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Research paper

Stem compression reversibly reduces phloem transport in *Pinus sylvestris* trees

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Manipulating tree belowground carbon (C) transport enables investigation of the ecological and physiological roles of tree roots and their associated mycorrhizal fungi, as well as a range of other soil organisms and processes. Girdling remains the most reliable method for manipulating this flux and it has been used in numerous studies. However, girdling is destructive and irreversible. Belowground C transport is mediated by phloem tissue, pressurized through the high osmotic potential resulting from its high content of soluble sugars. We speculated that phloem transport may be reversibly blocked through the application of an external pressure on tree stems. Thus, we here introduce a technique based on compression of the phloem, which interrupts belowground flow of assimilates, but allows trees to recover when the external pressure is removed. Metal clamps were wrapped around the stems and tightened to achieve a pressure theoretically sufficient to collapse the phloem tissue, thereby aiming to block transport. The compression's performance was tested in two field experiments: a ^{13}C canopy labelling study conducted on small Scots pine (*Pinus sylvestris* L.) trees [2–3 m tall, 3–7 cm diameter at breast height (DBH)] and a larger study involving mature pines (~15 m tall, 15–25 cm DBH) where stem respiration, phloem and root carbohydrate contents, and soil CO_2 efflux were measured. The compression's effectiveness was demonstrated by the successful blockage of ^{13}C transport. Stem compression doubled stem respiration above treatment, reduced soil CO_2 efflux by 34% and reduced phloem sucrose content by 50% compared with control trees. Stem respiration and soil CO_2 efflux returned to normal within 3 weeks after pressure release, and ^{13}C labelling revealed recovery of phloem function the following year. Thus, we show that belowground phloem C transport can be reduced by compression, and we also demonstrate that trees recover after treatment, resuming C transport in the phloem.

Keywords: belowground carbon transport, carbon-13, carbon partitioning, girdling, soil respiration, stem respiration.

Introduction

Tree belowground carbon (C) allocation is a major route of C flow in forest stands, often accounting for 35–80% of the C assimilated (Raich and Nadelhoffer 1989, Davidson et al. 2002, Giardina et al. 2003, Ryan et al. 2004).

Carbohydrates transported by the phloem to roots provide energy for a suite of physiological process of roots and attached microbial partners including mycorrhizal fungi (Björkman 1944, Colpaert et al. 1996, Binkley et al. 2006, Martin and Slater 2007, Högberg et al. 2009, 2010, Corrêa et al. 2011, Näsholm et al. 2013). Our understanding of belowground tree physiology and the importance of belowground C transport to ecosystem processes has greatly benefitted from girdling studies. These studies, where the phloem transport was terminated by physically removing the conducting tissue, have shown that the soil CO_2 efflux in boreal forests was reduced by 52–65%, implying that belowground C allocation of recently produced photosynthates dominates C-flow in these forest ecosystems (Högberg

et al. 2001, Bhupinderpal-Singh et al. 2003). Results from such girdling studies have been used to estimate the relative autotrophic and heterotrophic contributions to soil CO_2 efflux, and thus to calculate or model ecosystem C balances (Högberg et al. 2001, 2009).

Although girdling has led to key findings, it is destructive and irreversible and may have important side effects, including depletion of root starch reserves, causing an increased mortality rate of roots and their fungal symbionts (Marshall and Waring 1985). The increase in substrate enhances soil heterotrophic activity of decomposition, frustrating interpretations of soil CO_2 efflux (Högberg et al. 2001). Furthermore, the irreversibility of girdling necessitates use of different groups of trees serially, thus adding spatial to temporal sources of variation, further complicating extraction of the signal of treatment effect from background variance.

Localized chilling of stems or petioles have been proven effective in reducing C transport on several herbaceous species (Swanson and Geiger 1967, Giaquinta and Geiger 1973, Goeschl et al. 1984). This methodology, termed physiological girdling, is also effective on trees (Johnsen et al. 2007). It reduced soil CO_2 efflux of fertilized loblolly pine plots by 9%, which indicates lower C flux belowground (Palmroth et al. 2006). The methodology is based on lowering the temperature over the circumference of a section of phloem to increase viscosity of the phloem sap, thereby reducing its flow rate. The treatment had similar effects to girdling, but was shown to be reversible. Successful, reversible, blockage of phloem transport in 3-year-old oak trees (*Quercus robur* L.) was recently demonstrated using physiological girdling (De Schepper et al. 2011). Although numerous articles based on the method of chilling to reduce phloem transport have been published (Swanson and Geiger 1967, Gould et al. 2004, Zwieniecki et al. 2004, Peuke et al. 2006, De Schepper et al. 2011), Johnsen et al. (2007) is the only experiment to apply it to the stems of mature trees in a field setting. This may be because physiological girdling in the field requires rather extensive investments in terms of equipment and labour.

We hypothesized that applying an external pressure on the phloem tissue could also decrease phloem conductivity, compressing the sieve tubes and thus decreasing sap flow. If so, it would provide a simple, reversible alternative or complement to the above-described techniques. An external pressure that exceeds the turgor pressure of phloem cells would be required to compress them. Though the phloem is pressurized, we assumed that the tissue surrounding it is soft and would offer little resistance to compression against the relatively hard sapwood underlying it. Additionally, since the sieve cells are interconnected, we hypothesized that removing the pressure would allow sap from adjacent sieve cells to flow into and expand the compressed cells, re-establishing the flow path, thus making the treatment reversible. Alternatively, damaged but living phloem and cambial cells may re-establish phloem function once pressure is released.

The principle difference between stem compression and phloem chilling (Johnsen et al. 2007) is that while compression aims to reduce sieve tube conductivity by collapsing the sieve cells, chilling lowers sap temperature to increase the viscosity of the phloem sap. Both methods would result in a gradual steepening of the gradient in sap sugar concentration across the restriction, leading to an inflow of water and increasing pressure above the blockage. This pressure gradient could eventually counteract the block, although it would be necessary to account for the effect of rising sugar concentration on sap viscosity to be sure. On the other hand, compression may damage phloem tissue, slowing the recovery of phloem C transport, perhaps even until after growth of new phloem tissue.

The aim of the current study was to investigate whether belowground C transport of *Pinus sylvestris* (L.) trees can be reversibly reduced by physical compression of the phloem. To determine the effectiveness of the treatment, we used $^{13}CO_2$ canopy labelling and measured phloem ^{13}C contents as well as stem respiration, phloem sucrose content and soil CO_2 efflux. Hypothetically, starving the roots should also lead to reduced root and mycorrhizal vigour. For this reason, we also measured the concentration of carbohydrates in mycorrhizal root tips. Finally, we compared the effects of compression with those of traditional girdling.

Materials and methods

Study site

Two experiments were conducted in adjacent Scots pine stands: one containing large trees [~15 m tall, 15–25 cm diameter at breast height (DBH)] and the other containing smaller trees (2–3 m tall, 3–7 cm DBH). The smaller trees were more suitable for our whole-crown $^{13}CO_2$ labelling experiments and the older stand was better suited for studying the effects of treatments on soil CO_2 efflux. Both stands were located in northern Sweden (64°14′N, 19°46′E and 175 m above sea level). The soil is weakly podsolized sandy silt sediment, with a field layer consisting mainly of lichens in the young stand and Ericaceous dwarf shrubs under the mature trees. The dominant shrub species were *Vaccinium vitis-idaea* (L.), *Calluna vulgaris* (L.) Hull and *Vaccinium myrtillus* (L.). Both stands were established by natural regeneration after a clear-cut.

Stem compression technique

Steel bands resembling hose-clamps were tightened around the trees' stems at breast height to collapse the sieve cells of the phloem. The compression was provided by three clamps positioned directly adjacent to one another, compressing a section of the phloem 7.5 cm wide (Figure 1a). The clamps had a bolt on one side that, when tightened, pulled their ends together, reducing the

Figure 1. (a) The compression treatment. Three metal clamps (each 2.5 cm wide) were fixed to the stems at 1.3 m aboveground and tightened to a gauged pressure of 2.4 MPa. (b) The portable chamber and IRGA used to measure stem respiration at two heights on the stems, here attached to a control tree.

clamps' circumference. The open area between ends (beneath the bolt) was bridged by a sliding metal section, so that pressure was applied around the stem. Before clamps were attached to the stems, the thick outer bark was shaved off to facilitate an even pressure distribution around the tree. The stems were wrapped in Teflon tape to reduce friction as the clamps were tightened.

To ensure that all clamps were equally tightened, a torque wrench was used. Before the experiment, the maximum applied radial pressure was determined by placing four small pressure sensors (Tactilus free-form system, Sensor Products Inc., Madison, NJ, USA) at even intervals around the stem of one tree. We found that 2.4 MPa approached the maximum pressure that could be exerted on the stem before the clamps failed. This pressure is theoretically sufficient to overcome phloem turgor pressure (Hammel 1968, Sovonick-Dunford et al. 1981, Nikinmaa et al. 2014) and thus collapse the cells, terminating phloem sap flow. Thus, using the torque wrench, all clamps in the study were tightened to ~2.4 MPa. The clamps did not distribute the pressure evenly around the stem, resulting in a highest pressure (~2.4 MPa) directly under the bolt. Therefore, the bolts were positioned 120° in relation to each other, minimizing the pressure variation around the circumference and keeping the minimum pressure applied to >1.5 MPa (Figure 2).

Girdling was performed using a knife to completely remove the bark, phloem and cambium down to the sapwood, around the whole circumference of the stem. The vertical extent of girdling was 3–4 cm, which corresponds to about half the vertical width of the compression.

Experiment layout

Experiment 1: small trees Seven small Scots pine trees, between 2 and 3 m tall, were selected and randomly assigned to the

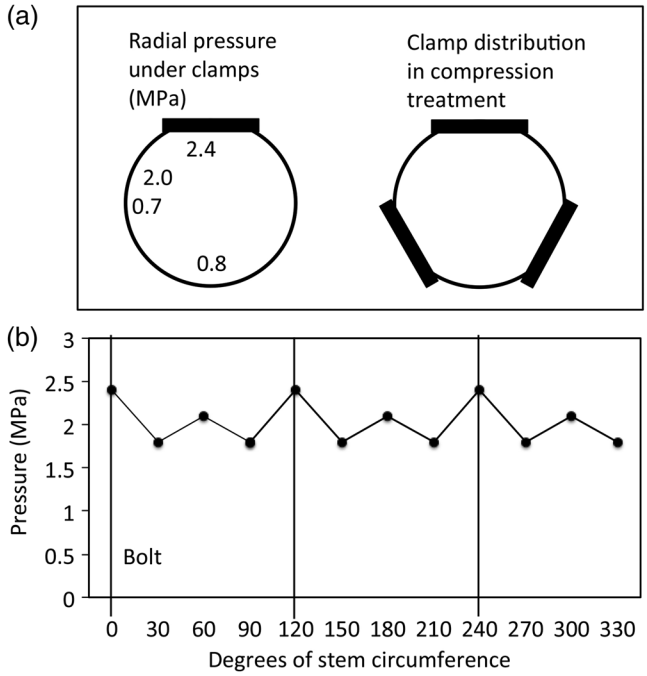

Figure 2. Radial pressure exerted on tree stems by the compression clamps. (a) The highest pressure of 2.4 MPa was found under bolts, dropping to 0.7 MPa by 90° to both sides and remaining so through the opposite side of the stem. The three clamps were arranged to minimize the effect of the pressure gradient. (b) A representation of the pressure distribution around the stem based on pressure measurements under one half of the circumference (shown in (a)). Vertical lines indicate the positions of the three clamps' bolts.

treatments. On 19 July 2013, three of the trees were compressed, three were left as untreated controls and one was girdled as an example of fully interrupted phloem C transport.

Each tree was then enclosed in a clear plastic chamber (~3.3 m^3), into which 1000 ml of $^{13}CO_2$ gas (99.9 atom% ^{13}C, BOC Spectra Gases, St Neots, Cambridgeshire, Great Britain) was injected. To avoid any labelled C being assimilated directly into the stem below the blockage, the stem was covered in black plastic from the ground up to the blockage height during the labelling. The ground inside the chambers was also covered in plastic to prevent $^{13}CO_2$ from diffusing into the soil and to prevent dilution of the ^{13}C fraction of CO_2 in the chamber by an efflux of unlabelled CO_2 from the soil.

The CO_2 concentration was monitored by an infrared gas analyser (IRGA; Vaisala CARBOCAP®, Vaisala Oyj, Helsinki, Finland) placed inside the chamber. Air temperature was controlled by a split type air conditioning unit (Argoclima S.p.A. Gallarate, Italy, Oscar twin 9A and 14A). The set-up was left in place until the IRGA showed that the CO_2 concentration inside the chamber levelled out, indicating that the tree was no longer drawing it down. Typically, this took 1.5 h and occurred at ~100 mol mol^{-1} below ambient concentration. To determine how much assimilated ^{13}C had passed below the compression, phloem samples were collected 1, 2 and 7 days after labelling, from above and below the blockage height.

The pressure was released after 24 days, and the following year (23 May 2014), a second labelling was performed to test if and to what extent phloem transport had resumed. During this second labelling, we noted that the girdled tree only managed to draw down the chamber CO_2 concentration by ~70 p.p.m., before it levelled out.

The values of ^{13}C content (expressed in atom% excess) were log-transformed to homogenize the variance and compared between treatments by analysis of variance (ANOVA). Means were then separated by a Tukey honest significant difference (HSD) test. Student's *t*-test was used to compare the two sampling heights within treatments.

Experiment 2: large trees Nine circular, 4-m radius plots were established in the mature Scots pine stand, each including 10–15 trees. Three plots were randomly assigned to each of the three treatments: untreated controls, traditional girdling and compression. All trees within a particular plot were given the same treatment. All treatments were initiated at the peak of the growing season, on 19 July 2013; the compressed trees were released after 27 days (on 15 August).

Stem respiration

Stem CO_2 efflux was measured on six trees per treatment, two in each plot, at two positions on the stem, separated 40 cm vertically, equidistant from the blockage located at 1.3 m aboveground. The aim was to quantify the effect of the blockage on respiration upstream and downstream of the blockage.

The chosen trees were fitted with equipment for attaching a custom-made chamber containing an IRGA (Vaisala CARBOCAP®, Vaisala Oyj) for repeatedly measuring stem respiration. The set-up consisted of a custom-made 'frame' of thick PVC plastic attached to the tree stem and sealed using foam gaskets of ethylene propylene diene monomer rubber and rubber putty (Casco häftmassa, Akzo Nobel Bygglim AB, Stockholm, Sweden). The backs of the frames were designed to follow the curvature of the stem, while the front edge was flat. The enclosed stem surface area was ~215 cm^2. The IRGA was inserted into a PVC chamber designed to be clipped onto a stem frame for measurement (Figure 1b). The air inside the IRGA chamber was stirred with a small fan. Before each measurement, the seal was checked using a syringe to introduce a slight overpressure through a septum in the chamber wall—any leak would then show up as a pressure drop on a U-tube manometer attached to the chamber side.

During a measurement, the chamber was sealed against the stem while the IRGA recorded the internal CO_2 level and air temperature. The chamber was left in place until the CO_2 concentration had reached 40 p.p.m. above ambient at the time of measurement. Stem respiration was measured on 10 days during the 66-day study period, focusing mostly on the period during and immediately following the compression. Simultaneous measurements were made above and below the treatment once per day for each tree (in total 18 trees) during each measurement occasion. The measurements were conducted between 10 a.m. and 2 p.m. and took ~3 h to complete. The order in which the trees were measured was chosen randomly for each sampling date. The data were expressed as the ratio of above/below blockage position before being tested statistically using ANOVA and Tukey's HSD test.

Phloem and fine root carbohydrate content

A 5-mm hole-punch was used to collect phloem samples from above and below the treatment height on three different days during the study period (26 July, after 7 days of compression; 13 August, after 25 days of compression; and 3 September, when the compression bands had been removed for 19 days). On each day, 13–15 samples were collected from each treatment and stem position. Soluble sugars were extracted by diffusion in pure deionized water (Devaux et al. 2009).

Before the experiment, this simple method was compared with exudations with solutions containing a chelating compound (polyphosphate buffer) to prevent callose from sealing the sieve elements. The test was performed on 20 samples taken from the same tree, 10 for each method. Phloem exudates (μg C mm^{-2} phloem) from the two methods did not differ significantly from one another (paired *t*-test, $P = 0.23$). The simpler deionized water exudation method has also been found accurate for analysis of stable isotope composition in organic compounds transported in the phloem (Gessler et al. 2004).

Fine roots were collected 4 weeks after the commencement of the compression treatment (on 14 August) and again 3 weeks after pressure release (on 11–13 September). To be certain that the roots indeed belonged to a particular tree, root sampling always began by digging along the roots at the tree's base until they branched into fine roots. All roots were collected from the humus layer just above the mineral soil. Roughly 2 ml of fine roots were collected per tree, and four to five trees per treatment were sampled on each occasion.

The collected roots were analysed for concentrations of soluble carbohydrates to determine the degree to which the treatments had depleted their C reserves. For the extraction of soluble sugars, 50 mg of freeze-dried and milled root samples were extracted twice in 0.5 ml of 80% ethanol containing 4 mM HEPES (pH 7.5 with KOH). Samples were thereafter shaken, heated at 80 °C for 30 min and centrifuged 15 min at 14,000 r.p.m. The supernatant was removed and kept cold while the pellet was extracted once more. The procedure was repeated twice thereafter with the ethanol concentration decreased to 50%. Supernatants were collected, mixed, evaporated and analysed. Soluble sugar concentrations were determined by ion chromatography (Metrohm, IC Net 2.3 with the column Metrosep Carb 1–250 run isocratically with the eluent 0.1 M NaOH at 1 ml min^{-1}). Root carbohydrate concentrations were log-transformed to homogenize the variance. We performed an ANOVA followed by mean separation using

Tukey's HSD test to compare sugar concentrations in phloem exudates and roots between treatments. Within-treatment comparisons were performed by Student's *t*-tests.

Soil CO_2 efflux

Soil CO_2 efflux from three soil collars placed in the centre of each plot was measured using a LiCor-6400xt (Li-Cor Biosciences, Lincoln, NE, USA). The collars' interior was kept free of ground and field vegetation. Measurements were taken on 10 occasions, once before treatments began (16 July), four times during the month of compression (22 July–14 August) and five times during the month following pressure release (16 August–19 September). Values from the three collars within a plot were averaged, and this average efflux rate was then used in further calculations to represent the plot's soil CO_2 efflux.

There was some variation in soil CO_2 efflux among plots before treatments began. To remove these differences among plots from the analysis, the efflux rate from each plot was normalized by multiplying it by a calibration factor:

$$(\text{Recalibrated efflux})_{\text{Day } t} = \text{efflux}_{\text{Day } t} \times \left(\frac{\text{efflux}_{\text{pre}}}{\text{mean control efflux}_{\text{pre}}} \right)$$

where $\text{efflux}_{\text{Day } t}$ is the measured soil CO_2 efflux on a given day, $\text{efflux}_{\text{pre}}$ is the measured efflux in the current plot, prior to treatment initiation and mean control $\text{efflux}_{\text{pre}}$ is the mean pretreatment efflux in the control plots. Thus, the measured efflux rates on each measurement occasion were recalibrated to account for non-treatment-related variation between plots. This recalibration relies on an assumption that non-treated respiration rates remain relatively the same among the plots over the study period. We believe this assumption to be reasonable based on the apparent homogeneity of the studied stand and the close proximity of the experimental plots to each other. The assumption of the non-treated rates changing in parallel is also supported by the, mostly, small standard errors shown for the mean of the control plots (see Figure 7a).

Recalibrated effluxes were then normalized in relation to daily control means, in order to show how compression and girdling affected soil CO_2 efflux relative to control plots. Repeated-measures ANOVA was employed to detect possible treatment effects during the course of the experiment. Where significant effects were found, means were separated by a Tukey HSD test ($\alpha = 0.05$).

Results

In Experiment 1, compression blocked the transport of ^{13}C label below blockage height (<0.0003 atom% excess; $P = 0.033$). In contrast, the label intensity in control trees was similar above and below the blockage height ($P = 0.9$), indicating that the label moved freely down the stems (Figure 3).

In Experiment 2, compression reduced sucrose concentration in phloem samples taken below the blockage to <50% of that above the compression ($P = 0.0016$) and 50% lower than the control trees ($P = 0.0006$). This reduction was similar to the effect of girdling ($P = 0.0118$; Figure 4). There were also treatment effects on total soluble C in the phloem: 1 week after treatment, total soluble C content below the blockage in compressed and girdled trees was ~25% lower than above ($P < 0.032$ and <0.017, respectively, Figure 4) and ~35% of controls ($P < 0.003$ and <0.0015, respectively). However, 19 days after the compression on trees had been released (46 days since the treatments began), soluble C contents of phloem in the compressed trees were statistically indistinguishable from the controls ($P = 0.301$). Conversely, at the same time the girdled trees

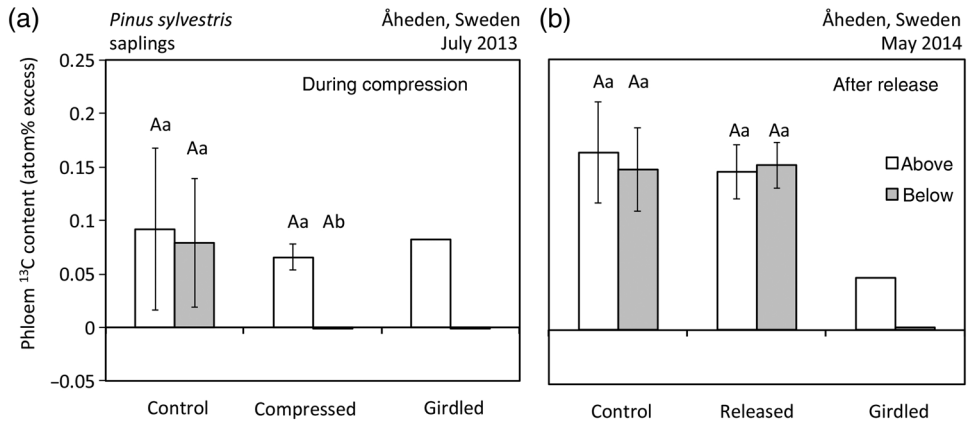

Figure 3. Phloem sap ^{13}C content (atom% excess; mean ± SE) in samples above the blockage position (open bars) and below it (filled bars). The considerable length of the control trees' error bars is due to one of the trees assimilating over 10 times more ^{13}C than the others. This is attributed to weather conditions as well as a larger amount of foliage. Different uppercase letters indicate differences between treatments (ANOVA). Different lowercase letters indicate a difference between stem positions within a treatment (Student's *t*-test). (a) Results from labelling the season of the compression treatment and (b) results from labelling the following spring.

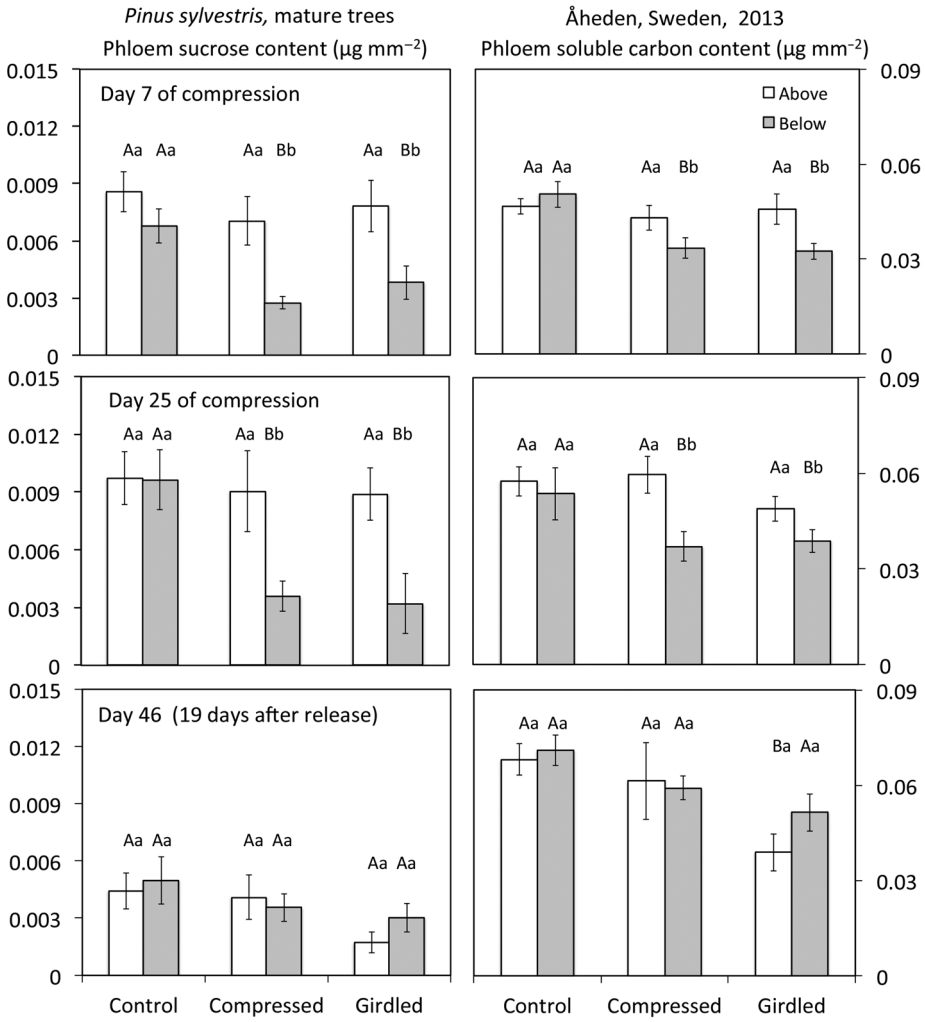

Figure 4. Sucrose and total soluble C concentration (µg mm^{-2}; mean ± SE) in phloem samples taken from stems above treatment (open bars) and below it (filled bars). Different uppercase letters indicate differences between treatments (ANOVA, Tukey's HSD test, $P < 0.05$). Lowercase letters indicate within-treatment differences (Student's *t*-test, $P < 0.05$).

displayed a 33% lower phloem C content above the girdle than controls ($P < 0.035$).

Both girdling and compression affected stem respiration and for both treatments, the effects were mainly in the form of an increased respiration above blockage, rather than a decrease below it. Stems of compressed trees displayed equally high rates of respiration above and below the compression for at least a week after pressure was applied, but after 13 days, the respiration above was 2.5 times greater than below the compression ($P = 0.02$). During the rest of the time, the compressions were in place, and for nearly 3 weeks after the pressure was released, the trees respired at nearly double the rate above the blockage as they did below (Figure 5). Examining the ratio of stem respiration above/below the blockage position (A/B ratio), the compressed trees' ratios were still significantly higher than the controls' 19 days after release. However, on the final measurement occasion, their ratio became similar to, and no longer significantly different from, control trees ($P = 0.112$). Girdling led to a threefold increase in the A/B ratio of stem respiration, and girdled trees continued to differ from the controls until the end of the study period ($P = 0.0001$).

Fine roots were collected in mid-August, when the phloem had been compressed for ~4 weeks, and then again in mid-September, ~4 weeks after pressure was released. In control trees, the sugar profile was dominated by glucose and fructose, at ~20 µmol g^{-1} fresh weight, followed by sucrose, at ~5 µmol g^{-1} fresh weight. In mid-August, no significant difference between treatments was detected ($P = 0.273$). In September, however, significantly less mannitol was detected in girdled trees than in the released (previously compressed) trees ($P = 0.0356$). No significant differences were detected in sucrose concentrations of fine roots. Additionally, compressed trees had significantly less glucose than controls ($P = 0.031$), and girdled trees had less glucose and fructose ($P < 0.001$). Roots of the control trees displayed the greatest variability in soluble C content, and girdled roots showed the smallest (Figure 6).

Soil CO_2 efflux increased almost twofold in control plots during the study period ($P = 0.016$, repeated-measures ANOVA). Initially, the mean efflux on control plots was just over 2 μmol m^{-2} s^{-1}, but within 3 weeks (6 August), it increased to ~5 μmol m^{-2} s^{-1} (Figure 7a). This was expected since belowground C partitioning is greatly increased during this part of the growing season (Högberg et al. 2001). Conversely, neither girdled nor compressed plots changed significantly over time ($P > 0.05$). However, after correcting the plots' rates for pretreatment inter-plot differences, significant treatment effects could be detected (Figure 7b) by repeated-measures ANOVA and a subsequent mean separation test (Tukey's HSD test, $\alpha = 0.05$): After 3 days of compression, soil CO_2 efflux in compressed tree plots had been reduced to 80% of control plots ($P < 0.05$). Compressed plots continued to have a mean soil CO_2 efflux of 60–66% of the controls' throughout the treatment period ($P < 0.05$). After pressure release, compressed plots were no longer statistically separated from control plots or girdled plots except on the fourth day after release, when their soil CO_2 efflux was significantly lower than the controls (repeated-measures ANOVA, Tukey's HSD test, $P < 0.05$), but not different from girdled plots. Girdling, on the other hand, reduced soil CO_2 efflux to 50% compared with control plots within 18 days ($P < 0.05$), and this effect remained throughout the entire measurement period (Figure 7b).

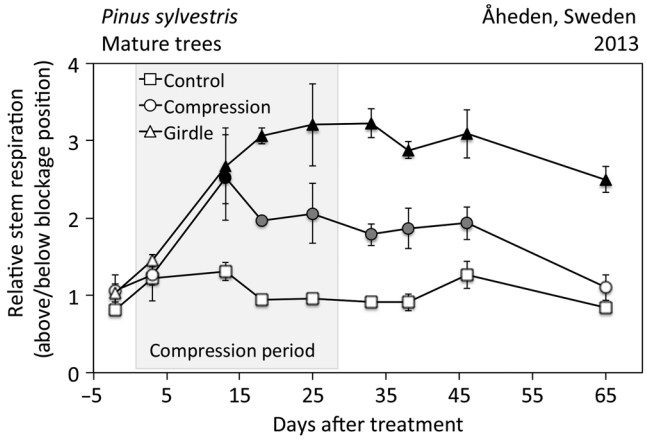

Figure 5. Stem respiration displayed as the ratio between the positions above and below treatment (mean ± SE). Different colours (black, grey, white) indicate significant differences between treatments on a particular day, and signify that a treatment was not significantly different from either of the others (ANOVA, Tukey's HSD test, $P < 0.05$). All treatments (compression and girdling) were initiated on Day 0, and the shaded area indicates the duration of the compression treatments (27 days).

Discussion

Stem compression successfully terminated belowground C transport in the smaller, ^{13}C-labelled trees used in the first experiment. Carbon transport resumed the following spring as shown by a second canopy labelling (Figure 3). In the larger trees of

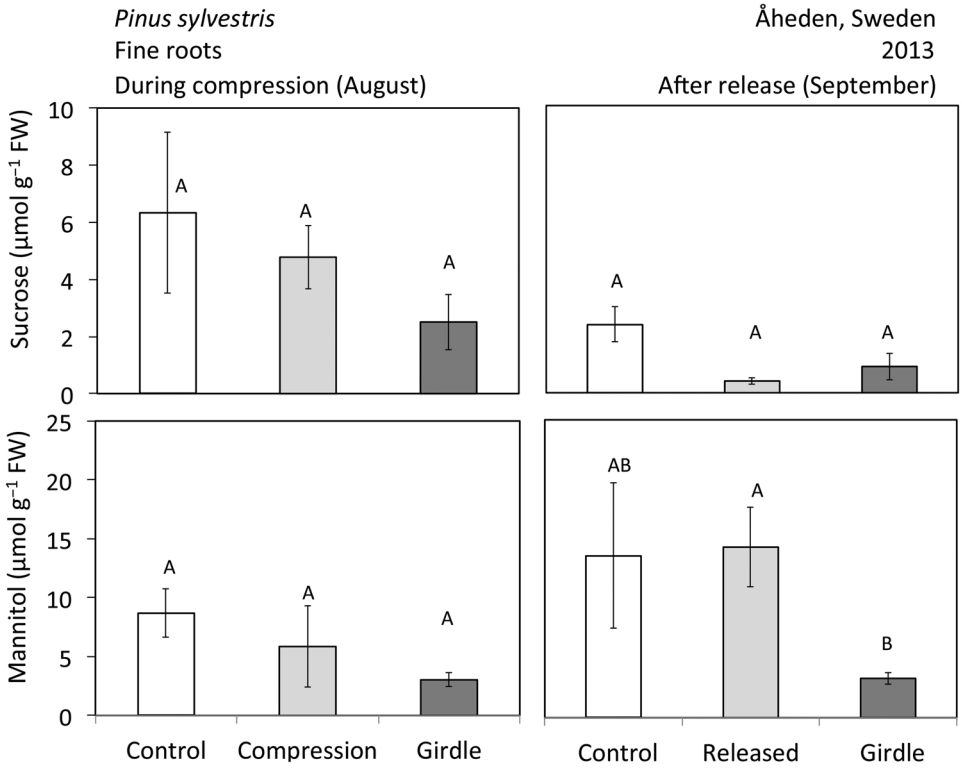

Figure 6. Mannitol and sucrose concentrations (μmol g^{-1} fresh weight (FW); mean ± SE) in fine roots of mature *P. sylvestris* trees. Samples were collected 7, 25 and 46 days after treatment initiation. Different letters indicate that treatments were significantly different (ANOVA, Tukey's HSD test, $P < 0.05$).

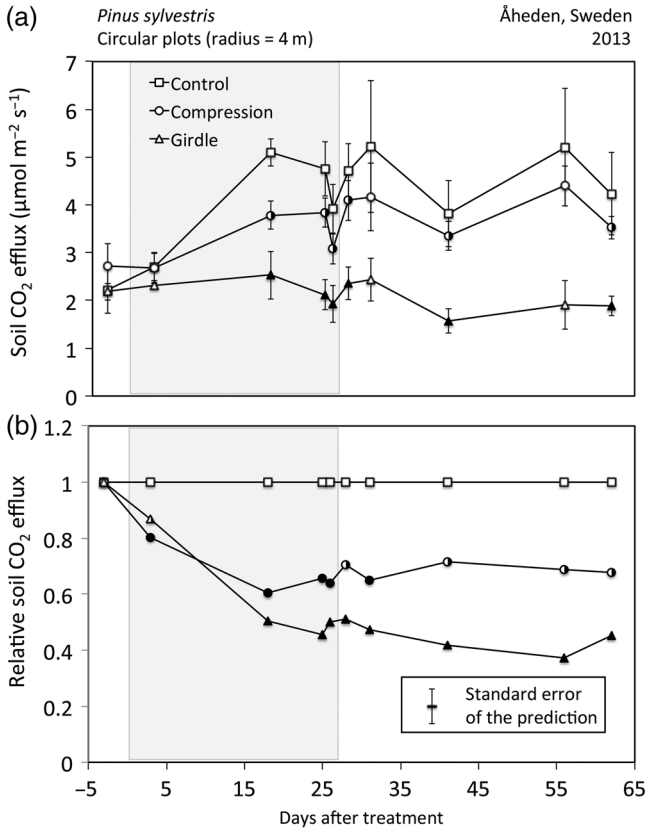

Figure 7. Soil CO_2 efflux rates of plots subjected to different treatments. (a) Measured efflux (µmol m^{-2} s^{-1}; mean ± SE). All treatments were applied on Day 0, and the shaded area indicates the compression duration (27 days). Treatment daily means were compared using repeated-measures ANOVA. Means were separated by a Tukey HSD ($\alpha = 0.05$) test where the ANOVA detected significant differences between treatments. Different colours (black and white) indicate significant differences between treatments, and two-toned markers signify that a treatment was not significantly different from either of the others. (b) Least-square means of the normalized CO_2 efflux relative to the control plots. Prior to normalization, the measured efflux rates were multiplied by a calibration factor to account for pretreatment variation between plots. According to repeated measurements analysis (means separated by Tukey's HSD test, $\alpha = 0.05$), the relative soil CO_2 efflux in compressed plots dropped significantly 3 days after pressure was applied. Girdled plots dropped to 50% of control plot efflux after 18 days. Different colours (black and white) indicate significant differences between treatments, and two-toned markers signify that a treatment was not significantly different from either of the others.

the second experiment, phloem sucrose concentrations below the blockage were equally reduced by compression and girdling (Figure 4), but stem respiration and soil CO_2 efflux of compressed trees were intermediate between control and girdled trees (Figure 5). Within 19 days of clamp removal, effects of compression on either stem respiration or soil CO_2 efflux were no longer detectable. Although the mechanism of phloem recovery cannot be inferred from the current study, the results indicate that compression did reversibly reduce phloem conductivity, as hypothesized. Functionality of the compressed phloem cells may then have been re-established after release, and/or new cells might have been produced to replace damaged sieve cells.

The effect of girdling on soil respiration, a 50% reduction, was consistent with previous studies in the same geographical area (Högberg et al. 2001), but was higher than a 26% reduction reported in a study of unfertilized *Pinus taeda* (Johnsen et al. 2007). Compression reduced soil CO_2 efflux by ~34% (Figure 7).

A variation on the same principle as employed in the current study was used by Björkman (1944), who studied mycorrhizal dependency on current photosynthate from potted seedlings by twisting a metal wire around their stems. On some of his seedlings, the wire was left in place for an entire vegetation period, and on others, it was removed after 3 months. The treatment successfully reduced phloem carbohydrate concentration and the abundance of mycorrhizal fungi was significantly lower. However, the question of reversibility was not emphasized. The damage inflicted on phloem and cambium in Björkman's experiment was probably greater than in our current study, thus might be considered partial girdling (Hamada et al. 2009).

Our study cannot determine conclusively why compression had a smaller effect on soil CO_2 efflux than girdling. Compression might produce a less complete blockage than girdling on large trees, especially between bolts positions where the pressure was lower (Figure 2). This contrasts with complete blockage in the small trees as was demonstrated by the ^{13}C labelling (Figure 3). In either case, compression could indeed be used to quantitatively manipulate the degree of flow reduction in the phloem, which would have useful implications and could be further developed for future research. Nevertheless, it seems that either a higher pressure or a more evenly distributed pressure is required in the larger trees to eliminate phloem conductivity.

The maximum pressure achieved by the three clamps used in this study was slightly higher than reported measurements of turgor pressures in white ash (*Fraxinus americana* (L.)) and red oak (*Quercus rubrum* (L.)), which ranged from a low of 0.5–1.2 MPa to a high of 1.4–2 MPa (Hammel 1968, Sovonick-Dunford et al. 1981). This is also similar to phloem turgor in Scots pine, as modelled by Nikinmaa et al. (2014). However, due to the clamp design, the maximum pressure was restricted to a radial section, while the rest of the circumference experienced pressure not quite sufficient for complete blockage of phloem flow (Figure 2a). Indeed, a previous attempt using a single clamp, thus compressing a 2.5-cm wide section of phloem but otherwise identical to the three-clamp version, did not produce clear reduction in phloem C transport. We speculate that this may be caused by uneven pressure distribution around the circumference of the stem, resulting in insufficient pressure on part of the phloem (Figure 2a).

The blockage was improved through the positioning of three overlapping clamps (Figure 2b). However, it has been shown that phloem C can laterally circumvent damaged tissue after partial girdling (De Schepper et al. 2013). Therefore, it is possible that the irregularity of pressure distribution may have allowed

some C to pass through the compression by travelling laterally around the highly pressurized bolt locations. This would have been more likely to happen in the larger trees since the area of maximum pressure behind the bolt represented a smaller proportion of the stem circumference than it did on the smaller trees in canopy labelling experiment. Thus, a different design may be necessary for a more complete blockage. Using more clamps, or developing clamps that are not deformed when tightened to ~2.4 MPa, are opportunities for methodological improvement in future work. Another improvement could be to reduce friction between clamp and stem during tightening to better protect the phloem from shearing forces.

It is possible that radial transport of C via ray parenchyma could act to circumvent the compressed sections, resulting in incomplete blockage. Such radial transport has been observed in girdling studies (Zwieniecki et al. 2004). It is usually not observed in phloem-chilling studies (Handley 1939, Johnsen et al. 2007, De Schepper et al. 2011), although there are exceptions (Gould et al. 2004). However, since the vertical extent of girdling in the current study was less than that of compression (3–4 cm, compared with 7.5 cm), it seems unlikely that radial C transport caused the difference in blockage between girdled and compressed trees.

Nevertheless, the doubling of stem respiration above stem compression relative to below it (girdling increased it threefold) and the halving of the phloem sucrose content below compression (same as girdling) suggests that compression was at least as effective in restricting phloem transport as chilling in Johnsen et al. (2007).

Potential applications

Stem compression should prove especially valuable in studies where the advantage of reversibility outweighs the possibility of incomplete phloem blockage or where it is important to avoid side effects like wounding responses. Other authors (Cernusak and Marshall 2001) have also expressed concern that girdling could lead to increased risk of xylem cavitation or fungal infections similar to those caused by mountain pine beetle (Hubbard et al. 2013). We note that girdling caused appreciably lower phloem ^{13}C even above the girdle the following year (Figure 3b), suggesting that a physiological feedback from girdling may have down-regulated photosynthesis.

Other unintended girdling effects include increased soil heterotroph activity and root decomposition. These effects have complicated studies concerning the influence of tree photosynthesis on ecosystem C balances (Bhupinderpal-Singh et al. 2003, Binkley et al. 2006), and they prevent repeated studies in a particular stand. Girdling has also been used to study the importance of root-exuded C on soil N dynamics (Dannenmann et al. 2009) and ectomycorrhizal species diversity (Pena et al. 2010). In such studies, stem compression can potentially provide a more versatile tool than girdling as the pressure could be manipulated to repeatedly reduce or restore the C flow through the phloem. The option of controlling the degree of reduction would also allow researchers to exert dynamic control over belowground C partitioning, analogous to the influence of dispensing fertilization of different amounts on N uptake. In combination, this would facilitate quantifying the response of plants and both associated and free-living soil organisms to controlled supplies of sugars and nitrogen. Considering the large spatial variation in C and N fluxes, the likelihood of successfully quantifying the effects of belowground C flow dynamics produced by phenological and seasonal environment variation is greatly enhanced if done quantitatively, reversibly and repeatedly in the same stands.

Further, physiological models have linked xylem–phloem water exchange to stomatal regulation and leaf gas exchange (Nikinmaa et al. 2013, 2014). Because water exchange between the phloem and xylem is driven by differences in osmotic potential, compression could provide a tool for studying these linkages. Finally, with refinement and measurements of responses to different pressures (perhaps combined with microscopy), we might gain greater understanding of phloem function (Knoblauch and Peters 2010, Turgeon 2010, Ryan and Asao 2014).

In conclusion, compressing the phloem of tree stems to reduce its conductivity could be used in place of stem girdling or chilling for the purpose of experimentally reducing belowground C transport. Our results indicate that phloem function partially recovered within 3 weeks after pressure release, and by the following year, phloem C transport has fully resumed. However, the pressures required may differ between tree species, and methodological improvement is required to ensure complete blockage. Additional studies focusing on quantitatively controlling the degree of reduction, and on the phloem's recovery time, will further enhance the usefulness of the technique.

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Conflict of interest

None declared.

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