Using direct phloem transport manipulation to advance understanding of carbon dynamics in forest trees

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Abstract

Carbon dynamics within trees are intrinsically important for physiological functioning, in particular growth and survival, as well as ecological interactions on multiple timescales. Thus, these internal dynamics play a key role in the global carbon cycle by determining the residence time of carbon in forests via allocation to different tissues and pools, such as leaves, wood, storage, and exudates. Despite the importance of tree internal carbon

dynamics, our understanding of how carbon is used in trees, once assimilated, has major gaps.

The primary tissue that transports carbon from sources to sinks within a tree is the phloem. Therefore, direct phloem transport manipulation techniques have the potential to improve understanding of numerous aspects of internal carbon dynamics. These include relationships between carbon assimilation, nonstructural carbon availability, respiration for growth and tissue maintenance, allocation to, and remobilization from, storage reserves, and long-term sequestration in lignified structural tissues. This review aims to: (1) introduce the topic of direct phloem transport manipulations, (2) describe the three most common methods of direct phloem transport manipulation and review their mechanisms, namely (i) girdling, (ii) compression and (iii) chilling; (3) summarize the known impacts of these manipulations on carbon dynamics and use in forest trees; (4) discuss potential collateral effects and compare the methods; and finally (5) highlight outstanding key questions that relate to tree carbon dynamics and use, and propose ways to address them using direct phloem transport manipulation.

Keywords: girdling, phloem compression, phloem chilling, carbon dynamics, carbon sink, carbon source, allocation, metabolism, wood growth, respiration

1. Introduction

Trees contain the majority of vegetation carbon worldwide (Pan et al., 2013), and forests, dominated and defined by trees, contribute the largest share to the global terrestrial gross primary productivity (Beer et al., 2010). While we have a mechanistic understanding of how carbon is fixed from the atmosphere, our understanding of the fate of assimilated carbon within a forest tree is much poorer (Friend et al., 2014). At one extreme, carbon is transported via the phloem to the lateral meristem, where it can be sequestered in lignified structural tissues for centuries, on the other hand it may almost immediately return to the atmosphere through respiration. Understanding the mechanisms underlying internal carbon dynamics and use in trees (i.e. why, when, and how much carbon is allocated to different tissues and functions), is fundamentally linked to a wide range of physiological processes, such as respiration for growth and tissue maintenance, allocation and remobilization of storage reserves, long-term sequestration in lignified structural tissues, and even feedbacks on carbon assimilation. An integral knowledge of carbon dynamics and use within mature forest trees is fundamental to address questions about the carbon cycle at the forest scale, because it contributes to debates about forest productivity (Körner, 2006), mortality (McDowell et al., 2008; Steinkamp and Hickler, 2015), and hence resilience in the face of environmental change. Indeed, at a global scale, the residence time of carbon in vegetation on land, which is largely a function of internal carbon dynamics and use, is the single largest source of uncertainty in projections of global vegetation carbon under future climate and atmospheric CO_2 (Friend et al., 2014). Most global vegetation models project a significant increase in plant growth and land carbon due to CO_2-fertilisation of photosynthesis (Cramer et al., 2001; Sitch et al., 2008). The prevailing modelling paradigm, also known as source-limitation (Fatichi et al., 2014), simply assumes that carbon gains due to the enhanced assimilation are proportionally passed down to growth in trees, thereby being sequestered for long-periods. However, this paradigm neither comprehensively reflects, nor is entirely

74 compatible with, current knowledge of the controls of plant growth (Körner, 2015). In fact,
75 an increasing number of studies ascribe primary importance to direct effects of
76 environmental conditions on the activity of sink processes, such as wood formation (Rossi et
77 al., 2008). Understanding carbon dynamics and use within forest trees is thus of paramount
78 importance, not least to inform models and improve projections of the global carbon cycle.
79
80 Our eco-physiological knowledge relies strongly on studies performed on herbaceous plants,
81 in which feedbacks and dynamics of sinks and sources of carbon have been extensively
82 researched (reviewed by White et al., 2016). While carbon dynamics and use within a tree
83 are likely dominated by a similar set of processes as in herbaceous plants, there are at least
84 two significant differences: trees have comparatively large stored reserves of nonstructural
85 carbon (e.g. Furze et al., 2018) and much longer signalling and transport pathways. Thus,
86 caution must be exercised when extrapolating from studies of herbaceous plants to infer
87 carbon dynamics and use in trees. Indeed, evidence from past phloem transport
88 manipulations suggests that, like herbaceous plants, trees actively manage source and sink
89 tissues (i.e. Poirier-Pocovi et al., 2018), thus they are not adequately represented by the
90 prevailing modelling paradigm, that models sink activity as a proportion of source activity
91 (Cramer et al., 2001; Friend et al., 1997; Inatomi et al., 2010; Ito and Oikawa, 2002; Krinner
92 et al., 2005; Sitch et al., 2003; Smith et al., 2001; Tian et al., 2011).
93
94 In trees and herbaceous plants alike, carbon — mainly in the form of sucrose — moves
95 along hydrostatic gradients in the phloem, from regions of high (normally near carbon
96 source tissues) to low (normally near carbon sink tissues) concentrations as a result of
97 osmotic effects (originally postulated by Münch (1927, 1930) and reviewed by De Schepper
98 et al., 2013b). Consequently, carbon asserts dual importance; first as a driver of phloem
99 transport and second as a transported resource for growth, energy supply, and signalling.

Although other tissues, such as the xylem, can transport substantial amounts of carbon for a part of the year in species such as *Acer saccharum* (Larochelle et al., 1998) and *Betula pendula* (Sauter and Ambrosius, 1986), carbon in trees is overwhelmingly transported via the phloem (Jensen et al., 2016). Since the phloem acts as the main conduit of transport between sources and sinks of carbon within trees, direct manipulations of phloem transport, such as girdling, phloem compression and phloem chilling (figure 1), are proposed as a cost-effective and logistically simple method to restrict flow from sources to sinks, and study the different impacts of variations in carbon availability on upstream and downstream regions. Some alternative experimental techniques, such as experiments using isotope labelling (Högberg et al., 2007) and CO_2 enrichment (Norby et al., 2004), to investigate carbon dynamics and use in mature, long-lived forest trees are complex and involve expensive and time-consuming approaches. The simple and more complex methods can be complemented by other techniques that manipulate source and sink strength, such as canopy shading (O'Brien et al., 2014), defoliation (Wiley et al., 2017, 2013) or genetic manipulations of translocation (Payyavula et al., 2011). However, for the scope of this review we focus on the three aforementioned methods of direct phloem transport manipulation (i.e. girdling, compression and chilling), because they are relatively easily implemented and still have a large unmet potential to contribute to our understanding of carbon dynamics and use in forest trees.

Direct phloem transport manipulation, in particular girdling, have a rich history with first mentions by Theophrastus more than two thousand years ago (Goren et al., 2004), and published studies on the subject going back more than 250 years (Fitzgerald, 1761). Since Fitzgerald's work on fruiting in 1761, girdling has also become a commonly practiced method in horticulture with many studies exploring its effects on reproduction (as reviewed by Goren et al., 2004). Despite its long and rich tradition, the horticultural focus on

126 reproduction, species and cultivars selected for reproductive properties, and managed
127 conditions (i.e. irrigation, fertilization, low competition) limits the transferability of gained
128 physiological knowledge to forest trees. While acknowledging the large body of horticultural
129 literature on phloem transport manipulations on reproduction, this review focusses on
130 studies performed to understand forest tree physiology. In turn, the issues discussed here,
131 such as unreported methods and tree mortality, may not apply as strongly to horticultural
132 studies. Across all disciplines, inconsistent methods and reporting have hindered the
133 unambiguous interpretation of numerous experimental outcomes. For example, basic terms
134 such as girdling, ring-barking, ringing, banding and notching have been used interchangeably
135 and inconsistently in the literature. Furthermore, previous definitions such as provided by
136 Goren et al. (2004) have focused the methods rather than mechanism. For example, Goren's
137 (2004) definition of "strangulation" prescribes the instrument (here a steel wire), but does
138 not define the pressure that needs to be applied. In general, important details have not
139 always been reported and norms vary between sub-field (horticulture versus silviculture).
140 Girdling studies often omit to report whether the cambium was removed or not, or whether
141 the phloem was fully, partially or not at all removed. This is important as the extent of
142 cambial and phloem removal strongly affects the interpretation of experimental outcomes,
143 as they control the potential for recovery of phloem flow in at least some species (Chen et
144 al., 2014; Pang et al., 2008; Zhang et al., 2011), as well as the effective reduction of phloem
145 transport (Asao and Ryan, 2015; De Schepper et al., 2013a; Jiménez et al., 2017). In fact,
146 partial application (i.e. around only part of the circumference), even over three quarters of
147 the circumference, has not resulted in a detectable reduction of phloem transport (Asao and
148 Ryan, 2015; De Schepper et al., 2013a; Hossain et al., 2004; Jiménez et al., 2017). Trees that
149 survive girdling have often been only partially girdled, or the cambium was only removed
150 over a sufficiently narrow strip, so that regrowth and recovery were possible (Annighöfer et
151 al., 2012). Nevertheless, most trees ultimately die due to stresses induced by complete

girdling of the main bole, although in extreme cases death can be postponed by decades (Noel, 1970). Hence, omitting crucial details, such as which tissues were destroyed during girdling, compromises the reproducibility and interpretability of studies manipulating phloem transport.

We hope that this paper stimulates greater reproducibility and interpretability of future direct phloem transport manipulations studies. To this end, we focus on three established techniques, namely (i) girdling, (ii) compression and (iii) chilling. We define these terms and describe their mechanisms and methodological details. We discuss the impacts of phloem transport manipulation, raise some caveats and challenges associated with these techniques and briefly compare them. Finally, we highlight a set of outstanding questions regarding carbon dynamics and use in trees and propose ways to address these questions using phloem transport manipulation.

2. Description of techniques to manipulate phloem transport in trees

Girdling, phloem compression and phloem chilling (figure 1) are the most common techniques to directly manipulate phloem transport and have been applied for very different purposes (see list of references and objectives over the last decade in supplementary table 1). While girdling has been used in numerous publications for a wide range of applications such as accelerating and investigating successional dynamics (Hardiman et al., 2013) or studies on priorities of nonstructural carbon allocation (Oberhuber et al., 2017), phloem compression and chilling have been pioneered only in recent years with regard to forest tree physiology (important aspects of key studies are listed in Table 1). To our knowledge, phloem compression has been applied only in one published study, on young and mature *Pinus sylvestris* (Henriksson et al., 2015), while phloem chilling has been reported in one laboratory study on young *Quercus robur* (De Schepper et al., 2011), as well as on mature

178 plantation-grown *Pinus taeda* (Johnsen et al., 2007a), and on individual branches of
179 naturally-growing mature *Acer saccharum* (Schaberg et al., 2017).
180
181 *2.1 Definitions*
182 **Girdling** (also known as barking, ring-barking, ringing, banding, cincturing, hacking, frilling,
183 scoring, stripping and notching) is the circumferential destruction of a band of surface tissue
184 with a specific width ($\mathbf{w_g}$ in figure 1) and depth (**d** in figure 1) around the entire woody
185 branch, stem or root.
186
187 Girdling stops phloem flow by simply destroying or removing the conduits. For girdling, the
188 depth of the wound and, more importantly the nature of the destroyed and damaged
189 tissues (bark, cambium, phloem, and/or xylem), determines the potential for recovery and
190 the effectiveness of the phloem transport disruption. In forestry, girdling is often performed
191 using chainsaws or axes to kill trees (Bauhus et al., 2009) or induce desired wood properties
192 (Gref and Ståhl, 1994; Taylor and Cooper, 2002). With such crude methods, the functioning
193 xylem is generally damaged (see figure 2b). In contrast, horticultural girdling is generally
194 performed with specifically designed tools, such as double-bladed knives, to improve crop
195 quantity and quality of fruits without substantial permanent damage and does not destroy
196 the cambium or xylem (Goren et al., 2004).
197
198 **Phloem compression** (also known as strangulation or wiring) as a reversible method of
199 phloem transport manipulation is the temporary application of a circumferential pressure
200 that exceeds the phloem internal pressure, but does not cause permanent damage to the
201 underlying cambium and xylem.
202
203 In contrast to girdling, which severs the phloem, compression restricts phloem flow by either
204 collapsing phloem cells, or damaging them without permanent harm to the underlying

205 cambium. The phloem cells appear able to resume normal function a few weeks after the
206 release of compression in *Pinus sylvestris* and *Pinus strobus,* due to the re-opening, repair or
207 replacement of collapsed and/or damaged phloem cells (Henriksson et al., 2015). Phloem
208 compression has been performed with heavy-duty steel bands (Henriksson et al., 2015) and
209 ratcheted polyester cargo webbing (see figure 2c). Phloem internal pressure, which needs to
210 be overcome for effective transport reduction, is believed to range between one and two
211 MPa (Wright and Fisher, 1980), but measurements of phloem internal pressure are difficult
212 and have only been performed on a few species (e.g. Nikinmaa et al., 2014; Sovonick-
213 Dunford et al., 1981).
214
215 **Phloem chilling** (also known as cold, cold-block or physiological girdling) as an effective
216 method of phloem transport manipulation is defined as a reduction of the phloem
217 temperature to just above the freezing point of water around the entire stem, branch or
218 root without actually freezing any living tissue.
219
220 Phloem chilling causes a temporary blockage of phloem flow within the chilled section due
221 to the exponential increase in sap viscosity with decreasing temperatures towards 0°C. This
222 blockage of phloem flow is only maintained for minutes to hours (Gould et al., 2004; Thorpe
223 et al., 2010). In fact, the increased resistance to flow in the chilled section is eventually
224 overcome by a stronger osmotic gradient resulting from accumulation and depletion of
225 sugar in upstream and downstream regions, respectively (Gould et al., 2004; Thorpe et al.,
226 2010). Despite the mass flow eventually resuming, a large difference in sugar concentration
227 across the zone of chilling persists until chilling is switched off (De Schepper et al., 2011;
228 Ntsika and Delrot, 1986; Peuke et al., 2006).
229
230 **3. Impacts of phloem transport manipulations on carbon dynamics and use**

231 Direct phloem transport manipulation has been shown to effectively modify the availability
232 of mobile carbon both upstream (i.e. in the region with a source activity previously
233 exceeding sink activity; see figure 2a) and downstream (i.e. the region with sink activity
234 previously exceeding source activity; see figure 2a) of the girdling, compression or chilling
235 zone. This manipulation can trigger several feedbacks that can alter the carbon dynamics
236 and use within a tree (figure 3).
237
238 A common outcome of phloem transport manipulation experiments is a reduction in the
239 rate of photosynthesis (De Schepper et al., 2011; De Schepper and Steppe, 2011a; Iglesias et
240 al., 2002; Myers et al., 1999), which is indicative of the fact that carbon source activity can
241 respond to sink activity. Three different hypotheses have been proposed to explain
242 photosynthetic inhibition (figure 3, interaction **B**).
243
244 The first hypothesis proposes that sugar accumulation in leaves results in end-product
245 inhibition (Iglesias et al., 2002), a common response to the removal or sinks (Paul and Foyer,
246 2001). To get high leaf sugar concentrations, a relative reduction in phloem loading
247 compared to carbon assimilation is a prerequisite. Most tree species, that have been
248 characterized, are symplastic loaders (Davidson et al., 2011; Liesche, 2017), and load the
249 phloem passively by diffusion (Jensen et al., 2013). For passive loaders, a high phloem sugar
250 concentration, resulting from a backlog above the treatment zone, would be sufficient to
251 reduce phloem loading and cause an accumulation of sugars in leaves, although evidence for
252 a short-term regulation of phloem export that is independent of rates of photosynthesis and
253 sugar remobilization needs to be acknowledged (Liesche, 2017). In fact, a mechanistic
254 pathway of photosynthesis inhibition, based on low sucrose export from leaves under high
255 phloem sucrose concentrations, has been characterized in sugar beet (Vaughn et al., 2002),
256 an herbaceous symplastic loader. For actively loading species, a reduction in leaf sugar

export may not be achieved at all, but will at least be delayed due to their capacity to continue leaf sugar export even against high phloem sugar concentration (e.g. Öner-Sieben and Lohaus, 2014). The effects on photosynthesis of sugar accumulation in leaves due to reductions in phloem loading have been extensively studied in herbaceous plants (Ainsworth and Bush, 2011), but have rarely been studied in trees. In girdled *Citrus reticulata,* leaf sugar accumulation occurred, resulting in photosynthetic inhibition correlated with a reduced quantum yield in photosystem II (Rivas et al., 2007), but details of the biochemical pathways have not yet been elucidated.

The second hypothesis to explain photosynthetic inhibition proposes that girdling reduces the export of abscisic acid from leaves, and the abscisic acid accumulation, not the sugar accumulation, triggers photosynthetic inhibition via reductions in stomatal conductance (Asao and Ryan, 2015). Accumulation of abscisic acid in leaves after girdling has been observed in four tropical tree species (*Hieronyma alchorneoides*, *Pentaclethra macroloba, Virola koschnyi* and *Vochysia guatemalensis;* Asao and Ryan, 2015) and in *Pinus radiata* (Mitchell et al., 2017). Girdling was also found to reduce stomatal conductance, which is a well know consequence of leaf-level accumulation of absicic acid, in *Pinus ponderosa* (Domec and Pruyn, 2008),.

The third hypothesis to explain photosynthetic inhibition proposes a mechanism related to increases in leaf starch concentrations (figure 3, interaction **F**), as observed after girdling in *Citrus unshiu* (Iglesias et al., 2002) and *Quercus petrea* (Maunoury-Danger et al., 2010). There is evidence for various pathways of high leaf starch concentrations directly damaging the photosynthetic apparatus, which results in reduced photosynthesis for herbaceous plants (Paul and Foyer, 2001). Significantly higher leaf starch concentrations after stem girdling have been observed in *Citrus reticulata* (Rivas et al., 2007), *Juglans regia* (Moscatello

et al., 2017a) and *Pinus taeda* (Myers et al., 1999), but not in *Populus deltoides* (Regier et al., 2010). For *Juglans regia* (Moscatello et al., 2017a), *Populus deltoides* (Regier et al., 2010) and *Pinus taeda* (Maier et al., 2010a) increases of stem starch concentrations above and decreases below the girdling zone were also apparent. Presumably, the increases above the girdling zone would eventually lead to increased leaf starch concentrations for passively loading pines. Interestingly, for *Pinus taeda* the changes in stem starch concentration were only significant when the girdling was performed in spring, as opposed to autumn (Maier et al., 2010a), indicating that photosynthetic activity during the peak growing season, or underlying seasonal fluctuations in nonstructural carbohydrate reserves (Furze et al., 2019; Richardson et al., 2013), might drive this change. Despite the evidence for changes in starch remobilization, evidence for the relationship between starch accumulation and photosynthesis inhibition in trees is merely correlative at the moment, but leaf chlorosis due to starch accumulation is a common symptom of girdling in the horticultural experiments (Goren et al., 2004). This leaves open the possibility that photosynthetic inhibition arises from concomitant changes in starch biosynthesis (figure 3, interaction **3**) and/or hydrolysis (figure 3, interaction **4**), which in turn are caused by changes in sugar availability in the phloem and leaves (hypothesis 1). The feedbacks, if driven by phloem sugar concentrations, are likely vary between passively versus actively loading species.

As implied above, phloem transport manipulation can induce the remobilization of nonstructural carbon reserves in adjacent tissues. Remobilization and use of nonstructural carbon reserves downstream of the girdling zone has been shown for *Pinus taeda* (Maier et al., 2010a)*, Populus deltoides* (Regier et al., 2010), *Prunus persica* (Jordan and Habib, 1996)*, Quercus petrea* (Maunoury-Danger et al., 2010), and *Scleronema micranthum* (Muhr et al., 2018). Using radiocarbon dating of the respired CO_2, Muhr et al. (2018) showed that such remobilized reserves after girdling were a decade old in the tropical species *Scleronema*

309 *micranthum*. This capacity to remobilize even decade-old reserves (Carbone et al., 2013;
310 Vargas et al., 2009) likely explains the large autonomy displayed by isolated woody organs
311 after girdling (Hoch, 2005) and phloem chilling (Schaberg et al., 2017).
312
313 Remobilization and storage of nonstructural carbon after phloem transport manipulation in
314 trees also partially drive alterations in respiratory rates (figure 3, interactions **C** & **F**), which
315 are consistently higher upstream and lower downstream in girdled (Domec and Pruyn, 2008;
316 Maier et al., 2010a; Maunoury-Danger et al., 2010), compressed (Henriksson et al., 2015),
317 and chilled stems (De Schepper et al., 2011). Leaf respiration has also been shown to
318 increase after girdling (Maunoury-Danger et al., 2010; Poirier-Pocovi et al., 2018) and
319 phloem chilling (De Schepper et al., 2011). These changes in respiration rates result from a
320 combination of changes in metabolic demand to fuel starch biosynthesis or hydrolysis (figure
321 3, interaction **F**), and changes in growth respiration (figure 3, interaction **6**). However, the
322 magnitude of respiration enhancement appears to exceed purely metabolic demands
323 (Domec and Pruyn, 2008; Maier et al., 2010a), and hence has been hypothesized to
324 constitute a mechanism for "burning off" surplus mobile carbon (figure 3, interaction **C**).
325 Domec & Pruyn (2008) have argued that such a "burning-off" of carbon could help to reduce
326 phloem sugar concentrations when a backlog develops after girdling. It is possible that this
327 metabolic burning is a safety mechanism to avoid damage to the photosynthetic apparatus,
328 due to high leaf sugar concentration. The excess respiration could use alternative metabolic
329 pathways, such as alternative oxidases (Amthor, 2000), that do not result in a net production
330 of ATP (Lambers and Ribas-Carbo, 2005). In conclusion, in phloem transport manipulations,
331 respiration is down-regulated downstream and up-regulated upstream of the treatment
332 zone, where it appears to exceed enhanced metabolic demand.
333

Direct phloem transport manipulation has also been shown to influence rates of wood formation, with girdling in trees always leading to enhanced wood formation upstream and complete cessation of growth downstream (Maier et al., 2010a; Wilson, 1968), which is also a common side-effect in horticultural girdling and compression treatments (Goren et al., 2004). For example, significant increases in rates of cell division, cell enlargement and cell wall thickening were found above the girdling zone, and decreased rates below the girdling zone in young *Pinus sylvestris* (Winkler and Oberhuber, 2017a). Phloem chilling has also been shown to lead to increases in wood growth upstream and decreases in wood growth downstream of the chilling zone (De Schepper et al., 2011). These results have been interpreted to imply carbon limitation of wood growth (figure 3, interaction **D**; (Winkler and Oberhuber, 2017a)), but they could also result from changes in hormonal signalling (figure 3, interaction **A**) or osmotically-induced changes in turgor due to variations in phloem sugar concentrations.

Finally, girdling has been used to block the export of current assimilates to roots, and hence reduce substrates for root growth, respiration, and exudation. This then permits partitioning of soil respiration into autotrophic and heterotrophic components (Högberg et al., 2001; Levy-Varon et al., 2012; Scott-Denton et al., 2006; Subke et al., 2004, 2011). The resulting partitioning estimates are likely biased, because of permanent damage to trees and enhanced fine root death. This enhanced decomposition due to root death has been hypothesized to partially (Högberg et al., 2009) or fully (Kuzyakov and Gavrichkova, 2010) compensate for reduced root respiration and exudation, leading to an underestimation of the autotrophic component. In contrast, Johnsen et al. (2007b) observed only a small, but statistically significant, reduction in soil respiration following phloem chilling in *Pinus taeda*. At the ecosystem scale, girdling has also been used to study the coupling between carbon assimilation and soil respiration on timescales ranging from a few hours to days (Mencuccini

360 and Hölttä, 2010), although such experiments need to be interpreted with care (Risk et al.,
361 2012; Stoy et al., 2007).
362
363 Contrary to the implied exclusive control of carbon dynamics and use in trees by source
364 activity (i.e. carbon supply), evidence outlined above paints a more nuanced picture with
365 complex feedbacks and dependencies. Wood growth, respiration and remobilization of
366 nonstructural carbon reserves are all affected by phloem transport manipulations and even
367 photosynthesis is downregulated.
368
369 **4. Collateral effects and practical challenges**
370 Despite all three manipulation techniques triggering similar effects on mobile carbon supply,
371 some peculiarities intrinsic to each method have been identified. These collateral impacts
372 need to be considered when choosing a method of direct phloem transport manipulation
373 and avoided, monitored and/or accounted for as much as possible.
374
375 *4.1 Collateral effects*
376 The degree of invasiveness is a primary aspect differentiating the three methods. Girdling
377 that damages the functioning xylem is by far the most invasive and least reversible, while
378 phloem chilling is the least invasive and most quickly reversible (figure 1). Wounding is likely
379 to result from girdling as well as overly-aggressive and persistent compression or chilling
380 treatments, which may ultimately lead to mortality. This damage triggers other (perhaps
381 undesirable) physiological responses, including the production and translocation of
382 signalling compounds, or the translocation of nutrients and water. In contrast to girdling,
383 phloem compression over days to months is believed to be reversible. For example, after
384 stem compression for a period of 27 days during the second half of the growing season, all
385 treated trees (*Pinus sylvestris*) had fully recovered phloem functionality in the following

386 spring (Henriksson et al., 2015). With phloem chilling, a potential risk is that temperatures
387 below freezing might cause permanent damage to unhardened tissues—for example,
388 freezing damage to the phloem and cambium could effectively girdle the tree.
389
390 Girdling has been shown to disrupt hormonal flows, including basipetal auxin transport
391 (Dann et al., 1985) and acropetal abscisic acid, cytokinin, gibberellin, amino acid and
392 amide transport (Cutting and Lyne, 1993; Havelange et al., 2000; Mitchell et al., 2017;
393 Skogerbo, 1992). However, the influence of phloem compression and chilling on the flow of
394 signalling compounds in trees is not known. Phloem compression might allow for some
395 leakage across the treatment zone, especially given the non-uniform pressure distributions
396 resulting from current methods. In fact, respiration rates downstream of the compression
397 zone do not drop as low as in girdled trees (Henriksson et al., 2015), which arguably results
398 from assimilate leakage. However, mobile carbon concentrations in the phloem downstream
399 of the girdling and compression zones are similar (Henriksson et al., 2015). By comparison,
400 phloem chilling only temporarily blocks phloem transport of mobile carbon, without
401 significantly reducing concentrations of inorganic solutes such potassium and amino acids in
402 downstream phloem sap, at least for the *Ricinus communis* (Peuke et al., 2006).
403
404 Phloem transport manipulation is clearly accompanied by collateral effects on adjacent
405 tissues, in particular water transport in the xylem. Phloem and xylem transport are known
406 to interact (Steppe et al., 2015), so consequences of phloem transport manipulations for
407 xylem transport are to be expected. In particular, it has been implied that leaving the xylem
408 exposed to the atmosphere may cause air seeding of cavitation (Ueda et al., 2014) or tissue
409 dehydration (Bloemen et al., 2014), but studies monitoring water transport after girdling
410 detected no significant difference to water transport in control trees within one year, if the
411 xylem remains intact and is sealed (Hubbard et al., 2013; Maier et al., 2010a). Nonetheless,

412 protecting the wound by sealing it with paraffin film (e.g. Cernusak and Marshall, 2001),
413 silicone grease (e.g. Christman et al., 2012) or aluminum foil (e.g. De Schepper and Steppe,
414 2011; Oberhuber et al., 2017) should be considered and reported. Similarly, phloem chilling
415 and compression have not had significant negative impacts on leaf water potential
416 (Rademacher et al., unpublished), suggesting that xylem water transport was not affected
417 detectably. However, the viscosity of water in the xylem also depends on temperature and
418 will be affected by chilling treatments. Whether this effect is substantial warrants more
419 research. Overall, current evidence suggests that phloem transport manipulation allows the
420 investigation of carbon dynamics without causing confounding effects driven by altered
421 water transport.
422
423 The timing and duration of phloem transport manipulation can influence experimental
424 outcomes. This is because the magnitude of phloem flow and the composition of phloem
425 sap vary diurnally, seasonally and interannually. Counterintuitively, preliminary evidence
426 for girdling suggests that the seasonal timing of the girdling did not affect mortality (Percival
427 and Smiley, 2015), or caused major differences in wood growth of girdled *Pinus sylvestris*
428 (Winkler and Oberhuber, 2017a). In contrast, wood growth increased significantly above and
429 decreased significantly below a chilling collar when phloem chilling was performed on ring-
430 porous *Quercus robur* during the peak growing season, but not when chilling was performed
431 during the early or late growing season (De Schepper et al., 2011). In *Quercus petraea*, also a
432 ring-porous species, early season girdling followed by recovery did not result in a difference
433 in wood growth compared to controls, but mid- and late-season girdling did (Maunoury-
434 Danger et al., 2010). Spring girdling in *Pinus taeda* resulted in greater effects on stem
435 respiration (e.g. increase above girdling and reduction below) than did autumn girdling
436 (Maier et al., 2010a). These seasonal differences corresponded to variations in stem soluble
437 sugar and starch concentrations in girdled *Pinus taeda* (Maier et al., 2010a), which was also

438 the case for girdled *Populus deltoides* (Regier et al., 2010) and chilled *Quercus robur* (De
439 Schepper et al., 2011), although it needs to be acknowledged that seasonal variations in
440 nonstructural carbon concentrations are common for various species (Furze et al., 2019;
441 Richardson et al., 2013). Overall, it is surprising, especially in locations with strong
442 seasonality, that the timing of application in relation to reproductive and phenological cycles
443 has not been unanimously observed to have strong effects on wood growth or tree
444 mortality, especially given the importance of temperature for cambial re-activation (Oribe
445 and Funada, 2017), hormonal signalling for cambial activity (Sorce et al., 2013), and the
446 observed effects of treatment timing on respiration and nonstructural carbon
447 remobilization. However, the limited number of studies with multiple timings and/or
448 durations of phloem transport manipulation does not currently allow clear attribution of
449 differences in outcomes to differences in phenology or morphology.
450
451 *4.2 Practical challenges*
452 In addition to potential collateral effects with each of the three methods, there are various
453 challenges associated with their implementation. The main challenge with girdling is the
454 limitation of tissue destruction to the desired depth. Precise methods such as incision by
455 razor blades take more time and require a good knowledge of the species' anatomy.
456 Specifically designed instruments from horticulture could facilitate consistent and precise
457 applications. Using a crude tool, such as an axe or chainsaw, is substantially quicker and
458 easier (and suitable if tree mortality is the desired consequence), but will increase collateral
459 effects through excessive tissue damage. If the cambium is not destroyed, regeneration of
460 the phloem and bark can eventually occur in some species (Chen et al., 2014; Pang et al.,
461 2008b; Zhang et al., 2011), while wound closure is normally driven by callus cells
462 proliferating from the edges until the callus tissue bridging the wound and re-differentiates
463 into phloem and cambium. The duration until full regeneration appears to depend on the

timing of the destruction, as observed in *Pinus taeda* (Maunoury-Danger et al., 2010). Given the wound closure dynamics, the dimensions of the wound are intuitively related to the probability of and duration until recovery, but we lack a quantitative understanding of this relationship. There are additional uncertainties associated with the timing of girdling (described above, section 4.1), which pose challenges when designing a girdling study.

Phloem compression equally has some challenges. First, it is desirable to apply pressure uniformly around the stem, branch or root, but this is difficult to achieve with current methods, because tree stems are rarely perfectly cylindrical. Further, if excessive pressure is applied and the cambium is permanently damaged, phloem compression may be equivalent to girdling. Second, fine tuning the pressure exerted by the collar is often difficult. In particular, when using ratcheted cargo webbing, the ratcheting mechanism used for tightening is, by design, not continuous. Third, the compression collar also exerts shear stresses, which can rupture the bark and underlying tissues. The likelihood of this damage can be minimized by pre-smoothing the bark and using, for example, polytetrafluouroethylene-based tape between the collar and the bark (e.g. Henriksson et al., 2015). In addition, stabilizing the collar, and tightening it slowly, is recommended to minimize unnecessary damage from shear stress. Any measures to minimize shear stress should be reported. Even when applied carefully, current techniques of phloem compression exert non-uniform pressure around the circumference. Metal bands, as pioneered by Henriksson et al. (2015), are particularly stiff, hence shear stress and surface irregularities result in substantial deviations from the desired pressure. The circumferential variation in pressure is generally less when using polyester cargo webbing, which can adapt to the shape of the stem, branch, or root. However, the metal ratchet used to tighten this webbing can itself be a source of shear stress and pressure variation over a smaller arc. One option is to use multiple collars, adjacent but rotationally offset, to homogenize circumferential pressure

490 variation. Pressure around the circumference should be measured and reported whatever
491 the exact setup. Fourth, weather and time may cause material fatigue of the compression
492 collar given the high forces involved. This fatigue may result in a loss of pressure, possibly
493 culminating in failure of the compression treatment. Fifth, relatively little is known about the
494 amount of pressure required to collapse the phloem without causing permanent damage,
495 leading to uncertainties about exactly how much pressure should be applied. Sixth, it is
496 possible that the applied compression could have additional side-effects on adjacent tissues,
497 such as the bark and xylem, or on phloem pressure gradients at the whole-tree level, with
498 consequence that have not yet been studied.
499
500 Setting up a phloem chilling experiment also involves multiple challenges and uncertainties,
501 as the method is new enough that answers to key questions are not yet documented in the
502 literature. First, the necessary width of the chilling zone ($\mathbf{w_x}$ in figure 1) is still unknown (e.g.
503 is there a minimum threshold to inhibit flow, is there a threshold above which collateral
504 effects increase disproportionately, do such thresholds depend on tree size or sap flow?).
505 Second, phloem chilling is most effective close to 0°C, but uncertainty remains how far and
506 how long positive temperature deviations can be tolerated before the treatment ceases to
507 be effective. Third, phloem chilling also reduces the temperature in adjacent tissues, such as
508 xylem, cambium, and bark, with unknown implications. Any temperature reductions at the
509 stem surface will propagate inwards and then predominantly upwards due to water
510 transport in the xylem, resulting in three-dimensional temperature gradients along the stem.
511 While the vertical gradients can be quantified using thermal imaging (e.g. figure 2d), radial
512 variations in temperature are more difficult to quantify and control for. Fourth, calculations
513 of heat exchange when designing a study are complicated due to large uncertainties
514 associated with the thermal properties of wood (Suleiman et al., 1999), variations in sap
515 flow and the water content of bark, phloem and xylem (Steppe et al., 2015). Fifth, the

516 thermal inertia of a chilling system is difficult to manage in field studies, particularly due to
517 diurnal and day-to-day variations in ambient temperature. Positive temperature excursions
518 during warm weather could result in flushes of carbon through the inadequately chilled
519 phloem, while negative temperature deviations might freeze unhardened, living tissue and
520 cause permanent damage (Vitasse et al., 2014). Consequently, the chilling capacity, and thus
521 energy requirements, needed to adequately maintain effective phloem chilling treatments,
522 even for medium-sized trees, in the field is substantial (e.g. roughly 60 kJ min^{-1} for a *Pinus*
523 *strobus* with a 30cm diameter at breast height assuming a maximal temperature difference
524 between target temperature and ambient air temperature of 30 K). Any chilling system
525 should include control loops that monitor phloem or bark temperature and adjust the
526 chilling accordingly to maintain optimal chilling temperatures (e.g. just above freezing).
527 Sixth, it is difficult to create a good interface—one that would maximize heat transfer—
528 between chilling collars and phloem tissue. For example, whereas copper tubing conducts
529 heat efficiently, it is difficult to shape to the rough exterior of a tree trunk, and the contact
530 area of round tubing with the trunk is comparatively small.
531
532 *4.3 Comparison*
533 Girdling is a simple and low-cost method to manipulate phloem transport in mature and
534 juvenile trees alike (e.g. Annighöfer et al., 2012). Phloem compression is, like girdling, a low-
535 cost way to manipulate phloem transport over a period of weeks and can be applied to trees
536 of various sizes (e.g. Henriksson et al., 2015). Unlike girdling, phloem compression is
537 reversible, less invasive, and even when applied to the main bole, non-lethal. Thus, in many
538 cases it is a good alternative to girdling because collateral effects are reduced. Phloem
539 chilling is logistically the most challenging, and by far the most expensive, method to
540 implement. However, the advantage of phloem chilling is that it provides an elegant switch
541 (i.e. a quickly reversible method to manipulate phloem transport) without the application of

542 mechanical force. Although phloem chilling is substantially more expensive than girdling or
543 phloem compression, it is far less expensive than other methods typically used in carbon
544 allocation studies (e.g. isotope labelling or CO_2 enrichment). Because of logistical
545 constraints, phloem chilling is better suited for intensive studies with a physiological focus
546 and a relatively small sample size. Indeed, published studies of phloem chilling have been
547 performed on just a few branches (Schaberg et al., 2017) or small trees (De Schepper et al.,
548 2011). The one phloem chilling experiment on mid-sized mature trees (n=12) took place in a
549 *Pinus taeda* plantation (Johnsen et al., 2007a). By comparison, girdling and phloem
550 compression can more easily be applied to larger sample and organ sizes or more dispersed
551 study designs (e.g. Högberg et al., 2001). The simplicity and low cost or girdling and phloem
552 compression facilitate studies beyond the historic confines of laboratories and plantations of
553 temperate forest trees (e.g. Asao and Ryan, 2015; Muhr et al., 2018).
554
555 *4.4 Reducing uncertainties associated with collateral effects*
556 Although phloem transport manipulation treatments can provide novel insights into tree
557 physiology, there is an urgent need to reduce uncertainties related to intrinsic collateral
558 effects of these techniques. Reporting has not been consistent enough and these treatments
559 (girdling excepted) have not been used widely enough yet to permit them to be considered
560 "established" or "standard" methods. Additional research into both the optimal
561 methodological details and the exact effects would be highly profitable and greatly inform
562 the appropriate design and application of future experiments. For example, we recommend
563 examining (i) whether, to what extent, and under what circumstances do these methods
564 allow leakage of carbon across the treatment zone, and on what time scales; (ii) what
565 substances continue to be transported, and what substances are blocked; (iii) what
566 determines the potential for recovery of phloem transport (i.e. reversibility) after the
567 treatment is stopped; and (iv) whether there are key treatment thresholds (e.g. wound

dimensions of girdling, the applied pressure of compression, or the magnitude and duration of chilling) that need to be exceeded or avoided?

5. Outstanding questions and unmet potential

Beyond questions concerning the methods themselves, key questions remain with regard to allocation and metabolic processes that determine the fate of carbon in trees. These remaining questions are fundamental to major uncertainties within our understanding of the global carbon cycle, such as the residence time of carbon in forests. In the following section, we discuss how phloem transport manipulation can be used to advance these important research avenues.

5.1 Photosynthesis inhibition

As discussed, phloem transport manipulation has been shown to lead to a backup of mobile carbon in source tissues, which correlates with observations of photosynthetic downregulation (e.g. De Schepper et al., 2011; Iglesias et al., 2002; Rivas et al., 2007), confirming simulated feedback effects (Hölttä et al., 2017). This accumulation of mobile carbon provides a framework to test these simulated mechanisms of potential feedbacks between sink and source activity in trees. Phloem transport manipulation could be used to establish a mechanistic understanding of phloem loading under high mobile carbon concentrations in the phloem for passively and actively loading species and the subsequent inhibition of photosynthesis by addressing (i) whether and how increased mobile carbon concentration in the phloem translates to increased mobile carbon concentrations in leaves, (ii) what is the response curve of photosynthesis to mobile carbon concentrations in leaves, and (iii) whether these patterns differ for passively and actively loading species?

593 The backup of mobile carbon in the phloem resulting from phloem transport manipulations
594 in passively loading species could be used to evaluate whether knowledge of leaf carbon
595 export and photosynthesis downregulation from herbaceous plants is transferrable to trees.
596 For example, phloem compression and phloem chilling could be used to isolate individual
597 branches of a tree for varying durations creating a gradient of mobile carbon. This would
598 allow the formulation of a response function of photosynthesis to mobile carbon
599 concentrations in branches and leaves. Repeating the treatments starting at multiple dates
600 throughout the season would further allow to account for variations due to phenological
601 and reproductive cycles. A better understanding of the mechanism of feedbacks inhibiting
602 photosynthesis under high mobile carbon concentrations is urgently needed for trees.
603
604 *5.2 Carbon remobilization dynamics*
605 Storage of assimilates, in the form of nonstructural carbohydrates, provides important
606 reserves that enable trees to survive periods of dormancy or unfavourable growing
607 conditions (Dietze et al., 2014; Rosas et al., 2013). Remobilization of stored reserves
608 supports metabolic processes and growth when the supply of recent assimilates is
609 insufficient. Phloem transport manipulation can be used to isolate stem sections from
610 current assimilates as well as from stored reserves in distant organs (e.g. roots and
611 branches). By forcing metabolic reliance on locally stored reserves, phloem transport
612 manipulation could be used to study remobilization dynamics. For example, Muhr et al.
613 (2018) found that control trees relied on recent assimilates to fuel stem respiration, whereas
614 girdled trees relied on progressively older stored reserves. Experimental phloem transport
615 manipulation could help answer a range of questions such as (i) under what circumstances
616 does remobilization occur; (ii) what is the age and availability of stored reserves for
617 remobilization; and (iii) does remobilization use reserves in reverse chronological order - the
618 “last in, first out” paradigm (Carbone et al., 2013; Lacointe et al., 1993) - of when they were

stored; (iv) how much mixing between younger and older reserves occurs in different tissues?

Starch biosynthesis has been shown to be enhanced with high local mobile carbon concentrations above the zone of girdling, whereas starch hydrolysis is amplified with low local mobile carbon concentrations below the zone of girdling (Maier et al., 2010b; Maunoury-Danger et al., 2010; Moscatello et al., 2017b; Regier et al., 2010). But, it is not known whether these changes are driven by local carbon availability, or by co-transported signalling compounds. Previous phloem transport manipulation studies have typically used just a single phloem transport bottleneck on each treated tree (with the exception of De Schepper et al. (2007) and Winkler and Oberhuber (2017b)), but by creating two bottlenecks - a restriction above and a restriction below an effectively isolated stem section - it would be possible to cut off transport both *down* from the canopy and *up* from stored reserves in roots. This would lead to a hypothesized gradient in carbon availability, from highest above the top restriction, to intermediate below the bottom restriction, to lowest between the two restrictions. Wood growth and maintenance respiration of the isolated section would be dependent on the remobilization of local reserves, and radiocarbon dating could be used to determine the age of stored reserves used to support respiration (by trapping emitted CO_2; e.g. Muhr et al. 2016, 2018) and new tissue production (by cellulose extraction; e.g. Carbone et al 2013). This experiment would also enable inferences about internal mixing of stored reserves and the affinity of remobilization for more recently stored substrates (Carbone et al., 2013; Richardson et al., 2015). To the extent that the transport of carbon and signalling compounds may be differentially affected by girdling, phloem compression, and phloem chilling, such an experiment might yield different results depending on the phloem transport manipulation that is applied. While girdling has been recommended to address whether 'regular' allocation is maintained under carbon limitation (Hartmann and Trumbore,

645 2016), and what the effects on carbon remobilization are (Muhr et al., 2016), we argue that
646 phloem compression and phloem chilling are better suited, because collateral effects are
647 minimized. Regardless of the approach, phloem transport manipulations provide a way to
648 elucidate changes in carbon allocation under both high and low carbon availability, and thus
649 potentially offering insights that cannot be obtained from costly CO_2 enrichment
650 experiments.
651
652 *5.3 CO_2 emissions from respiration*
653 Respiration—the metabolic oxidation of glucose to CO_2, H_2O, and energy— can rapidly
654 return recently assimilated and stored nonstructural carbon to the atmosphere. Phloem
655 transport manipulation provides a valuable tool to investigate the rate of respiration in
656 relation to locally available carbon, metabolic activity associated with growth of new tissues,
657 and remobilization of stored reserves. Carbone et al. (2013) used radiocarbon dating to
658 show that the substrate for growing season stem respiration was recently assimilated
659 carbon compounds, whereas during the dormant season there was reliance on older stored
660 reserves. Further insights could be provided by combining studies like this with phloem
661 transport manipulation (Muhr et al., 2018). Specific questions could include: (i) does
662 respiration increase proportionally with metabolic demand; (ii) what proportion of emitted
663 CO_2 is respired locally versus transported in xylem sap from distant tissues (Bloemen et al.,
664 2014; Tarvainen et al., 2014; Teskey et al., 2008); and (iii) are younger (more recently
665 assimilated) substrates used preferentially over older substrates?
666
667 *5.4 Effects of carbon availability on wood growth*
668 Wood formation is the key process through which atmospheric carbon is sequestered in tree
669 biomass (Rathgeber et al., 2016). By creating local gradients of carbon availability, phloem
670 transport manipulation can be used to help understand the extent to which wood formation

671 is limited by carbon, as long as concurrent osmotically-induced changes in turgor and other
672 signalling compounds are accounted for. Specific questions that could be addressed by
673 phloem compression and phloem chilling are: (i) how much is wood growth limited and/or
674 controlled by carbon availability, and does this relationship vary with tree age or size; (ii) is
675 the response of wood growth to carbon availability linear or non-linear; and (iii) how do
676 rates of cell division, cell expansion and cell wall thickening change in response to carbon
677 availability?
678
679 Measurements of wood formation and structure in young *Pinus sylvestris* after girdling are
680 consistent with carbon limitation of wood growth (Winkler and Oberhuber, 2017a).
681 However, modelling (Hayat et al., 2017) and theoretical arguments (Körner, 2006, 2003)
682 suggest that with increasing size, tree growth switches from being limited by carbon source
683 activity to being limited by sink activity. In future studies of the controls of tree growth,
684 phloem compression and phloem chilling would have a distinct advantage over girdling,
685 because they are reversible and less invasive. In particular, they appear to restrict carbon
686 transport but may allow the passage of other compounds, that might stimulate or even drive
687 wood growth. For example, phloem chilling of *Ricinus communis* was shown to maintain a
688 gradient of sugar concentrations across the zone of chilling without significantly impeding
689 the phloem transport of signalling compounds and inorganic solutes (Peuke et al., 2006).
690 When phloem chilling is used, stem temperatures in close proximity to the sample locations
691 for xylogenesis need to be monitored, as they directly affect cambial activity (Oribe and
692 Funada, 2017). Alternatively, compression could be used to avoid collateral effects of
693 temperature on cambial activity.
694
695 *5.5 Root respiration, root exudates and transport of carbon to the rhizosphere*

696 The irreversible and invasive nature of girdling, when applied to the main bole, which blocks
697 the downstream flow of current assimilates into the soil, has been harnessed to reduce
698 carbon fluxes to soils across large areas and numbers of individuals. Previous studies have
699 evaluated the impacts of girdling on mycorrhizal abundance and community structure
700 (Dannenmann et al., 2009), and have used girdling to partition soil respiration into
701 autotrophic and heterotrophic components (Bhupinderpal-Singh et al., 2003; Högberg et al.,
702 2001; Subke et al., 2004). However, phloem compression may improve partitioning
703 estimates by reversibly restricting nonstructural carbon transport to roots, without inducing
704 fine root mortality (Henriksson et al., 2015), although leakage and the large reserves
705 of nonstructural carbon reserves in roots could still lead to underestimation of the
706 autotrophic component (Aubrey and Teskey, 2018). In fact, phloem chilling has been used to
707 reduce carbon transport to soils and had a similar effect as girdling on a *Pinus taeda*
708 plantation, but was reversible (Johnsen et al., 2007a). Thus, compared to girdling of the
709 main bole, non-lethal methods to manipulate phloem transport belowground have the
710 potential to minimize effects of fine root mortality, enhanced decomposition, and
711 subsequent effects on microbial communities (Kaiser et al., 2010; Pena et al., 2010; Rasche
712 et al., 2011), which arguably lead to biases in studies using girdling (Högberg et al., 2009;
713 Kuzyakov and Gavrichkova, 2010). Some questions that non-lethal and reversible techniques
714 could answer are: (i) how long can root nonstructural carbon reserves buffer reductions in
715 the phloem flow of assimilated carbon; (ii) what is the temporal coupling between carbon
716 assimilation and soil respiration; and (iii) how long does it take for soil respiration to recover
717 after a temporary blockage of carbon fluxes?
718
719 **6. Conclusions**
720 We have reviewed the three most common direct methods to manipulate phloem transport,
721 and have assessed and evaluated their effectiveness and potential to answer questions

722 related to carbon dynamics and use in forest trees. In particular, non-lethal methods of
723 phloem compression and phloem chilling have the potential to further contribute to the
724 understanding of carbon dynamics and use in trees. We propose some key experiments
725 which could help develop a more mechanistic understanding of the fate of assimilated
726 carbon, and the timescales on which it returns to the atmosphere. In addition, we argue that
727 accurate interpretation, reproducibility and assessment of experimental results are reliant
728 on the recording, reporting and publishing of relevant methodological details, and ancillary
729 measurements. Full and accurate reporting is essential if we are to correctly interpret these
730 experiments and unravel the mechanisms underlying growth and metabolic responses to
731 phloem transport manipulation. Such a quantitative understanding of how activities of
732 carbon sources (recent assimilates from photosynthesis, as well as stored nonstructural
733 carbon reserves) and sinks (growth and maintenance respiration, and new tissue growth) in
734 trees are controlled by environmental conditions and internal feedbacks, is required for
735 accurate projections of forest growth and hence the land carbon cycle under future
736 conditions, especially of elevated atmospheric CO_2.
737

738 **Author contribution statement**

739 ADR and TTR planned and defined the scope of the paper. TTR drafted the
740 paper and produced the figures. DB and JL provided images for the figures. TTR
741 and AHE assembled the tables. All co-authors discussed ideas, provided
742 feedback, edited the manuscript draft, and approved the manuscript for
743 submission.
744

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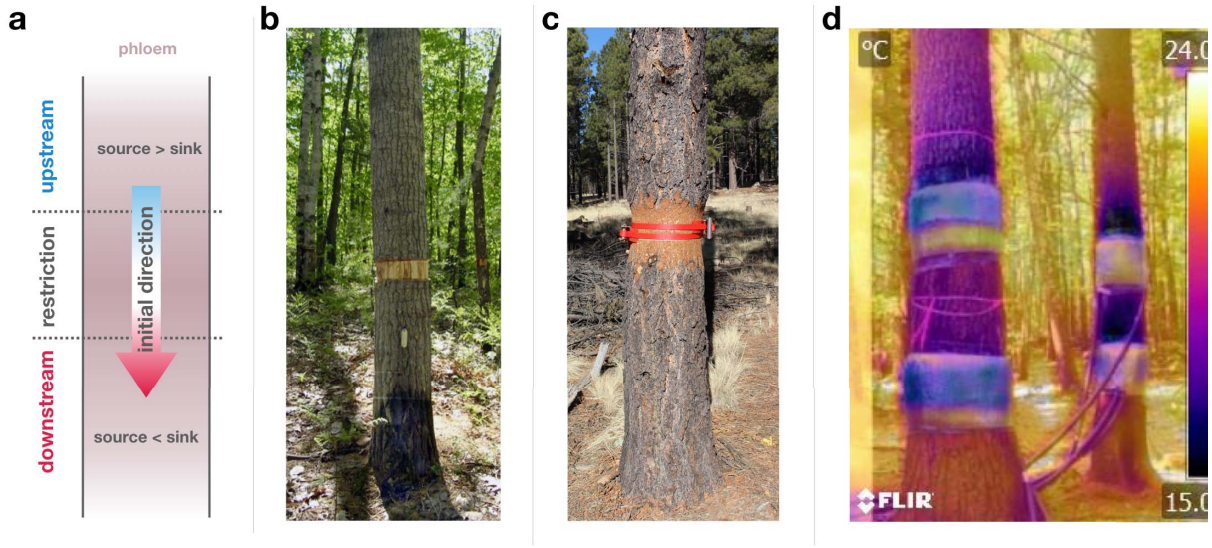
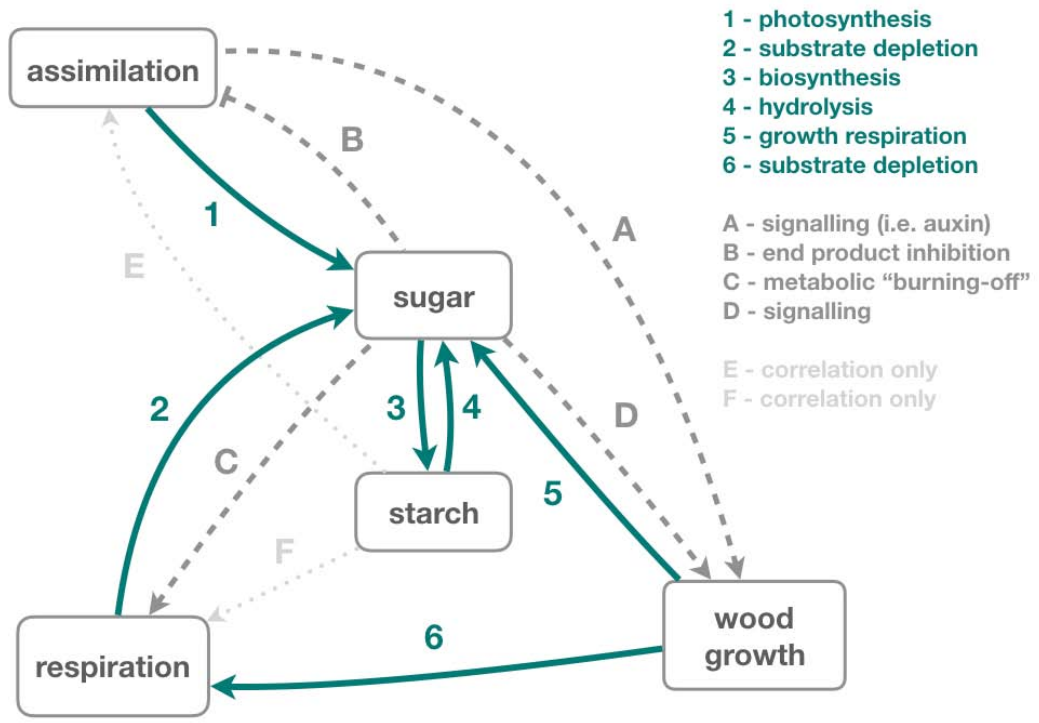
Figures and captions

Figure 1 – provides a summary of key facts about different phloem transport manipulations. In particular, panel **a** illustrates a cross-sectional woody organ being girdled, compressed and chilled. The table in panel b summarizes key facts for the three most applied methods ranked from the most invasive (girdling) to the least invasive (phloem chilling). $\mathbf{w_g}$, $\mathbf{w_c}$ and $\mathbf{w_x}$ mark the width of the zone for which tissue is affected during girdling, compression and chilling, respectively. **d** marks the depth of girdling with four distinct categories (subscripts $_{1\text{-}4}$), that determine which tissues are affected. $\mathbf{d_1}$ includes destroying of bark, phloem, cambium and part of the functioning xylem, $\mathbf{d_2}$ spares the xylem, $\mathbf{d_3}$ only destroys the bark and phloem and $\mathbf{d_4}$ only the bark. $\mathbf{P_c}$ is the normal pressure applied during compression. $\mathbf{T_x}$ is the temperature experienced by the phloem during chilling.


a
phloem
upstream
source > sink
restriction
initial direction
downstream
source < sink
b
c
d
°C
24.0
FLIR
15.0


assimilation
sugar
starch
respiration
wood growth
1
2
3
4
5
6
A
B
C
D
E
F
1 - photosynthesis
2 - substrate depletion
3 - biosynthesis
4 - hydrolysis
5 - growth respiration
6 - substrate depletion
A - signalling (i.e. auxin)
B - end product inhibition
C - metabolic “burning-off”
D - signalling
E - correlation only
F - correlation only

(Vaughn et al., 2002). (C) Respiration also seems to respond to locally high sugar concentrations by “burning-off” some sugar. (D) sugar concentrations have been observed to correlate with wood growth but there is no evident causal relationship. Equally, changes in starch concentrations often correlate with changes in (E) assimilation and (F) respiration, but causal linkages are unclear.

1284

1285 Table 1. Summary table of selected publications on phloem-transport manipulation
1286 experiments with regard to forest tree physiology. Girdling publications were selected based
1287 on their relevance to the topic of intra-tree carbon dynamics. To our knowledge, all phloem
1288 chilling and phloem compression experiments on trees are summarized here. **Method** list
1289 whether the study used girdling, chilling or compression. **Reference** for the study. **Location**:
1290 Forest = Forested area left to grow without significant management for a significant amount
1291 of time; Plantation = plantation and open field conditions; Laboratory = fully controlled
1292 conditions in a laboratory or greenhouse. **Objectives** of the study. **Species** studied. **Age and**
1293 **size**: **age**: tree age or stand age; **height** = (average) height of tree(s) studied, **dbh** = (average)
1294 stem or branch (*) diameter at the zone of treatment. **Timing:** Day of year when treatment
1295 was performed. **Sample size:** number of treated trees. **Measurements:** all measurements
1296 reported in the publication. **Methodological details: Depth of girdle** = cambium removed
1297 means bark, phloem and cambium are removed only; phloem removed means only bark
1298 including the phloem is removed. **Coolant temperature** = average temperature of the
1299 coolant, **phloem temperature** = mean temperature reported for the phloem. **Delta T** = the
1300 mean difference between air temperature and phloem temperature. **Type of collar** = type of
1301 compression collar. **Min. achieved pressure** = minimum pressure achieved by the
1302 compression collar. **Width** = width of the compression collar. **Offset =** Number and
1303 rotational offset of the collars.