots

averaged 82% ($n = 5$) and 6–7 year plots averaged 97% ($n = 2$). We assume that trees that show visual evidence of death and are no longer growing do not transfer new photosynthate below ground and that the end of diameter growth is a reasonable estimate of the time that transfer of photosynthate from above- to belowground tissues ceased.

Stem girdled chronosequence

The Niwot Ridge forest (NWT, http://niwot.colorado.edu) has not been significantly affected by mountain pine beetle, although the beetle has been present for several years (Mitton & Ferrenberg 2012). This chronosequence was located at 40° 1′58″N, 105°32′38″W at 3050 m elevation. The forest has mean annual temperature of 1.5 °C and mean annual precipitation of 800 mm year^{-1}. Soils are on a late-Pleistocene glacial moraine with generally thin (mean 60 cm) sandy/rocky Inceptisols covered by a thin (~3–9 cm) organic horizon (Cole & Braddock 2009). At NWT, researchers in earlier studies investigating rhizodeposition and soil biological processes (Scott-Denton *et al.* 2006; Weintraub *et al.* 2007) established plots of *P. contorta* that were stem girdled in 2002, 2003 and 2004. Plots were similar in diameter and tree density to the FEF plots. For the present study, additional plots were girdled in 2008, 2009, 2010 and 2011, and three plots were left undisturbed as controls. Tree stems were girdled by removing the bark, phloem and cambial tissue from the perimeter of a tree bole, thus terminating the transfer of photosynthate from aboveground to belowground tissues. There were 24 total study plots at NWT, three for each disturbance year and three controls. All were similar in topography, stand age and parent material (Trahan *et al.* 2015).

Carbon isotope composition of soil respiration

The $\delta^{13}C$ of CO_2 produced by respiration in the soil was determined by collecting soil gas from within the soil profile and subsequent laboratory measurement. In 2011 three soil gas wells were installed in all plots by placing 6.4 mm (outer diameter) stainless steel tubes into the soil to three depths: the interface of the organic and mineral soil horizons (O/A interface, which we denote 0 cm depth), 10 cm, and 30 cm below the O/A interface. The top end of each tube was then sealed with a tube union (B-400-6 Swagelok, Solon, OJ, USA) and septum (Microsep F-138, Grace Division Discovery Science, Deerfield, Il, USA). Prior to soil gas sampling on each date, gas in the headspace of the well was removed by inserting a needle through the septum and removing twice the volume of gas contained in the tube. A 15 mL sample of soil gas was then drawn through the septum with a syringe and transferred to an evacuated 12 mL septum-capped glass vial (Labco exetainer, Labco Ltd., Lampeter, Ceredigion, UK). Soil gas samples were collected from all plots on 6–10 dates during the warm seasons of 2011 and 2012, for a total of 1926 samples.

Using a conservative value of soil porosity (20%) each sample of soil gas would occupy a spherical volume within the soil with a radius <2.7 cm. Given this small volume, contamination of gas samples at the shallow O–A horizon because of sampling-based advection during sampling was likely to be negligible. In treatment plots (girdled or beetle-killed), reduced root water uptake may have resulted in wetter soils and somewhat larger sample volumes, but we were cautious to avoid sampling immediately following rain events when this effect would have been largest. We have recently published a comparison of an automated version of this method with direct measurement of $\delta^{13}C$ of CO_2 in the soil surface flux with automated chambers (Bowling *et al.* 2015). In that study the automated gas well sampling involved considerably more sampled volume (>2 L) without problem at 10 and 30 cm. However, we acknowledge that experimentally induced advection may have influenced our shallowest sampling depth in the present study, particularly in locations where the organic horizon was shallower than 3 cm.

Upon return to the laboratory, the mole fraction of CO_2 in the soil gas samples was measured by injecting 0.5 mL of soil gas into a closed-loop infrared gas analyser system (LI-7000, Li-Cor Biosciences Inc., Lincoln, NE, USA) as described by Moyes *et al.* (2010). The $\delta^{13}C$ of the CO_2 in these samples was measured relative to Vienna Pee Dee Belemnite by continuous-flow-isotope ratio mass spectrometry (Finnigan GasBench II & DELTAplus Advantage, Thermo Scientific Inc., West Palm Beach, FL, USA).

The isotope ratio of soil respiration (δ_J) was determined from these data using the method of Davidson (1995):

$$\delta_J = \frac{C_s(\delta_s - 4.4) - C_a(\delta_a - 4.4)}{1.0044(C_s - C_a)},$$

where C_a and C_s are the mole fractions of CO_2 in air (just above the soil surface) and soil gas, respectively, and δ_a and δ_s are the $\delta^{13}C$ of CO_2 in air and soil gas, respectively. In a forest, C_a and δ_a vary in time with atmospheric stability and biological activity, but the variability is negligible compared with the much larger difference between these quantities in the air and the soil (Bowling *et al.* 2015). Because our objective was to compare δ_J between disturbance treatments along the chronosequences, C_a and δ_a were assigned constant values of 420 ppm and −9.5‰, respectively, based on measurements 10 cm above the soil surface during the same period at Niwot Ridge (Bowling *et al.* 2014).

Bulk soil carbon and carbon isotope composition

In June of 2011 and 2012, soil cores (2 cm diameter) were collected from the O and A horizons at random locations within each plot (NWT forest, $n = 9$ per treatment). Cores from the organic layer integrated the entire depth of the organic horizon, and A horizon cores the top 10 cm of mineral soil. These were dried to constant mass at ~60 °C, sieved to 2 mm, then ground in a stainless steel ball mill (Retsch MM200, Retsch GmbH, Haan, Germany). The carbon content and $\delta^{13}C$ of these soils were determined by continuous flow elemental analysis and isotope ratio mass spectrometry. Samples were combusted in a Fisons 1110 CHN elemental analyser (CE Elantech, Inc., Lakewood, NJ, USA), and the isotope ratios of combustion gases were determined using a Finnigan DELTAplus

Advantage mass spectrometer (Thermo Scientific Inc., West Palm Beach, FL, USA).

Statistical analysis

We used multi-level repeated measures ANOVA (Bates *et al.* 2015), with sample date as a random variable to test for differences in δ_J and bulk soil $\delta^{13}C$ values between treatment and control plots. The categorical variable in this test was the time in years since beetle or girdling disturbance. We then subjected these ANOVA models to post-hoc analysis using the Tukey's honest significant difference (Tukey's HSD) test (R Core Team 2015) using an alpha value of 0.05 to indicate significant pairwise differences between all treatments, including the control plots.

RESULTS

The $\delta^{13}C$ of soil respiration (δ_J) changed significantly across both beetle mortality and stem girdling chronosequences, but these changes were small relative to the total variability in observed δ_J. The patterns can be summarized as (1) enrichment of δ_J relative to controls in near-surface soils in the first 2 years after disturbance; (2) depletion of δ_J relative to controls in deep soils in the first 2 years after disturbance; (3) depletion of δ_J (or no change) relative to controls during years 3–7; (4) enrichment relative to controls at all depths in years 8–10 and (5) high variability within a season relative to differences between mortality classes.

Seasonal mean soil respiration at beetle-impacted plots at FEF was depleted by ~1‰ in ^{13}C relative to control plots ($P < 0.05$) in years 3–7 following tree mortality (Fig. 1). Differences during this period were statistically significant ($P < 0.05$) at 0, 10 and 30 cm depths in both sampling years in many, but not all mortality classes. Changes in the seasonal means of δ_J at the stem girdled forest were more complex (Fig. 2), in part because of the larger number of disturbance years represented. The 3–7 year post-disturbance period was not well represented in the NWT chronosequence, but there was some consistency in the depletion of δ_J relative to controls (for example at the 0 cm depth in years 3–4) that was observed at FEF. At NWT, surface soils produced a pulse of enriched respiration immediately following stem girdling (Fig. 2). The δ_J in the shallowest soils (0 cm) tended to be enriched by ~0.5–1.0‰ relative to controls during the first year after disturbance in both sampling years, but these differences were not statistically significant and were not observed at other depths. This transient enrichment may have occurred at FEF and the lack of plots of 1–2 years post-disturbance did not allow us to observe it (Fig. 1). There was also a transient depletion (statistically significant) in δ_J observed at 30 cm depths at NWT from one to two years after girdling (Fig. 2). In plots 8–10 years after disturbance at NWT, δ_J was significantly enriched relative to controls at all depths (Fig. 2). This later enrichment was not apparent at the oldest plots (7 years) at FEF (Fig. 1).

These differences in the seasonal means of δ_J across the chronosequences (Figs 1 & 2) were smaller than variation in any given treatment within a season. At FEF, δ_J varied within a season by 1 to 6‰ depending on depth, time since

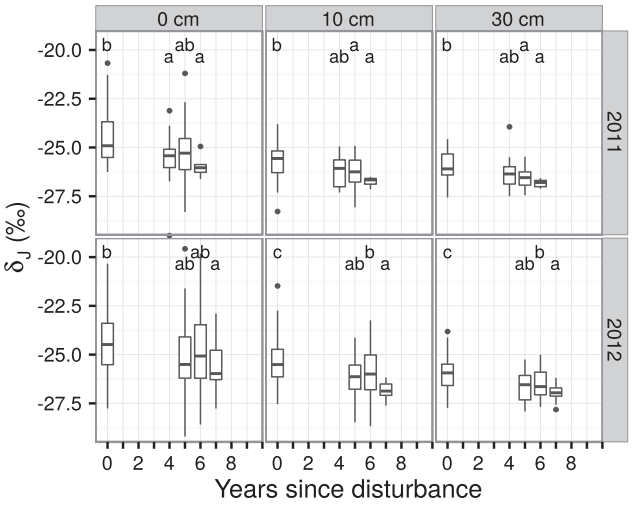

Figure 1. The $\delta^{13}C$ of CO_2 in soil respiration (δ_J) from soil gas collected at three depths in the beetle-impacted forest (FEF). Boxplots show the statistical distributions for 2011 and 2012 separately, including range (bars), 25th and 75th percentiles (top and bottom of box), and median (line within box) of the data for each treatment ($n = 6, 5, 5$ and 2 plots per treatment from control to oldest treatment, sampled throughout the warm season). Outliers are shown as filled black circles. Time since disturbance is shown on the abscissa (0 year indicates control plots with no mortality). Letters signify significant pairwise differences between treatments using Tukey's HSD test ($\alpha = 0.05$).

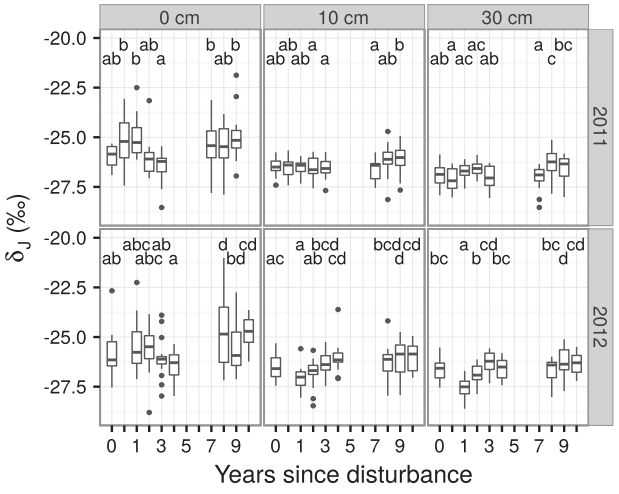

Figure 2. The $\delta^{13}C$ of CO_2 in soil respiration (δ_J) from soil gas collected at three depths in the stem girdled forest (NWT). Symbols and letters are as in Fig. 1, but $n = 3$ plots per treatment. Time since disturbance of <1 year indicates sampling during the same year as girdling (2011 only).

disturbance and sampling year (Figs 3 & S1). Similar seasonal variability was observed in the stem girdled plots at NWT (Figs 4 & S2) in both sampling years. In 2011, there was a tendency at both sites for δ_J to become more enriched as the season progressed (Figs S1 & S2), at most sampling depths. However, this seasonal pattern of enrichment was not repeated in 2012 (Figs 3 & 4). We were unable to find any pattern in soil

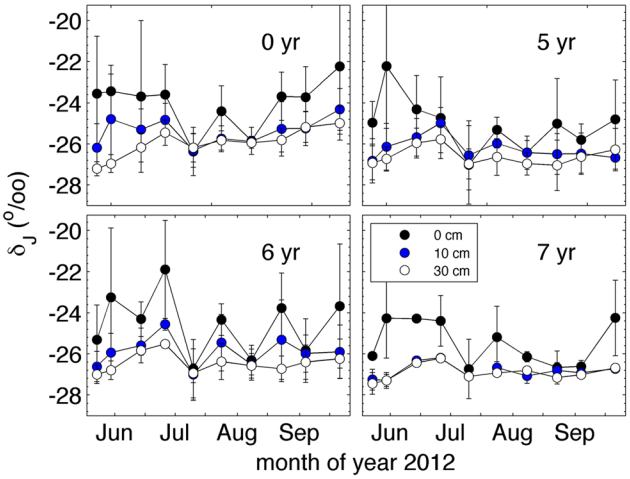

Figure 3. The seasonal pattern of δ_J in the beetle-impacted forest (FEF) during 2012. Symbols and error bars are the mean and standard deviation of measurements from each plot ($n = 6$, 5, 5 and 2 plots per treatment from control to oldest treatment, per sampling date). Time since disturbance is shown in different panels (0 year indicates control plots). Data for 2011 are shown in Fig. S1.

temperature or moisture that could explain within-season variation in δ_J in either chronosequence (data not shown).

At both forests we observed more enriched $\delta^{13}C$ in soil respiration at shallower depths. This pattern is clear in Fig. 3, where δ_J was usually 2–3‰ more enriched at 0 cm than at 30 cm, with the 10 cm depth in between. The pattern was consistent in both years across most sampling dates (Figs 3 & 4, S1 & S2). Additionally, the shallower gas wells had much greater variability in δ_J, both seasonally and along the chronosequences (error bars in Figs 3 & 4, S1 & S2).

Organic soils had higher carbon content and more negative $\delta^{13}C$ compared with mineral soil at Niwot Ridge (Figs S3 & S4), but few significant differences in the carbon content or isotope ratio of bulk soil were evident across the chronosequence. Bulk soil carbon content of organic and mineral soils did not show trends with time since disturbance (Fig. S3). In 2012, we observed a significant decline in organic soil carbon content immediately after girdling and a significant increase in bulk mineral soil carbon content at year 8. There was no significant change in the $\delta^{13}C$ of either soil horizon across the chronosequence (Fig. S4). Overall, there was no coherence between the $\delta^{13}C$ of the bulk soil carbon (Fig. S4) and the $\delta^{13}C$ of soil respiration (Figs 2 & S2).

DISCUSSION

Decreased soil respiratory efflux is usually observed following stem girdling (e.g. Högberg *et al.* 2001; Subke *et al.* 2011) because the carbon transport from aboveground to belowground plant tissues is ended. However, following beetle-induced mortality some studies have documented decreases in respiration while others have found no change (Morehouse *et al.* 2008; Brown *et al.* 2012; Speckman *et al.* 2015), perhaps because of variability in time since disturbance. We have previously shown that the soil surface CO_2 flux decreased 40–100% after forest mortality by bark beetle infestation at FEF and by stem girdling at NWT (Moore *et al.* 2013; see also Scott-Denton *et al.* 2006). Because both disturbances should eventually cease the autotrophic contribution to soil respiration, in this study we

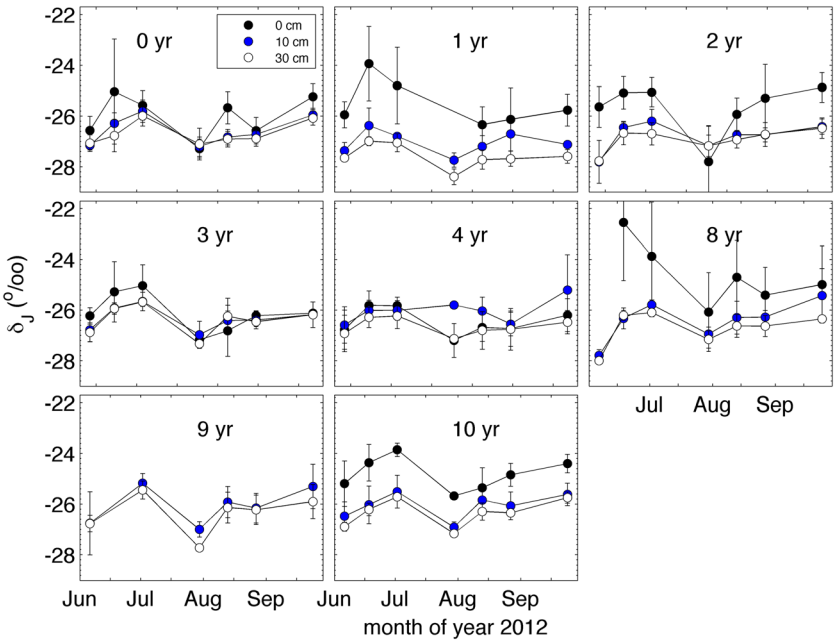

Figure 4. The seasonal pattern of δ_J in the stem girdled forest (NWT) during 2012, as in Fig. 3 ($n = 3$ plots per treatment per sampling date). Data for 2011 are shown in Fig. S2.

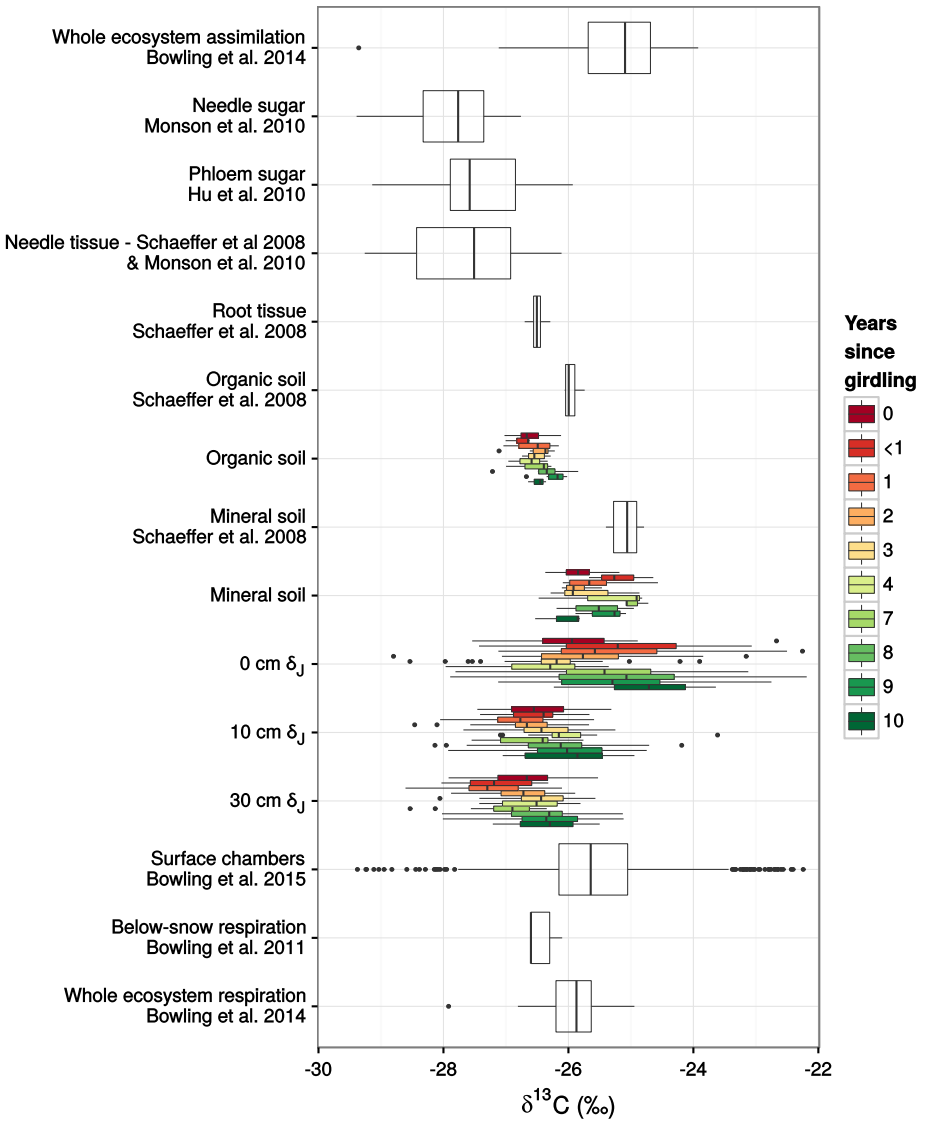

Figure 5. Comparison of $\delta^{13}C$ of carbon pools and fluxes at the stem girdled forest (NWT). Data from earlier studies are shown with white boxes and those from the current study with coloured boxes, where colours indicate time since disturbance. Boxplot symbols are as described in Fig. 1.

anticipated changes in the $\delta^{13}C$ of soil respiration (δ_J) following disturbance. We predicted that soil respiration would be enriched in ^{13}C relative to controls both early (1–2 year post-disturbance) and late (8–10 years) in our chronosequences.

These expectations are most easily evaluated by first describing what is known about isotope composition of ecosystem carbon pools and fluxes. At the NWT, several previous studies have examined the $\delta^{13}C$ of carbon pools and fluxes in the undisturbed forest, and these are compared with our results in Fig. 5. On average, whole-ecosystem assimilation (GPP) was enriched in ^{13}C relative to all other pools and fluxes. Conifer needle tissue and leaf and phloem sugars (Schaeffer *et al.* 2008; Hu *et al.* 2010; Monson *et al.* 2010) were the most ^{13}C-depleted carbon pools measured. Leaf (needle) respiration has not been measured at the forest, but across a wide spectrum of plants has been found to be enriched by 4–6‰ in ^{13}C relative to leaf tissue (Ghashghaie & Badeck 2014). Leaf, soil and woody tissue respiration must ultimately balance mass between GPP and leaf carbon if there is no change in isotopic composition of pools over time.

Soil carbon pools at NWT were enriched relative to plant pools and were progressively more enriched in order from root tissue, to organic soil, to mineral soil (Schaeffer *et al.* 2008). Bulk soil $\delta^{13}C$ observations from the present study followed the same pattern, with mineral soil enriched by about 1‰ relative to organic soil, but offset by ~0.5‰ in comparison to the earlier sampling (Fig. 5 and Schaeffer *et al.* 2008), which perhaps resulted from spatial variability. Our δ_J observations were enriched relative to leaf and phloem carbon measurements from the recent past, although there is significant potential for seasonal and inter-annual variability in the isotopic composition of these carbon pools (Brüggemann *et al.* 2011). The $\delta^{13}C$ of soil respiration in the present study generally agreed with earlier observations of warm-season respiration from surface chambers, below-snow respiration and whole-ER (Fig. 5). Note that the earlier studies were

conducted in undisturbed forest and that the changes associated with stem girdling (colours in Fig. 5) all fell within the range of respiration measurements from the undisturbed sites. In other words, while stem girdling substantially decreased soil respiration (Moore *et al.* 2013), the resulting isotopic effects were smaller than the natural (undisturbed) range of variability (Fig. 5).

Isotopic enrichment of surface soil respiration in early year post-disturbance

We observed a pulse of ^{13}C-enriched respiration within the near-surface soils (0 cm depth) in the two years following stem girdling at NWT (Fig. 2). Possible explanations for this include the turnover of enriched mycorrhizal and other rhizosphere biomass, reductions in depleted rhizosphere respiration or likely a combination of both. Microbial biomass carbon and nitrogen (N) declined in the 2 years following girdling (Trahan *et al.* 2015), suggesting that turnover of the microbial community was occurring. This was concomitant with a decrease in bulk soil C:N (from ~14 to ~8), and in microbial biomass C:N (~13 to ~10), which are consistent with a shift from a fungal-dominated community to a more bacterial community (Cleveland & Liptzin 2007).

The pulse of ^{13}C-enriched respiration is in agreement with our first prediction. Enriched δ^{13}C in mycorrhizal biomass (relative to plant and soil carbon pools) has been observed in several studies (Hobbie *et al.* 1999; Högberg *et al.* 1999; Wallander *et al.* 2004; Boström *et al.* 2008; Werth & Kuzyakov 2010), most likely because of preferential use of carbon substrates from host roots and fractionation during carbon transport and respiration (Hobbie *et al.* 1999; Werth & Kuzyakov 2010; Brüggemann *et al.* 2011). Root respiration tends to be ^{13}C-depleted relative to other plant and soil biomass (Ghashghaie & Badeck 2014), so reductions in root respiration are likely to have further contributed to enrichment of the bulk soil CO_2 flux. This is consistent with the results of Bhupinderpal-Singh *et al.* (2003), who noted a pulse of ^{13}C enriched soil respiration (~2–3‰ relative to controls) at 0.5 to 1.5 years after girdling in a Scots pine forest. Following stem girdling, Ekberg *et al.* (2007) found significant ^{13}C enrichment in the soil dissolved organic carbon (DOC) pool between 1 and 2 years after the disturbance. Both studies attributed these isotopic changes to the turnover of enriched mycorrhizal or other rhizosphere biomass and we concur.

Isotopic depletion of deep soil respiration in early year post-disturbance

In the present study, 1–2 years after disturbance, soil respiration at the girdled forest was depleted in ^{13}C relative to control plots at 10 and 30 cm depths (NWT; Fig. 2). We did not anticipate this isotopic shift, but it was almost certainly caused by a change in the carbon sources driving root, rhizosphere and soil respiration. As discussed, decreased root respiration after girdling probably would lead to ^{13}C-enrichment in soil respiration rather than depletion. Respiration of stored root carbon reserves, however, may explain the observed depletion in soil respiration. Trees allocate carbon to storage as carbohydrates or lipids for use when more labile carbon forms are unavailable. Cessation of the labile carbon supply to roots triggers the mobilization of root carbon stores for use in respiration by dying roots. Stored carbon can be used to drive a portion of root respiration for at least 2 years after it is initially fixed (Hopkins *et al.* 2013; Lynch *et al.* 2013). In *Pinus*, a large fraction of carbon reserves are stored in the form of lipids (Hoch & Körner 2003; Hoch *et al.* 2003), and synthesis of lipids leads to a ^{13}C-depleted carbon storage pool (DeNiro & Epstein 1977; Melzer & Schmidt 1987). Because our forests were dominated by *Pinus contorta*, it is plausible that before dying, roots respired ^{13}C-depleted CO_2 derived from stored lipids for 2 or more years after girdling.

Isotopic depletion of soil respiration in middle years post-disturbance

During years 3–7 after beetle-induced mortality, soil respiration at the Fraser forest was depleted in ^{13}C relative to control plots at all depths (FEF; Fig. 1). This is another isotopic shift we did not anticipate, but we find the most likely cause to be the addition of newly dead root and leaf (needle) tissue to the pool of carbon available to decomposers. Evidence from this and other studies suggests that respiration of root carbon reserves and decomposition of mycorrhizal and rhizosphere biomass decline within 2–3 years of disturbance (NWT; Fig. 2), and roots likely died following this (Bhupinderpal-Singh *et al.* 2003; Subke *et al.* 2011). At FEF and other forests nearby, researchers have found significantly increased leaf litter and organic layer depth in the 4–7 years following beetle-induced mortality (Klutsch *et al.* 2009; Trahan *et al.* 2015) as forests transition from the red phase to the gray phase and needles drop (Edburg *et al.* 2012). Once available for decomposition, root and needle litter carbon could be respired for a few years, contributing CO_2 to the bulk soil respiration flux around the middle of the decade after disturbance (Gholz *et al.* 2000; Harmon *et al.* 2009; years 3-7, Gaudinski *et al.* 2010). Conifer needles are depleted in ^{13}C relative to most ecosystem carbon pools (Fig. 5), and their decomposition might lead to the observed depletion in the overall soil respiration flux (Fig. 1). It is worth noting again that at the FEF site the low-mortality 'control' plots experienced 34% tree mortality, and potentially, an elevated level of litterfall during this time. Our chronosequence of stem-girdled plots does not include this 3–7 year time period, so we have no similar evidence of needle decomposition and subsequent depletion in δ_J from the Niwot forest.

Isotopic enrichment of soil respiration in later years post-disturbance

Both the enrichment and depletion of δ_J observed in early and middle years after disturbance were temporary and followed by another period of enrichment relative to control plots in years 8–10 (Fig. 2). This was only observed at the stem girdled

forest, perhaps because the beetle-killed forest had a shorter chronosequence record. Coarse roots and woody debris are slower to decompose (Chen *et al.* 2001; Kueppers *et al.* 2004), and so decomposition of woody biomass probably contributed a large proportion of CO_2 to soil respiration only in later years. In general, woody and coarse root tissues are ^{13}C-enriched relative to plant leaves and sugars (Fig. 5; Leavitt & Long 1982; Bowling *et al.* 2008) and their decomposition should lead to enriched respiration. No significant patterns in bulk soil carbon content or $\delta^{13}C$ were observed across the NWT chronosequence (Figs S3 & S4), indicating that any disturbance-induced perturbation to the soil organic matter pool lagged behind the soil CO_2 flux response. Overall, these results are consistent with our second prediction, indicating a shift away from ^{13}C depleted, recently fixed carbon that began with the release of carbon from root reserves and decomposing rhizosphere biomass, and continuing with the decomposition of dead needles, fine roots and eventually larger roots and woody debris.

Patterns of $\delta^{13}C$ of soil respiration with depth

It is widely known that the $\delta^{13}C$ of bulk soil organic matter tends to be more enriched with depth (Nadelhoffer & Fry 1988) as we have found (Figs 5 & S4). Specific mechanisms for this remain unknown, but several hypotheses exist including microbial fractionation during litter decomposition, preferential microbial decomposition of particular compounds, microbial carboxylation of heavier CO_2 within the soil gas or the ^{13}C Suess effect (Ehleringer *et al.* 2000; Boström *et al.* 2007).

In the present study, patterns of $\delta^{13}C$ with depth in bulk soil and in soil respiration were qualitatively opposite. Soil respiration was more depleted at depth than near the surface, while the $\delta^{13}C$ of bulk soil was more enriched at depth as usual (Fig. 5). Our data indicate that respiration measured *insitu* was depleted relative to $\delta^{13}C$ of the bulk soil (Fig. 5), which stands in contrast to some incubation studies (Boström *et al.* 2007). To our knowledge, increasing depletion in the $\delta^{13}C$ of soil respiration with depth has not been reported before and is consistent with respiratory carbon loss leading to the ^{13}C enrichment of soil organic matter. These contrasting depth profiles of the isotopic composition of carbon pools and fluxes may indicate that discrimination against ^{13}C during respiration increases in deeper soils. Alternatively, soil carbon pools accessible to decomposing heterotrophs become proportionally smaller and increasingly depleted in ^{13}C with depth, while a more recalcitrant, microbially derived carbon pool becomes proportionally larger and more enriched in ^{13}C with depth.

Isotopic compositions of autotrophic and heterotrophic respiration

Stable isotopes are one of many potential means to separate the autotrophic and heterotrophic components of soil respiration [refer to reviews by Kuzyakov (2006) and Paterson *et al.* (2009)]. The disturbances we have investigated in this study are arguably a powerful means to test if there are systematic differences in the $\delta^{13}C$ of respiration by plants and their root symbionts and by the heterotrophic soil community. We found substantial decreases in the rate of soil respiration following beetle-induced mortality and stem girdling across our chronosequences (Moore *et al.* 2013), a clear indication that the contribution of roots and root symbionts to soil respiration was severely limited following forest mortality (Högberg *et al.* 2001). However, the differences in δ_J in different mortality classes were generally quite small (Figs 1–4, S1 & S2). This finding is in agreement with other studies that have highlighted that the carbon isotope difference between autotrophic and heterotrophic respiration is too small to provide a generally useful tool to partition between them in the absence of isotope labelling or photosynthetic pathway (C3–C4) shifts (Hanson *et al.* 2000; Formánek & Ambus 2004; Kuzyakov 2006).

This point is further supported by the within-season variability in δ_J (Figs 3 & 4, S1 & S2), which was much larger than the differences between treatments (Figs 1 & 2). We were not able to explain what caused this variation based on our observations, but the seasonal changes were much larger than the differences between mortality classes (which should reflect the difference in $\delta^{13}C$ of autotrophic and heterotrophic respiration). Forest mortality leads to changes in a wide variety of biophysical and environmental characteristics including light, temperature, moisture, wind, evaporation, sublimation, precipitation accumulation and interception (Edburg *et al.* 2012; Pugh & Small 2012; Pugh & Gordon 2013; Vanderhoof *et al.* 2013; Biederman *et al.* 2014). These factors could lead to changes in $\delta^{13}C$ of soil respiration via altered photosynthetic discrimination of surviving trees or via the complex isotopic interactions among other ecosystem carbon reservoirs (Brüggemann *et al.* 2011). These complexities lead to difficulty in interpreting the isotopic signals present in ecosystem CO_2 fluxes and organic matter in the absence of isotope labelling. Indeed, there is considerable debate on whether natural abundance isotopic approaches are well suited to discern the dynamic links in the plant-soil-atmosphere system (Kuzyakov & Gavrichkova 2010; Mencuccini & Hölttä 2010; Brüggemann *et al.* 2011; Hopkins *et al.* 2013). Our study offers a reasonably coherent picture of the soil carbon cycle response to beetle attacks and girdling in subalpine conifer forests, but underscores the challenges with using natural abundance carbon isotopes to examine forest carbon cycling processes.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's website.

Figure S1. The seasonal pattern of δ_J in the beetle-impacted forest [Fraser Experimental Forest (FEF)] during 2011. Symbols and error bars are the mean and standard deviation of measurements from each plot ($n = 3$ plots per treatment per sampling date). Time since disturbance is shown in different panels (0 year indicates control plots). Data for 2012 are shown in Fig. 3 of the main article.

Figure S2. The seasonal pattern of δ_J in the stem girdled forest [Niwot Ridge forest (NWT)] during 2011, as in Fig. S1. Data for 2012 are shown in Fig. 4 of the main article.

Figure S3. Carbon content of bulk soil in the organic (top) and mineral (bottom) horizons at the stem girdled forest in 2011 and 2012. Time since disturbance is shown on the abscissa (0 year indicates control plots, <1 year indicates sampling during the same year as girdling). Letters signify significant pairwise differences between treatments using Tukey's HSD test ($\alpha = 0.05$).

Figure S4. The $\delta^{13}C$ of bulk soil in the organic (top) and mineral (bottom) horizons at the stem girdled forest in 2011 and 2012, as in Fig. S3. Letters signify significant pairwise differences between treatments using Tukey's HSD test ($\alpha = 0.05$).