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Physiological girdling of pine trees via phloem chilling: proof of concept

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ABSTRACT

Quantifying below-ground carbon (C) allocation is particularly difficult as methods usually disturb the root–mycorrhizal–soil continuum. We reduced C allocation below ground of loblolly pine trees by: (1) physically girdling trees and (2) physiologically girdling pine trees by chilling the phloem. Chilling reduced cambium temperatures by approximately 18 °C. Both methods rapidly reduced soil CO_2 efflux, and after approximately 10 days decreased net photosynthesis (P_n), the latter indicating feedback inhibition. Chilling decreased soil-soluble C, indicating that decreased soil CO_2 efflux may have been mediated by a decrease in root C exudation that was rapidly respired by microbes. These effects were only observed in late summer/early autumn when above-ground growth was minimal, and not in the spring when above-ground growth was rapid. All of the effects were rapidly reversed when chilling was ceased. In fertilized plots, both chilling and physical girdling methods reduced soil CO_2 efflux by approximately 8%. Physical girdling reduced soil CO_2 efflux by 26% in non-fertilized plots. This work demonstrates that phloem chilling provides a non-destructive alternative to reducing the movement of recent photosynthate below the point of chilling to estimate C allocation below ground on large trees.

Key-words: allocation; below ground; carbon; chilling; feedback inhibition; non-destructive; respiration; root exudation; soluble C.

INTRODUCTION

Below-ground carbon (C) allocation is one of the least understood processes in tree physiology, and its quantification is necessary for accurately modelling forest net primary productivity and net ecosystem productivity (Landsberg 2003). Recently, a number of studies have estimated below-ground C allocation by physically girdling groups of trees and quantifying any short-term decrease in total soil CO_2 efflux within the treated area. This method assumes that girdling will immediately prohibit the transfer below ground of C recently fixed via photosynthesis, that the decrease in soil CO_2 efflux represents the quantity of C transfer halted by the girdling and that there is minimal disturbance to the root–mycorrhizal–soil continuum. In a study using large plots of 45–55-year-old Scots pine (*Pinus sylvestris* L.) (Högberg *et al*. 2001; Högberg, Nordgren & Ågren 2003), physical girdling in late summer decreased soil CO_2 efflux by 37% in 3 d and 56% within 14 d. An early-summer girdling of 40-year-old Norway spruce (*Picea abies* [L.] Karst), in a long-term fertilization experiment in the same region, reduced soil CO_2 efflux after 2 weeks by 44 and 15% in non-fertilized and fertilized stands, respectively (Olsson *et al*. 2005). Other girdling studies using eucalyptus (Binkley *et al*. 2006) and European chestnut (Frey, Hagedorn & Giudici 2006) resulted in decreases in soil CO_2 efflux ranging from 16 to 44%. However, as has been discussed previously (Högberg *et al*. 2001; Olsson *et al*. 2005), the physical girdling approach provides conservative estimates as girdling is destructive and irreversible.

Our goal was to cease C allocation below ground in a manner that results in minimal damage to trees and is reversible. We sought to halt phloem transport by temporarily chilling tree stems and to compare the efficiency of this technique with physical stem girdling. The chilling technique has previously been exploited only on herbaceous plants growing in controlled environments (Swanson & Geiger 1967; Giaquinta & Geiger 1973; Goeschl *et al*. 1984). We chilled the phloem of 10 trees forming a patch in an even-aged, mid-rotation loblolly pine stand receiving annual fertilization. We also compared chilling responses with those from physically girdled tree plots in fertilized and non-fertilized stands. Both experiments were conducted in the spring and late summer/autumn. The soil CO_2 efflux and stem respiration (R) were measured over time.

MATERIALS AND METHODS

Site

The Southeast Tree Research and Education Site (SETRES) (Albaugh *et al*. 1998), a nutrition/irrigation experiment, and the adjacent SETRES2 (Retzlaff *et al*. 2001), a genotype × fertilization interaction study, are located in the Sandhills of Scotland County, North Carolina

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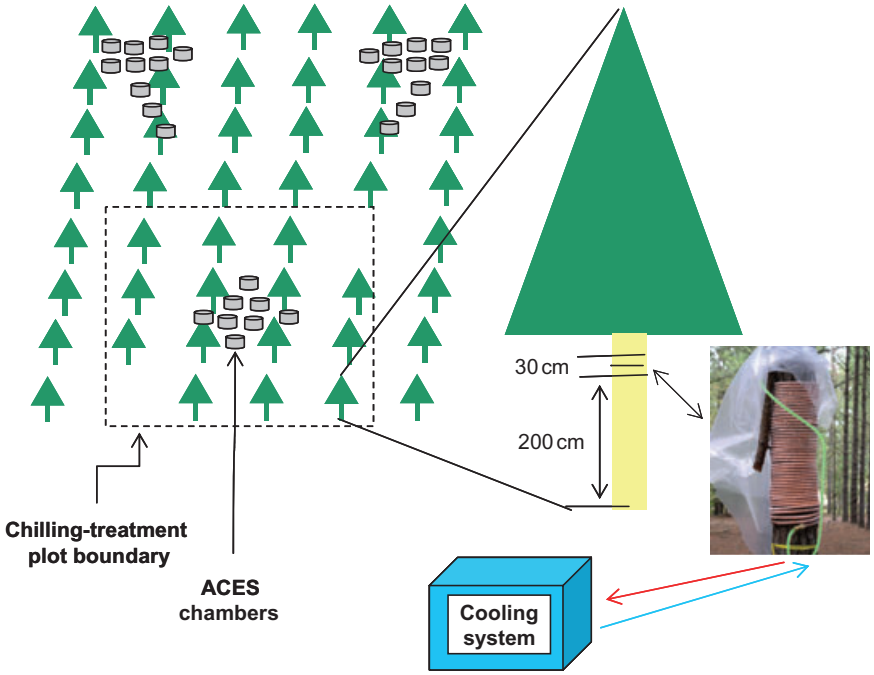

Figure 1. Illustration of cold-block installation, including a picture of the stem chilling coil, and the layout of automated carbon efflux system (ACES) chambers used to sample soil CO_2 efflux in the treatment plot and exterior treatment plot (diagram not to scale).

(34°55′N, 79°30′W). The summers are warm and humid, and winters are moderate. Annual precipitation (1000–1200 mm) is evenly distributed during the year. The experiments are on infertile, excessively drained sandy sites; SETRES was hand planted in 1985 and SETRES2 in 1993. Only the fertilized plus irrigation treatment at SETRES were used for the chilling studies conducted in the autumn of 2003 and spring of 2004, and the fertilized and non-fertilized treatments from SETRES2 were used for the physical girdling studies in the spring and in the autumn of 2005.

Chilling technique

We installed the cold-block system on 10 adjacent trees forming a 9×8 m patch in the stand (Fig. 1) An adjustable cold-block collar was fitted on each tree at 200 cm above ground level. Collars were made of 2 mm copper tubing coiled around the circumference 30 times to enclose ~ 30 cm section of the stem. Chilling was applied by circulating anti-freeze chilled to ~ 2 °C through the cold-block collars connected to the tubing that passed through a cooling system (portable freezer). The anti-freeze was circulated from and to an insulated container using submersible pumps. Cycling the pumps on and off as needed controlled the temperature of the anti-freeze reaching the cold-block collars. Two thermocouples were inserted under the copper tubing approximately 1 cm past tree bark, and were monitored constantly.

Physical girdling

Four 10×10 tree plots were established in both the spring and the autumn (girdled non-fertilized, control non-fertilized, girdled fertilized, control fertilized). A small chainsaw was used to girdle trees by removing all bark and phloem in an approximately 2.5 cm band around each tree at approximately 1.5 m above ground level.

Soil CO_2 efflux

The soil CO_2 efflux was measured with the automated carbon efflux system (ACES) (constructed at USDA Forest Service Lab, RTP. NC, US Patent 6,692,970). ACES is a chamber-based, multi-port respiration measurement system (Butnor, Johnsen & Maier 2005) that uses open system, dynamic soil respiration chambers measuring 25 cm in diameter (491 cm^2) equipped with air and soil thermocouples (inserted to a depth of 5 cm). Chambers were designed with pressure equilibration ports to ensure that minute differences in chamber pressure do not compromise the quality of the respiration measurement (Fang & Moncrieff 1996). Two ACES were utilized, each having 15 sample chambers and one null calibration chamber that were measured sequentially for 10 min each, allowing a complete run every 2 h and 40 min or nine complete runs per day. When not being actively sampled, the other 15 chambers were refreshed with reference air to prevent any build-up of CO_2 in the chambers.

In the chilling studies, chambers were spaced so that a total of eight were located within the area surrounded by the chilled trees, and 20 were outside of the chilling region up to 25 m away (Fig. 1). In the physical girdling studies, eight chambers were placed within each girdled plot and seven were placed within the control plots. To compare the treatments and experiments, the soil CO_2 efflux data were normalized per experiment/treatment combination by dividing the daily mean soil CO_2 by the highest mean daily rate observed per experiment/treatment combination.

Stem R

Stem R was measured using the ACES and the teflon stem chambers (Maier & Clinton 2006) by placing the chambers approximately 10 cm above and below the point of chilling or physical girdling. In the chilling study, six chilled and three non-chilled trees were measured and in the physical girdling study, four girdled trees and three control trees were measured. Stem R was only measured on fertilized plots.

Net photosynthesis (P_n)

P_n was measured using the LiCOR 6400 (Li-Cor Biosciences, Lincoln, NE, USA). During the chilling studies, measurements were taken on five occasions on single fascicles immediately after detachment from the upper half of the crown (Gough *et al*. 2004). Measurements were taken between 1000 and 2400 h at 1600 μmol m^{-2} s^{-1} photosynthetically active radiation and ambient temperature. On each occasion, 10 trees from the chilling plot and five non-chilled trees were sampled (except on the third measurement date where five trees per treatment were sampled). In the physical girdling study, measurements were taken, as previously mentioned, on six occasions on five trees per treatment combination.

Root exudate capture and analysis

Soluble C was captured *in situ* onto XAD-7 (Sigma-Aldrich, St. Louis, MO, USA) non-ionic carbonaceous resin capsules (Johns & Skogley 1994; Morse, Yevdokimov & DeLuca 2000). Fresh mass of the capsules averaged 3.53 g with an SD of 0.13. During the chilling study, duplicate resin capsules were placed at approximately 10 cm deep adjacent to each of the ACES chambers. Capsules were in place for 14 d during the chilling period, and new capsules were in place for 13 d after the chilling ceased. Compounds captured on the resin capsules were treated with trifloroacetic anhydride (TFAA). The TFAA served the dual purpose of extracting the organic material from the resin and converting polar groupings (i.e. the hydroxyl groups in carbohydrates) into non-polar groupings. The derived material was taken up in chloroform, and a subsample from each capsule was measured for total C and N by combustion analysis and was also analysed by liquid-state proton and fluorine nuclear magnetic resonance (^{1}H and ^{19}F NMR).

RESULTS AND DISCUSSION

There were no responses to physiological (via phloem chilling) or physical girdling when the experiments were conducted in the spring. We hypothesize that this is because above-ground growth was rapid at this time and below-ground processes were predominately provided C via starch reserves, which are highest in the spring (Sampson *et al*. 2001).

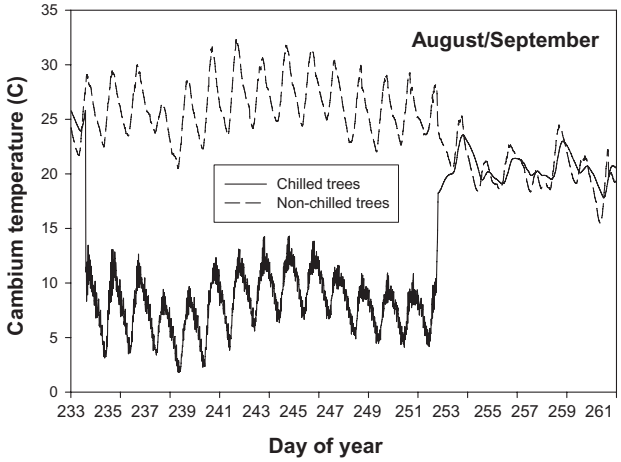

Figure 2. Cambium temperatures and temperature differential of non-chilled and chilled loblolly pine trees before, during and after chilling experiment.

Our stem chilling protocol was highly effective and consistent; cambium temperatures of chilled trees were, on average, 17.5 °C lower than ambient (Fig. 2). In the autumn, both physiological and physical girdling rapidly reduced soil CO_2 efflux indicating the dependence of below-ground R on the availability of current photosynthate. Chilling reduced soil CO_2 efflux in approximately 3 d while in the physical girdling experiment, the response took 6 d (Fig. 3). When the chilling was ceased, the recovery in soil CO_2 efflux was almost immediate. Analyses of least square means using linear relationships shown in Fig. 4 indicate that both physiological and physical girdling reduced soil CO_2 efflux similarly (chilling: –9%, $P = 0.04$; physical girdling: –7%, $P = 0.05$) in the fertilized treatments; the reduction was much greater (–26%, $P < 0.0001$) in the non-fertilized plot subjected to physical girdling.

Chilling and physical girdling also increased stem R (Fig. 5) above the point of the girdle, although the effect was far greater during the physical girdling study. This further indicates that both physiological girdling, via phloem chilling, and physical girdling created a bottleneck for the downward movement of C recently gained via photosynthesis, stopping or reducing its movement below ground. P_n measurements (Fig. 6) also supported the reduced below-ground C movement by phloem chilling because P_n decreased 35% relative to the controls in all treatments 11–13 d after the onset of chilling and physical girdling, consistent with feedback inhibition (Myers, Thomas & Delucia 1999). Again, this effect was rapidly reversed once the chilling was stopped. Immediate full recovery of soil CO_2 efflux, stem R and P_n indicates that chilling caused no or minimal damage to phloem cells and the root–mycorrhizal–soil continuum.

Although not measured during the pre-chilling, during the chilling period the total soluble C captured in resin capsules placed within the chilling treatment was 39% of the amount measured outside the chilling zone; following the chilling treatment, the amount captured in the chilling

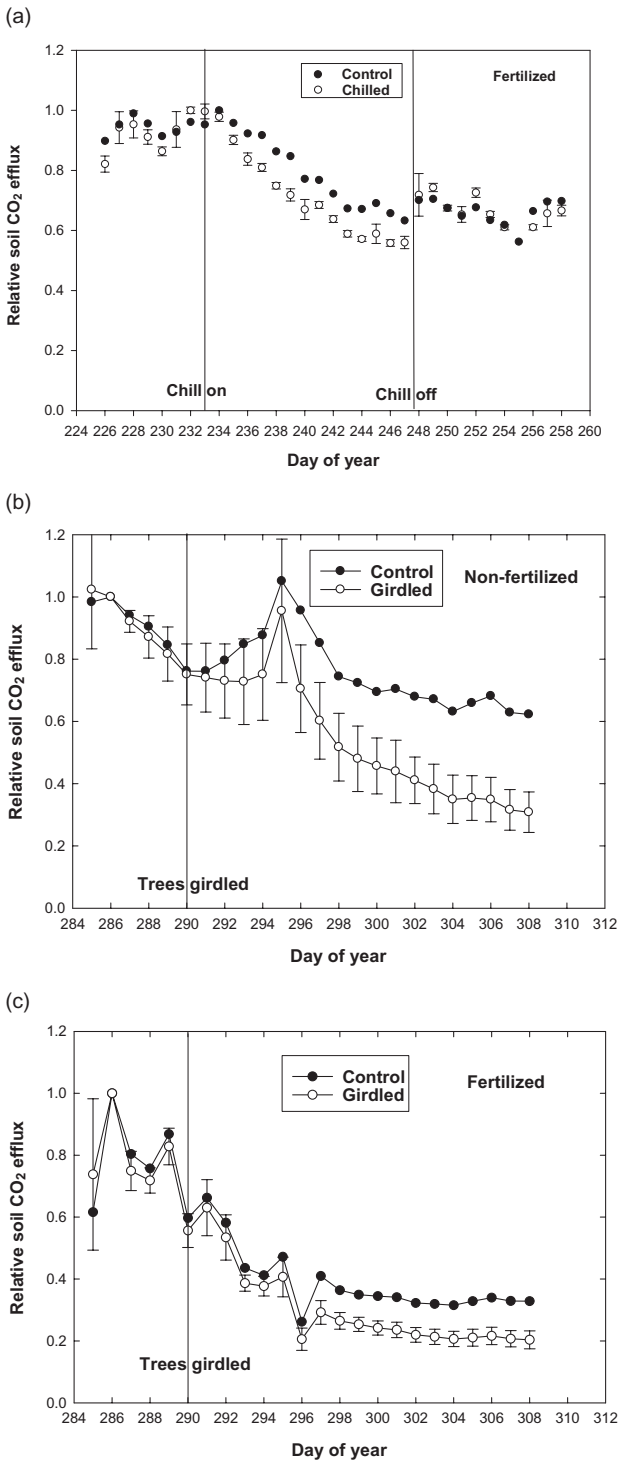

Figure 3. Relative soil CO_2 efflux in loblolly pine stands: (a) within and outside chilled tree plots before, during and after chilling experiment; and before and after physical girdling in (b) fertilized and (c) non-fertilized plots. Ninety-five per cent confidence intervals are shown for chilled and physical girdling treatments.

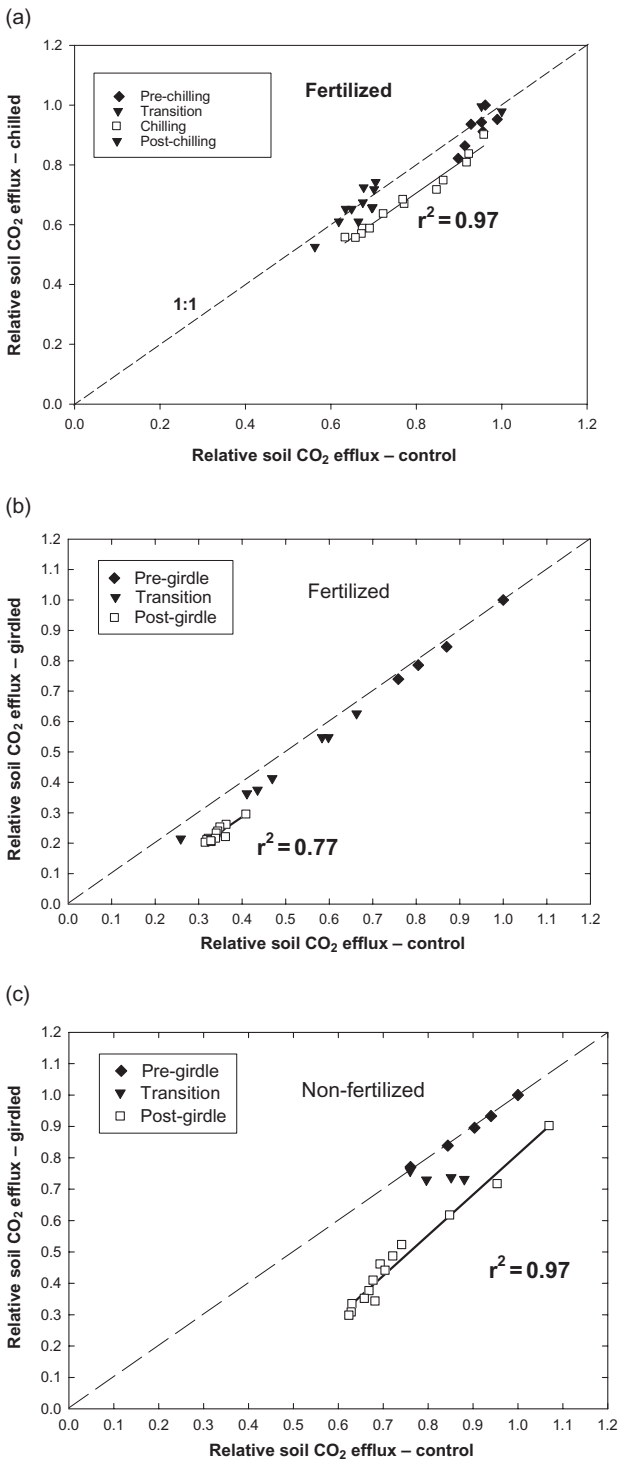

Figure 4. Treatment relative soil CO_2 efflux versus control relative soil CO_2 efflux for (a) chilling study and girdling study on (b) fertilized and (c) non-fertilized plots of loblolly pine. A 1:1 relationship is shown as well as regression lines during chilling and after physical girdling.

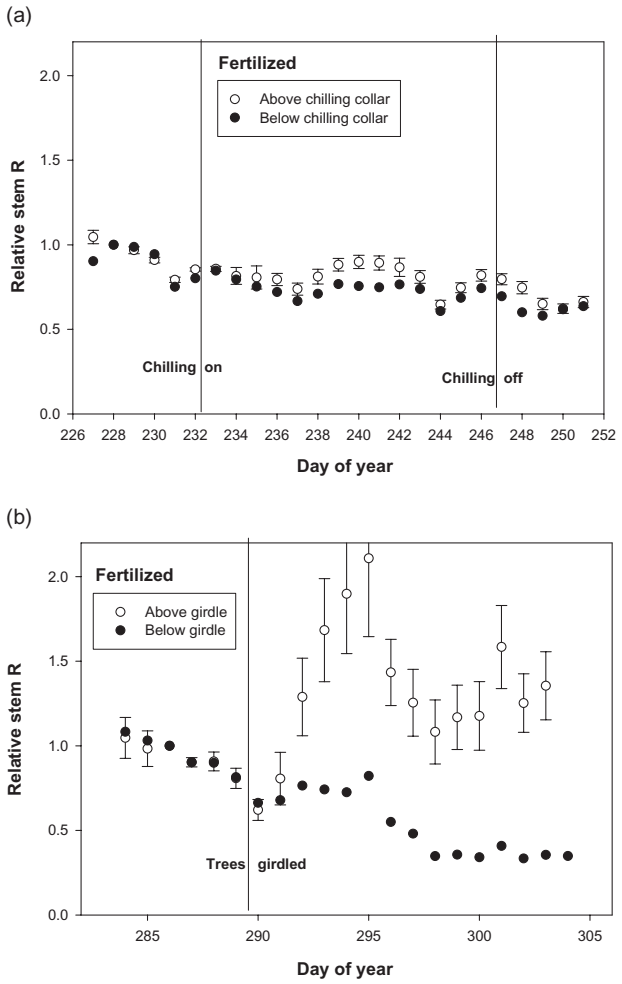

Figure 5. Stem respiration (R) of fertilized loblolly pine trees: (a) of chilled trees above and below the point of chilling before, during and after chilling; and (b) of physically girdled trees above and below the girdle before and after girdling. Ninety-five per cent confidence intervals are shown for chilled and physical girdling treatments.

zone was 71% of that outside the zone (Fig. 7). Similarly, girdling has also reduced soluble soil C (Högberg *et al*. 2001; Scott-Denton, Rosenstiel & Monson 2006). Particularly important, during the chilling period, was the drop in labile material (data not shown). Typical labile components in soil include carbohydrates and amino acids, and are readily utilized by microbes (Morse *et al*. 2000; Högberg & Högberg 2002). These results indicate that chilling the phloem may have reduced C that was allocated below ground and was exuded by roots.

Carbon allocation varies with season as a function of changing source/sink relationships (Thornley 1997), and we believe that this explains the different responses between spring and autumn experiments. The chilling approach used here can be used to quantify such effects and should provide generalized information if tied to growth phenology. Carbon partitioning also varies genetically (Johnsen & Seiler 1996) and is affected by resource availability. Partitioning to fine roots is reduced by increasing the availability of soil nutrients via fertilization (Haynes & Gower 1995; Albaugh *et al*. 1998). Elevated CO_2 may also impact C partitioning both as a direct effect and due to resulting nutrition imbalance. Loblolly pine stands subjected to free-air carbon enrichment (FACE) initially grew faster under elevated CO_2, but the effect only persisted for 3 years after which FACE trees and control trees were growing at the same rate (Oren *et al*. 2001). Fertilization of half of the FACE plot resulted in an interactive growth response greater than the additive combination of fertilization and elevated CO_2. In the same study, elevated CO_2 also increased soil CO_2 efflux, but fertilization resulted in marked decreases (Butnor *et al*. 2003). The smaller reduction in soil CO_2 efflux due to girdling in fertilized versus control plots in our study supports the contention that total C allocation below ground is reduced as soil fertility is increased. From the results of the FACE

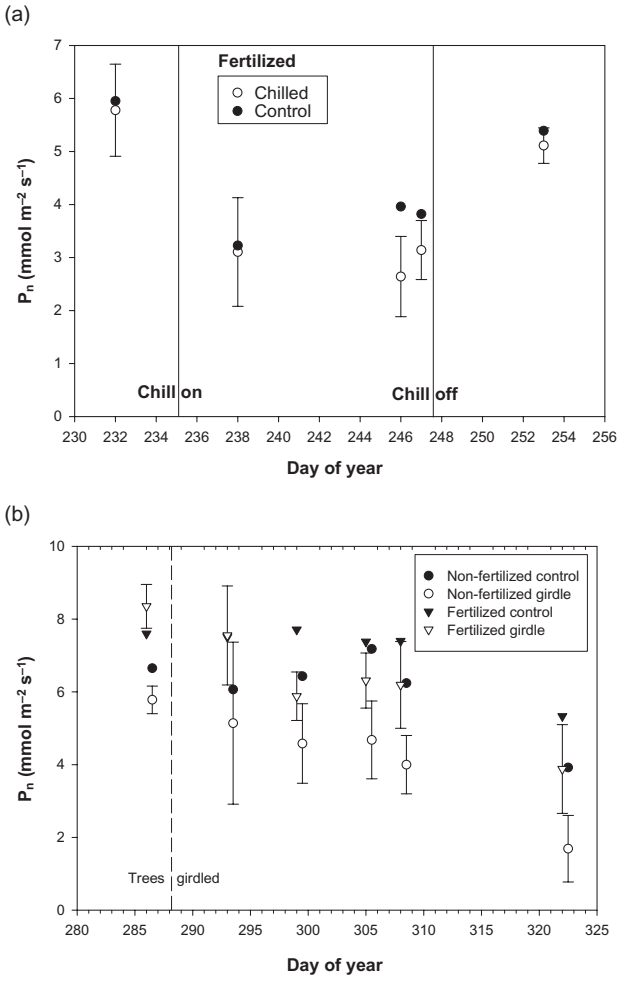

Figure 6. Net photosynthesis (P_n), (a) before, during and after chilling period, and (b) before and after physical girdling. Ninety-five per cent confidence intervals are shown for chilled and physical girdling treatments. Note: Although all treatments were on the same days, fertilized and non-fertilized treatments are offset to improve clarity.

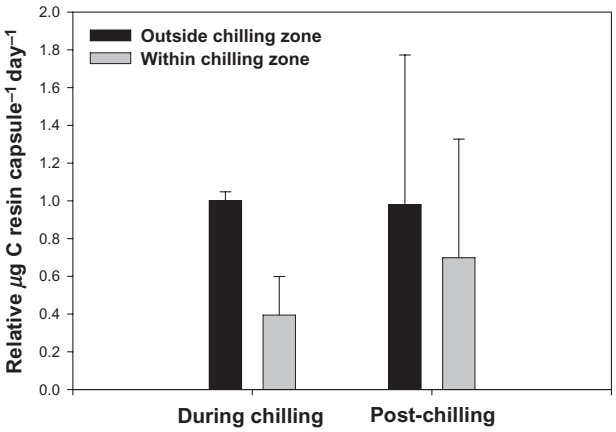

Figure 7. Soluble C (with 95% confidence intervals captured on resin capsules per day during and after chilling period, within and outside chilled tree plots.

experiments and our chilling study, it appears that while under nutrient limitations, trees have reduced C sinks above ground and more C is allocated below ground into additional fine root biomass (unpublished data), and increased root C exudation and increased respiration by mycorrhizal fungi (Schäfer *et al*. 2003; Hobbie 2006; Scott-Denton *et al*. 2006).

This experiment indicates that reversible physiological girdling of field-grown trees is possible and results in blockage of phloem transport below ground. Although the magnitude of decreases in soil CO_2 efflux was similar in the fertilized stands subjected to chilling or physical girdling, it is still unclear what percentage of C partitioned below ground was decreased via chilling because different stands were measured in different years. On one hand, the much smaller increase in stem R above the chilling collar compared with the physical girdle indicates that chilling was less effective than physical girdling. On the other hand, soil CO_2 efflux decreased 4 d sooner with chilling versus physical girdling. In addition, because loblolly pine fine roots have been shown to extend up to 5.8 m from individual trees (Johnsen, Kress & Maier 2005), the reduction in soil CO_2 efflux may have been muted, as a result of the contribution of non-chilled trees. In future studies, we plan to use trenched plots with isolated root systems and examine soil and stem R as function of temperature to better quantify the effectiveness of the chilling technique.

Although we chilled trees for 2 weeks, our data suggest that the procedure could be applied for as little as 3 d to minimize experimental artifacts such as depleting root starch reserves and altering the root–mycorrhizal–soil continuum. The phloem-chilling methodology reported here, if applied seasonally and under varying environmental conditions, can help provide robust estimates of C allocation. Phloem chilling should permit repeating measurements so that in addition to quantifying seasonal variation, responses to varying environments within seasons can be assessed. Such intensive investigations would be difficult to impossible using physical girdling. Such data will greatly improve the prediction of seasonal C allocation with process-based models.

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