SHORT COMMUNICATION

Whole-crown ^{13}C-pulse labelling in a sub-arctic woodland to target canopy-specific carbon fluxes

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Key message Whole-crown ^{13}C-pulse labelling can target tree canopy C fluxes in regions with dense understorey cover and investigate how increased photosynthetic C inputs may affect whole-ecosystem C fluxes.

Abstract Climate change-driven increases in plant productivity have been observed at high northern latitudes. These trends are driven, in part, by the increasing abundance of tall shrub and tree species in arctic ecosystems, and the advance of tree-lines. Higher plant productivity may alter carbon (C) allocation and, hence, ecosystem C cycling and soil C sequestration. It is important to understand the contributions that the newly established canopy forming overstorey species makes to C cycling in these ecosystems. However, the presence of a dense understorey cover makes this challenging, with established partitioning approaches causing disturbance and potentially introducing measurement artefacts. Here, we develop an in situ whole-crown ^{13}C-pulse labelling technique to isolate canopy C fluxes in areas of dense understorey cover. The crowns of five mountain birch (*Betula pubescens* ssp. *czerepanovii*) trees were provided with a $^{13}CO_2$ pulse using portable field equipment, and leaf samples were collected from neighbouring con-specific trees and hetero-specific understorey shrubs on days 1–10 and 377 post-crown labelling. We found effective and long-term enrichment of foliage in labelled trees, but no evidence of the ^{13}C-signal in con- or hetero-specific neighbouring trees or woody shrubs. This method is promising and provides a valuable tool to isolate the role of canopy tree species in ecosystems with dense understorey cover.

Introduction

Trends of increasing plant productivity, or "greening", have been observed in recent decades across large parts of the northern hemisphere (Epstein et al. 2012, 2016; Reichle et al. 2018). At high latitudes, these trends are partly driven by the increasing abundance of tall shrubs in tundra ecosystems (Myers-Smith et al. 2011; Bjorkman et al. 2018) and the northern advance of treelines (Hagedorn et al. 2014;

Rees et al. 2020) with the presence of trees increasing at higher latitudes and up slopes (Hofgaard et al. 2013). This has the potential to alter the relative contribution of tall and short-stature plants to ecosystem carbon (C) cycling. With 'greening' and an increase in primary productivity, inputs of recently fixed C to Arctic and northern ecosystems are expected to increase (Pearson et al. 2013). However, we have limited understanding of the potential fate of this increased C, or how it impacts on below-ground processes, which may alter ecosystem C cycling and soil C sequestration (Wookey et al. 2009; Street et al. 2018, 2020). Therefore, understanding the knock-on effects of increased C inputs and changes to plant community composition in northern ecosystems necessitates the ability to partition canopy and understorey C fluxes to forecast and understand the drivers of C fluxes at ecosystem level, including various plant, microbial, and soil C pools. One way to partition canopy and understorey C fluxes is through targeted stable isotope labelling of C assimilated by canopy forming trees.

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Labelling of plants using stable (^{13}C) or radioactive (^{14}C) isotopes has been used to investigate C cycling in terrestrial ecosystems and to trace C allocation and remobilisation in plants (Reviewed by Brüggemann et al. 2011). Using $^{14}CO_2$ in pulse labelling is advantageous, because very small amounts of $^{14}CO_2$ can be supplied to the foliage due to the very weak environmental background for ^{14}C compared with ^{13}C in the atmosphere and in the plant–soil system. However, using $^{13}CO_2$ for pulse labelling is much more cost-effective than using $^{14}CO_2$, and avoids deployment of radio-isotopes in the environment (Epron et al. 2012). Numerous studies have focused on C dynamics in plants and soils using $^{13}CO_2$ pulse labelling as a powerful tool in ecophysiology to analyse C fluxes (Dawson et al. 2002; Bowling et al. 2008; Epron et al. 2011), estimate turnover of soil organic matter (e.g., Lichtfouse et al. 1995; Klumpp et al. 2007; Theis et al. 2007), partition C fluxes and pools (e.g., Hoch and Keel 2006; Plain et al. 2009; Subke et al. 2012; Street et al. 2013, 2018), and evaluate C fluxes to soil biota (e.g., Simard et al. 1997; Teste et al. 2010; Epron et al. 2011) and through food-webs (e.g., Leake et al. 2006; Pollierer et al. 2007, 2012; Högberg et al. 2010). The technical complexity of pulse labelling experiments means that such experiments are challenging to implement in the field. Pulse labelling of short-stature Arctic shrub species has been accomplished using relatively small ^{13}C labelling chambers (Deslippe and Simard 2011; Street et al. 2018); however, pulse labelling larger shrubs and trees at remote high-latitude field locations present greater challenges and have rarely been attempted (Masyagina et al. 2016).

Methods for C-pulse labelling of trees have been extensively reviewed by Epron et al. (2012). Pulse labelling of whole areas of forest can be achieved using free-air CO_2 enrichment (FACE) techniques (e.g., Pepin and Körner 2002; Klein et al. 2016). However, this method does not provide insight at the level of individual trees, nor does it partition canopy and understorey fluxes. Pulse labelling of whole trees has been achieved using large labelling chambers housing individual trees in temperate (Plain et al. 2009; Drake et al. 2019; Gao et al. 2021), tropical (Glaser et al. 2012), and arctic (Masyagina et al. 2016) regions, or whole patches of boreal forest (Högberg et al. 2008). Such approaches have provided valuable insight into tree C dynamics, but due to the size of labelling chamber, these methods involve substantial investment in infrastructure and personnel. Whole-ecosystem labelling presents challenges for tracing the short-term fate of newly fixed C from plants to soil. Physical diffusion of $^{13}CO_2$ into soil pores during the pulse period complicates the detection of biological signal from assimilate transfer to roots (Subke et al. 2009). Furthermore, large chambers generally label both trees and forest-floor species, making it harder to examine the significance and fate of tree-mediated inputs in isolation from

understorey communities. At higher latitudes, with dense understorey cover contributing significantly to total leaf area index (Parker et al. 2020) and ecosystem gross primary productivity (Kulmala et al. 2019), mechanistic understanding requires approaches that identify the relative contribution of tree versus understorey C inputs to the ecosystem C cycle. This distinction cannot be made using methods which label both canopy and understorey communities together.

One approach to tackle this challenge is to pulse-label a fraction of a whole tree, such as a single branch (Hoch and Keel 2006; Masyagina et al. 2016). Labelling only a fraction of a whole tree inevitably results in a high level of dilution of the ^{13}C signal by the $^{12}CO_2$ fixed by the rest of the tree. Another approach is to inject ^{13}C-carbonate into tree xylem (Powers and Marshall 2011). This approach avoids the need for large chambers, but a large proportion of the C label delivered to the tree xylem has been found to diffuse primarily out of stem tissues (Żelawski et al. 1970), thereby limiting the value of this technique for tracing photosynthates into roots, mycorrhizal fungi, and soil.

Once isotopic labelling of recently fixed C in trees has been achieved, the isotopic signal can be used to trace C allocation into various plant tissues (e.g., leaves, stems, or roots), within which the main C sinks are growth and storage in the form of non-structural carbohydrates (NSCs), respiration, symbiotic associations with mycorrhizal fungi, and exudation (Ericsson et al. 1996). This makes isotopic labelling of trees in high-latitude regions a valuable method, not only to understand C cycling at the ecosystem level, but also to improve our limited understanding of how long-term storage pools (NSCs) buffer against seasonal variation in C supply through extended winter periods, maintain important mycorrhizal relationships, and how these C pools respond to environmental change (Hartmann and Trumbore 2016).

Here, we present a method for in situ whole-crown ^{13}C-pulse labelling of short-stature (2–3 m) trees, using portable field equipment and delivery of $^{13}CO_2$ to individual trees. This provides a tool to trace the fate of carbon fixed by canopy forming trees, which create the forest overstorey, isolated from the short-stature understorey plant community.

Materials and methods

For full methods, see Supplemental Materials.

Site description and plot set-up

All studied plots were in the sub-Arctic mountain birch (*Betula pubescens* Ehrh. ssp. *czerepanovii* (Orlova) Hämet Ahti) treeline forest at Nissunsnuohkki, south of Abisko, Sweden (ca. 68°18'56.2"N 18°49'18.2"E). In these forests, mountain birch trees form the *canopy*, defined here as the

upper vegetation layer formed by mature tree crowns, and the majority of the *understorey* is formed by short-stature woody shrubs, predominantly <50 cm in height.

Five single-stemmed trees (to be ^{13}C labelled, henceforth referred to as 'target trees') 2–3 m tall, with mean basal area of 37.0 ± 29.2 cm[2] (mean $\pm$ SD), were selected $\geq$ 50 m apart. Each plot included three selected trees: the 'target' tree, a 'trenched' control tree, and an 'un-trenched' control tree. The average distance between the target tree and selected control trees was 3.85 ± 0.56 m (mean $\pm$ SD). An approximately 25 cm-deep trench severing roots, and mycorrhizas was cut in a semi-circle at the mid-point between the target tree and one control tree (this became the 'trenched' control tree, henceforth referred to as such). The other control tree in the plot remained undisturbed (this became the 'un-trenched' control, henceforth referred to as such). Both trenched and un-trenched controls were included to account for any potential ^{13}C translocation via common mycelial networks (CMNs), below-ground, which has previously been observed in arctic *Betula* species (Deslippe and Simard 2011; Deslippe et al. 2016).

Plots were selected to have a representative understorey with three common species [*Betula nana* L., *Vaccinium vitis-idaea* L., and *Empetrum nigrum* L. ssp. *hermaphroditum* (Hagerup) Böcher] present. Four *Betula nana* plants (50–560 cm from target tree), and four patches of *Vaccinium vitis-idaea* (40–440 cm from target tree) and *Empetrum nigrum* (15–520 cm from target tree), were marked for leaf sampling, two on either side of the trench.

Isotope labelling

The crown of the target tree, defined here as all branches and leaves of the individual tree, was covered in a large $(140 \times 178 \times 230$ cm) clear plastic bag (Gardener's Dream Ltd, Glasgow, UK) and sealed to the main stem at the base of the crown, below the lowest branches. Two battery-operated fans inside the crown bag facilitated air movement during labelling. A temperature probe (Traceable®, Webster, USA) was secured to a branch and shielded from direct solar radiation to monitor temperature inside the labelling bag throughout the labelling process (Online Resource Fig. S1).

Immediately after sealing the crown bags, each of the five target trees was labelled using $^{13}CO_2$ produced in situ by adding 90 ml 2% HCl to 2 g $Ca^{13}CO_3$; thus, 0.257 g [13]C (99 atom % enriched with ^{13}C, Sigma-Aldrich Ltd, Dorset, UK) contained in a beaker outside the crown bag (Fig. 1). The $^{13}CO_2$ was led into and out of the crown bag via infrared gas analysers (PP Systems International, Amesbury, MA, USA) monitoring the concentration of CO_2 produced (Online Resource Fig. S1) and creating a closed loop (Fig. 1b). Crown $^{13}CO_2$ labelling was conducted for approximately 1 h 15 min on 20 July 2018 between 08:00 and 17:00 h,

with diffuse high clouds allowing sufficient light for high photosynthetic rates without long periods of direct sunlight.

Foliar and air sampling

10–15 mature leaves were sampled from the selected trees and shrubs prior to labelling and on days 1, 3, 6, 10, and 377 post-labelling. All foliar samples were frozen in the field within 1 h of sampling (Waeco Dometic CDF-26 21 L Portable Compressor Fridge Freezer) to reduce metabolic processes, and remained frozen at − 20 °C for $\geq$ 24 h and dried at 60 °C for 72 h before milling to ensure homogenisation.

Air samples, each of which was 24 ml, were taken from within the crown bag during ^{13}C-pulse labelling. During the ^{13}C-pulse labelling period, air samples were also taken from within the canopy of the control trees to detect any leaks from the labelling bag. After ^{13}C-pulse labelling, air samples were collected during foliar sampling, within the canopy of all three trees at each plot.

Stable isotope analysis

Milled crown leaf samples (*Betula pubescens*) and all air samples pre-pulse and on days 1–10 post-^{13}C-pulse, were analysed at the NERC (Natural Environment Research Council) Life Science Mass Spectrometry Facility at the Centre for Ecology and Hydrology (CEH) in Lancaster, UK, by Elemental Analyser-Isotope Ratio Mass Spectrometry (EA-IRMS). All understorey samples and crown samples from day 377 post-^{13}C-pulse were analysed at the Soil Microbial Ecology Lab at the University of Manchester with a Picarro G2201-i CRDS. For full descriptions of analysis methods, including standards used, please refer to the Supplementary Materials.

Statistical analyses

All analyses were carried out using R Version 3.4.0 (R Core Team 2017). $\delta^{13}C$ enrichment of trenched and un-trenched control trees over time was modelled using a linear mixed-effects model 'nlme::lme' (Pinheiro et al. 2012; R Core Team 2017) with sampling day as a fixed variable and 'plot' as a random variable. Exponential decay of $\delta^{13}C$ enrichment over time in target trees was modelled using a self-starting non-linear asymptotic regression (stats::SSasymp; (R Core Team 2017)). Pre-pulse data and day 377 data are presented as summarised raw data and tested using a paired Student's t test within each plot. $\delta^{13}C$ enrichment of the understorey over time is presented as summarised raw data. The difference in $\delta^{13}C$ enrichment between trenched control and un-trenched control samples was tested using a paired Student's t test within species, with Bonferroni corrections.
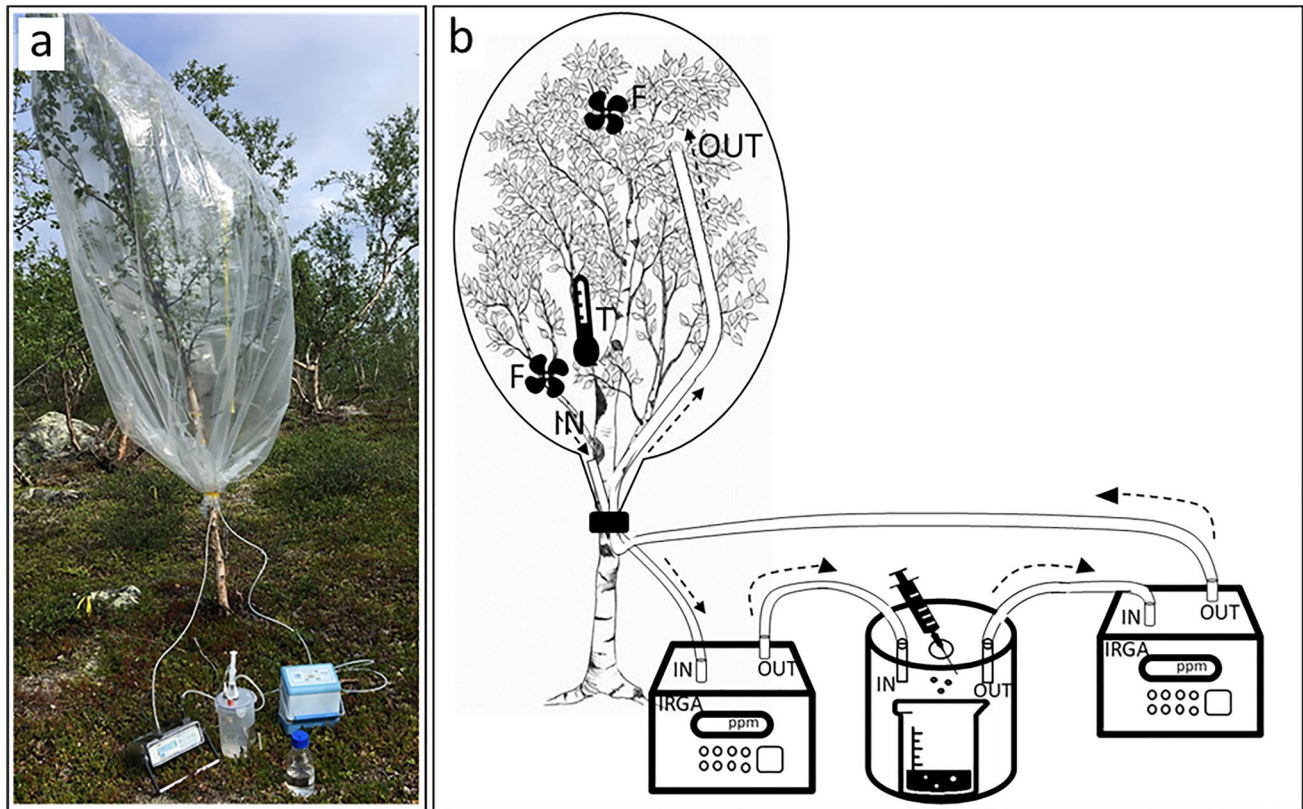
Fig. 1 a Field pulse labelling set-up being deployed in situ. **b** Schematic diagram of $^{13}CO_2$ pulse labelling set-up. A clear plastic bag fitted around target tree with two fans (F), one thermometer (T) in the crown space, and gas flow to and from the bag. Gas flows through two infrared gas analysers (IRGA) either side of $^{13}CO_2$ generated by adding HCl to $Ca^{13}CO_3$ using a syringe. Dashed lines indicate direction of cyclic gas flow

Results and discussion

Using a whole-crown bag and $\delta^{13}C$-enriched CO_2 (Fig. 1), we successfully labelled five replicate mountain birch trees in situ in a Swedish sub-Arctic treeline forest (Fig. 2). The foliage of target trees assimilated 0.18 ± 0.06 g (mean $\pm$ SD, $n = 5$) ^{13}C based on enrichment of day 1 samples (Supplemental Materials Eq. 1).

There was no difference in $\delta^{13}C$ values between trenched and un-trenched control trees either pre- or post-^{13}C labelling ($P = 1.00$ on all sampled days) suggesting no (or undetectable) tree-to-tree transfer of ^{13}C via CMNs. Although ^{13}C enrichment was not detected in the foliage of control trees, it is possible that the ^{13}C signal could have been transferred to the roots or stem tissues (Klein et al. 2016); however, these were not sampled here. More targeted experiments, examining contrasting plant and fungal tissues, are needed to draw any firm conclusions.

Leaves of the target tree had significantly higher $\delta^{13}C$ enrichment throughout the initial 10-day post-pulse period than natural abundance samples and both the trenched and un-trenched control trees ($P < 0.001$) (Fig. 2). This demonstrates successful ^{13}C labelling of the target tree with minimal or undetectable leakage or transfer of the ^{13}C signal to surrounding mountain birch trees. Air sampling during the ^{13}C-pulse labelling shows very high $\delta^{13}C$ enrichment inside the crown bag but none outside the bag near control trees ($P < 0.001$) (Fig. 3). This pulse labelling method can therefore be deployed with a high degree of accuracy, labelling only selected target trees. Air samples from the subsequent 10 days post-^{13}C-pulse trended higher in $\delta^{13}CO_2$ in crowns of target trees, but were not significantly enriched compared to neighbouring trees ($P > 0.05$) (Fig. 3), which means that any $^{13}CO_2$ respired from target trees was undetectable in free-mixing treeline forest air. This also strongly suggests that any leaf enrichment detected stemmed from the original ^{13}C-pulse, and not significantly from fixed and subsequently respired $^{13}CO_2$ from the target tree.

We found no evidence of the ^{13}C signal in any of the surrounding understorey shrubs on days 1–10 or 377 (Fig. 4) relative to natural abundance samples ($P > 0.1$). $\delta^{13}C$ values on day 377 were significantly higher than on day 10 for all three understorey species (*B. nana*: $P < 0.001$, *E. nigrum*: $P < 0.001$, *V. vitis-idaea*: $P = 0.039$) (Fig. 4). This small but

Fig. 2 $\delta^{13}C$ of leaf biomass for each treatment type (target, trenched, un-trenched, $n = 5$) pre-, 1–10 days, and 377 days post-pulse labelling. Pre-pulse samples are taken as natural abundance $\delta^{13}C$. Error bars and ribbons are ±1 standard deviation, and results within sample day are off-set to aid comparison. The inset magnifies the y-axis range for trenched and un-trenched control samples as well as target tree samples pre-pulse and on day 377, to aid visualisation of differences between trees
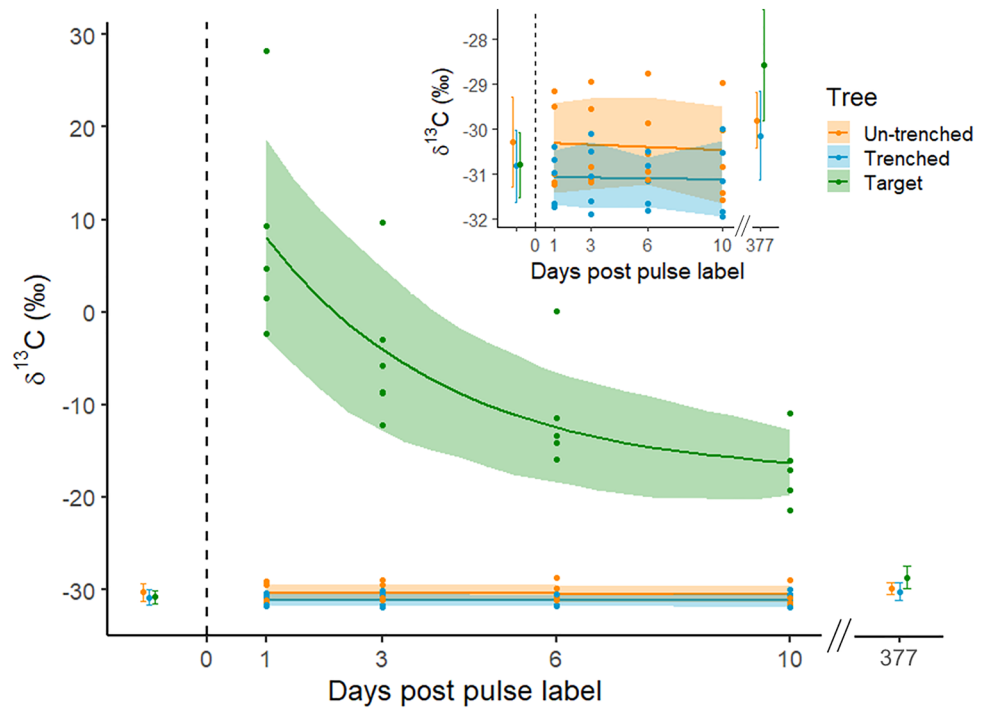
Fig. 3 Mean (points) $\delta^{13}C$ values in air samples taken at all three trees (target, trenched, un-trenched, $n = 5$) during and after ^{13}C-pulse labelling. Error bars are ±1 standard error; $n = 5$. Mean values within sample day are off-set to aid comparison. Note the contrast in y-axis scales during and post-^{13}C-pulse (the latter are expanded to aid data visualisation)
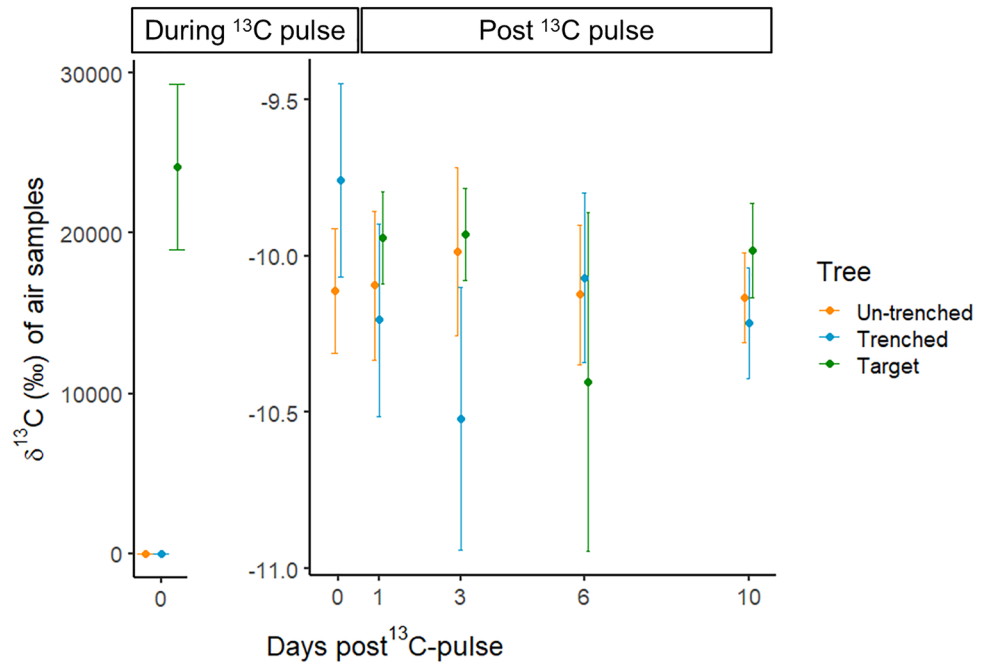
statistically significant increase in ^{13}C enrichment is consistent in all understorey shrub species >1 year post-pulse. However, because the response was observed in shrubs from both trenched and un-trenched areas, it is unlikely that the ^{13}C enrichment is caused by the pulse itself (e.g., re-fixation of $^{13}CO_2$ respired from target tree roots), and more likely reflects interannual variation. Interannual variation in ^{13}C natural abundance may occur due to interannual variation

in abiotic factors such as precipitation, snow depth, and temperature (Blok et al. 2015; Gamm et al. 2018), which can influence stomatal aperture and alter C fractionation patterns.

These results show that this pulse labelling technique is able to target specific members of the plant community in isolation, thereby facilitating the partitioning of understorey and canopy tree C fluxes (not directly measured here). The

Fig. 4 Mean $\delta^{13}C$ values in understorey species up to 10, and 377 days, post-pulse labelling of the target tree. Colour illustrates whether there was a trench severing roots and mycorrhizas between the pulse labelled tree and where the understorey was sampled. Error ribbon and bars are ±1 standard deviation, $n = 5$. Mean results for day 377 are off-set to aid comparison
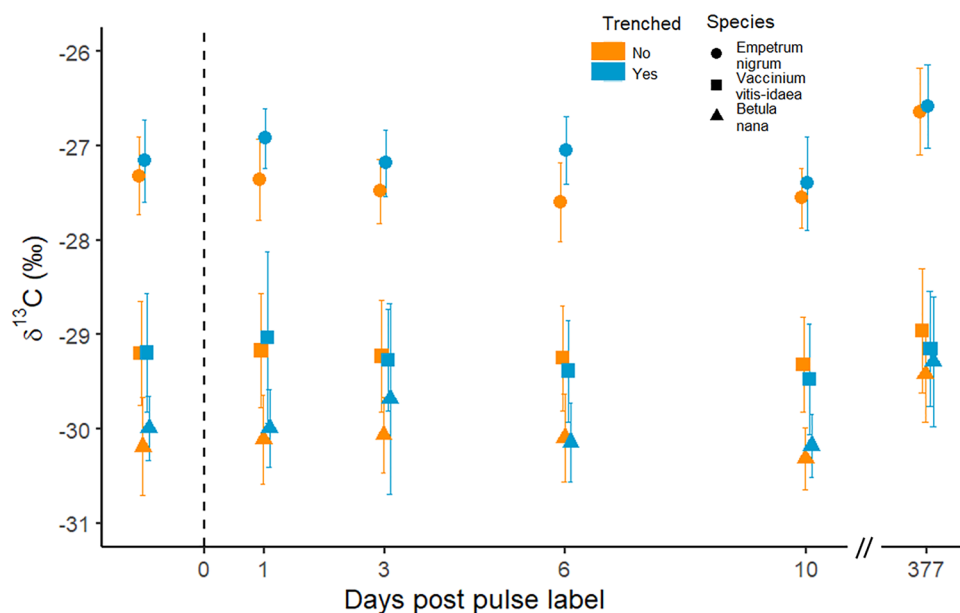
success of this method in isolating canopy C fluxes in forests with dense understorey cover is promising, with considerable scope for application in other forest ecology contexts. Understanding of C cycling and forest function could be advanced substantially by: (1) determining the fate of C fixed by canopy trees through tracing the ^{13}C into rhizosphere, mycorrhizal, microbial, or soil C pools (Brüggemann et al. 2011), and (2) identifying linkages between individual plants within forests by tracing C fixed by one tree into other plants (Simard et al. 2012).

One timely application could be to investigate the effects of climate change-driven Arctic 'greening', expansion of shrubs, and northward advance of treelines (Myers-Smith et al. 2011; Rees et al. 2020), which increases above-ground plant productivity and C inputs below-ground. As different species allocate recently fixed C between plant and soil pools differently (Street et al. 2018), it remains largely unknown how shifts in species abundance will alter below-ground processes and affect soil C sequestration (Wookey et al. 2009; Parker et al. 2021). Most research in this region, to date, has dealt with the mountain birch forest as a whole (e.g., Sjögersten and Wookey 2009; Hartley et al. 2010, 2012; Parker et al. 2015), or focussed on canopy species (Friggens et al. 2020; Parker et al. 2020). However, to understand changes in C pathways between plants, microbes and soil organic matter, we need methodologies that can selectively target contributions of key species without physically disturbing understorey plants or below-ground connections. At high latitudes, where biomass is more evenly distributed between canopy and understorey (Kulmala et al. 2019), and climate change may alter the relative dominance of the two, it is increasingly important to disentangle the contribution of these two plant community components to whole-ecosystem

C fluxes to forecast the potential effects of such a shift in dominance. The specificity of this labelling technique is complementary to manipulative techniques such as 'stem girdling' (Högberg et al. 2001; Parker et al. 2020) or trenching (e.g., Bond-Lamberty et al. 2004; Street et al. 2020), but offers advantages in terms of minimal disturbance and the opportunity to determine the medium-term fate of specific C inputs. Thus, it will be valuable in partitioning the relative influence of canopy and understorey C fluxes within plant communities with potentially interactive effects on belowground processes.

Stable isotope pulse labelling creates, by definition, a transient signal, and by day 377 post-^{13}C labelling, the foliage of target trees was no longer significantly $\delta^{13}C$-enriched relative to both the trenched ($P = 0.14$) and un-trenched ($P = 0.079$) control trees (Fig. 2). When comparing day 377 samples with the natural abundance pre-pulse samples, there was no significant difference in ^{13}C enrichment between natural abundance and day 377 for both trenched ($P = 0.35$) and un-trenched ($P = 0.24$) control trees. However, target trees were significantly enriched on day 377 compared to natural abundance $\delta^{13}C$ pre-pulse samples ($P = 0.027$) which suggests that a fraction of the ^{13}C label may have been preserved in the target trees >1 year after the ^{13}C-pulse. This indicates that a fraction of the ^{13}C fixed during pulse labelling was retained in long-term C storage pools of target trees, likely as reservoirs of non-structural carbohydrate (NSC) (Martínez-Vilalta et al. 2016). The detection of the ^{13}C within new leaves more than 1 year after labelling suggests that the pulse likely generated a strong signal in NSCs. As this is a deciduous species, detection of enhanced ^{13}C in annual foliage provides compelling evidence of translocation of NSC from stem and/or root tissues to leaves in the year following

assimilation (Hartmann and Trumbore 2016). Isolating specific compound classes may open up new applications of this method over multiple growing seasons in the future, and thus, the chance to improve understanding of how C reserves are used in arctic tree and shrub species adapted to surviving extended winters.

Conclusions

We present a method for in situ whole-crown ^{13}C-pulse labelling mountain birch trees in a sub-arctic treeline forest. Using this method, we successfully labelled the foliage and long-term C storage pools (>1 year post-^{13}C-pulse) of five mountain birch tree crowns, whilst detecting none of the ^{13}C signal in the foliage of neighbouring trees or in any of the sampled understorey shrubs. This method of whole-crown pulse labelling provides a valuable tool to trace the fate of recently fixed C from individual canopy trees into various plant, microbial, and soil pools, and to investigate how increased photosynthetic C inputs and shifts in canopy and understorey dominance in northern ecosystems may affect whole-ecosystem C fluxes in a changing world.

Declarations

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