Intra-annual dynamics of non-structural carbohydrates in the cambium of mature conifer trees reflects radial growth demands

Sonia SIMARD[1,2], Alessio GIOVANNELLI[3], Kerstin TREYDTE[1], Maria Laura TRAVERSI[3], Gregory KING[1,4], David FRANK[1,4], Patrick FONTI[1]

[1]Swiss Federal Research Institute WSL, Zürcherstrasse 111, 8903 Birmensdorf, Switzerland

[2]German Research Centre for Geosciences GFZ, Telegrafenberg, 14473 Potsdam, Germany

[3]CNR - IVaLSA, Via Madonna del Piano 10, 50019 Sesto F.no (Firenze), Italy

[4]Oeschger Center for Climate Change, University of Bern, Bern, Switzerland

Running head: NSC IN THE CAMBIUM OF MATURE CONIFERS

Corresponding author:

Sonia Simard
German Research Centre for Geosciences GFZ
Telegrafenberg
14473 Potsdam
Germany
email: simard@gfz-potsdam.de
phone number: +49 331 288 1899

This document is the accepted manuscript version of the following article:
Simard, S., Giovannelli, A., Treydte, K. K., Traversi, M. L., King, G. M., Frank, D., & Fonti, P. (2013). Intra-annual dynamics of non-structural carbohydrates in the cambium of mature conifer trees reflects radial growth demands. Tree Physiology, 33(9), 913-923.
https://doi.org/10.1093/treephys/tpt075

Summary

The presence of soluble carbohydrates in the cambial zone, either from sugars recently produced during photosynthesis or starch remobilized from storage organs, is necessary for radial tree growth. However, considerable uncertainties on carbohydrate dynamics and the consequences on tree productivity exist. This study aims to better understand the variation of the different carbon pools at intra-annual resolution by quantifying how cambial zone sugar and starch concentrations fluctuate over the season and in relation to cambial phenology. A comparison between two physiologically different species growing at the same site, i.e., the evergreen *Picea abies* and the deciduous *Larix decidua* and between *Larix decidua* from two contrasting elevations is presented to identify mechanisms of growth limitation.

Results indicate that the annual cycle of sugar concentration within the cambial zone is coupled to the process of wood formation. The highest sugar concentration is observed when the number of cells in secondary wall formation and lignification stages is at a maximum, subsequent to most radial growth. Starch disappears in winter while other freeze-resistant non-structural carbohydrates (NSC) increase. Slight differences in NSC concentration between species are consistent with the differing climate sensitivity of the evergreen and deciduous species investigated. The general absence of differences between elevations suggest that the cambial activity of trees growing at the treeline was not limited by the availability of carbohydrates at the cambial zone but instead by environmental controls on growing season duration.

Keywords: NSC, cambium, *Larix decidua*, *Picea abies*, intra-annual analysis, tree line, phenology

Introduction

Tree growth and survival depends not only on their capacity to produce and use carbohydrates, but also on efficient carbohydrate storage and remobilization. Carbohydrates are supplied by fixing atmospheric carbon (C) during photosynthesis and are vital in almost all plant physiological processes including the maintenance of existing tissue, the formation and enlargement of organs, and all associated metabolic processes. Investigating ecosystem productivity under changing climatic conditions requires understanding how various physiological and environmental factors constrain plant growth. In this regard, studies of non-structural carbohydrates (NSC; defined here as soluble sugars + starch) have been widely used to assess the source-sink balance of trees (Barbaroux and Bréda 2002; Damesin and Lelarge 2003; Fischer and Holl 1992; Gruber et al. 2012; Michelot et al. 2012; Oberhuber et al. 2011; Woodruff and Meinzer 2011). Stored NSCs can be viewed as reservoirs refilled when C demand for growth and maintenance is low and called upon during periods of high C requirements. Under this functional interpretation of NSCs, accumulation of large C pools is inconsistent with the hypothesis of carbon supply limiting growth, and rather suggests “sink” limitations (Hoch and Körner 2012; Körner 2003; Millard et al. 2007). Large pools of stored C have been observed in mature coniferous and deciduous trees with, for example, C reserves in deciduous trees sufficient to replace the entire canopy several

69 times (Hoch et al. 2003). Trees growing under conditions of high
70 environmental stress such as towards their cold thermal limits of growth
71 (Fajardo et al. 2013; Hoch and Körner 2003; Hoch and Körner 2012; Hoch et
72 al. 2003), following severe water stress (Breda et al. 2006; Gruber et al.
73 2012), or defoliation (Hoch 2005; Palacio et al. 2008), also showed high levels
74 of stored NSCs associated with, or despite, reduced growth.

75 However, in the context of on-going climate change and concurrent increases
76 in tree mortality, the role of carbon allocation has been revisited with several
77 studies concluding that significant amounts of mobile carbohydrates in various
78 tree tissues may not be simply interpreted as a sink-limitation (McDowell and
79 Sevanto 2010; Millard and Grelet 2010; Ryan 2011; Sala et al. 2010; Wiley
80 and Helliker 2012). Data obtained by interrupting the supply of new
81 photosynthates via phloem girdling (BhupinderpalSingh et al. 2003), or
82 following girdling and defoliation (Hoch 2005), has revealed significant NSC
83 pools that remain unused by trees. This may reflect a condition intermediate
84 to the “source” and “sink” limitations. Namely, trees appear physiologically
85 capable of incorporating stored photosynthates into permanent tissues, yet do
86 not do so. It is still unclear to what extent these data (i) indicate the inability of
87 trees to supply the growing tissues with carbohydrates, (ii) reflect a
88 prioritization of resources as a safety margin in the face of environmental
89 stochasticity, or (iii) some other underlying mechanism. Regardless of the
90 cause, active carbon storage competing with growth has been evidenced in
91 different species (Chapin et al. 1990; Genet et al. 2010; Silpi et al. 2007).
92 Consequently, if a significant fraction of the C pool is actively stored (thus
93 competing with growth) or sequestered (i.e., unavailable for any further

94 physiological processes), then the observed overabundance of C in trees is
95 not a useful indicator for a sink limitation (Millard and Grelet 2010). Therefore,
96 under a long-term perspective, competing requirements for NSCs (e.g.
97 respiration, defense and export, maintenance of hydraulic integrity) might
98 cause a source limitation (Epron et al. 2012; Hartmann et al. 2013; Sala et al.
99 2012; Wiley and Helliker 2012).

100 Despite abundant literature on NSCs, the processes and pathways related to
101 NSC allocation and storage within trees remain poorly understood (Epron et
102 al. 2012; Sala et al. 2012; Wiley and Helliker 2012). Progress is hampered by
103 the scarcity of field data necessary for model testing, with studies in natural
104 mature forests particularly needed (Barbaroux & Breda, 2002; Hoch et al.,
105 2003; Gough et al., 2009, Richardson et al. 2013). NSC concentrations have
106 been measured in diverse tree organs including stems, branches, foliage and
107 roots (Barbaroux and Bréda 2002; Damesin and Lelarge 2003; Fischer and
108 Holl 1992; Gruber et al. 2012; Michelot et al. 2012; Oberhuber et al. 2011;
109 Uggla et al. 2001; Woodruff and Meinzer 2011). In these studies, a general
110 conclusion was that high variation in intra-annual NSC content was observed
111 nearer to sites of active growth (e.g., apical and root meristems), while low
112 variation was recorded within the storage tissues (e.g., sapwood, coarse
113 roots, ray parenchyma). Investigations at the primary sink location would be
114 expected to yield better insights into the seasonal carbohydrate supply and
115 demand in trees.

116 Due to technical challenges associated with the sampling and isolation of
117 NSC in the cambial zone, very few studies have reported on carbon stock

118 measurements where secondary growth occurs (Deslauriers et al. 2009;
119 Giovannelli et al. 2011; Sundberg et al. 1993; Uggla et al. 2001). The cambial
120 zone in tree stems is composed of a thin layer of meristematic cells and only
121 recently has a procedure been developed to solve the challenge of separating
122 this tissue for NSC extraction (Giovannelli et al. 2011).

123 Here we use this new procedure to contribute to the debate on carbon
124 dynamics in trees by supplying a detailed description of the intra-annual
125 carbon fluctuation (soluble NSCs and starch content) in the cambial zone of
126 mature conifer trees. We aim at elucidating the mechanisms controlling
127 growth and at better understanding the effective sink strength of the cambium
128 and its variability over time. Therefore we perform measurements in a
129 deciduous and an evergreen conifer species at specific phenophases of wood
130 formation, as well as at two sites at contrasting elevations. In addition, we
131 quantify the individual carbon sugars as glucose, fructose, sucrose, raffinose,
132 pinitol, and starch to better assign a functional meaning to the seasonal
133 variations in NSC concentrations. This design allows us to address specific
134 questions related to growth limitation and carbon demand, such as (i) Is
135 secondary growth limited by the availability of carbon or constrained by
136 environmental conditions acting upon the sink function? (ii) How does carbon
137 supply in the cambium respond to the carbon demand for stem growth and
138 concurrent foliage production? (iii) Is there a species- and/or site-specific?
139 Strategy in the production, storage and supply of NSCs/individual sugars in
140 terms of carbon allocation to growth, management of reserves, and protection
141 against environmental stress?

MATERIAL AND METHODS

Study sites and field activities

Our study was performed in the Lötschental (46°23'40"N, 7°45'35"E), a southwest-northeast oriented inner-alpine valley in the central Swiss Alps. The valley bottom is surrounded by steep forested slopes primarily composed of mixed, evergreen Norway spruce (*Picea abies* Karst.) and deciduous European larch (*Larix decidua* Mill.). The climate of the region is cool and relatively dry, with a mean annual temperature of 6°C, ranging from -3°C (January) to 15°C (July) and a mean annual precipitation exceeding 800mm (data from MeteoSwiss for the period 1987-2006).

Field activities were conducted in 2010 at two sites, about 1km apart from each other, with contrasting elevations. The high elevation site is located at the upper tree line at ~2200 m asl, on a south-facing slope and consists solely of larch (this site is abbreviated S22L to reflect the aspect (**S**outh), elevation (**22**00 asl), and species (**L**arch). The low elevation site is near the valley bottom close to the north-facing slope at 1300 m asl and is a mixture of larch and spruce (similarly abbreviated N13L and N13S). The mean temperature difference between sites, as monitored from April to October 2010, was 3.5°C (average maximum and minimum temperature difference of 6 and 2.5°C, respectively). Hydrological conditions are generally dryer at the low elevation site due to a combination of less precipitation and higher evaporative demand. The soils of both sites are ~60 cm deep podzolic cambisols characterized by

165 significant coarse stone content and low clay amounts. Field activities
166 involved i) collecting stem samples to follow NSC dynamics in cambial tissue,
167 and ii) weekly monitoring of foliar and wood formation to document the
168 progress of growth at the time of cambial sampling.

169

170 *NSC sampling and biochemical analysis*

171 NSC sampling was performed on five different dates on 45 mature trees in
172 total, with 15 trees per species and site (S22L, N13L, and N13S). Sampling
173 dates were selected to target five relevant phases of annual ring formation,
174 i.e., 1) when cambial division / and earlywood cell enlargement are highly
175 active, 2) when earlywood cells are in both phases, enlargement and wall
176 thickening, 3) after cellular division has stopped but enlargement and wall
177 thickening phases continue, 4) when only latewood cells are conducting
178 secondary wall thickening and, 5) during dormancy of cambium (Table 1). The
179 sampling dates were estimated based upon data from 2007-2009 (King et al.
180 in. prep.; Moser et al. 2010).

181 Two samples per tree and sampling date were taken at about 50 cm height
182 using 37 mm diameter metal punchers. The samples, comprising phloem,
183 cambium and xylem, were kept on ice during fieldwork, stored at -22°C once
184 in the laboratory, and freeze-dried. Subsequently the samples were prepared
185 for biochemical analysis according to the protocol described in Giovannelli et
186 al. (2011). Accordingly, samples were split along the tangential plane in the
187 cambial zone and then the differentiating phloem and cambial cells from the

188 phloem side sample were gently scraped with a razor blade to obtain a
189 powder from the cambial tissue. Due to the low amount of cambium powder
190 per sample, blocks of five trees per site and species were pooled to obtain
191 enough material for sugar extraction. After pooling and homogenizing an
192 equal amount of cambium powder per tree, a 40 mg subsample was used for
193 sugar extraction. NSCs were extracted from the cambial powder using
194 chemical procedures described in Giovannelli et al. (2011). The sugar content
195 was determined by High-Performance Liquid Chromatography (HPLC)
196 analysis equipped with a SHODEX SUGAR Series SC 1011 8x300mm
197 column (Showa Denko, Germany) preceded by a pre-column Guard Pak
198 Insert Sugar Pak II (Waters). The mobile phase was water, Milli Q grade, at
199 0.5 ml min^{-1}. Soluble carbohydrate identification was verified using
200 carbohydrate standards (Sigma, USA) and quantified by means of an internal
201 standard. Concentrations of glucose, fructose, sucrose, pinitol and raffinose
202 were thus obtained. The remaining pellet after soluble NSC extraction was
203 used for starch quantification. The starch content was measured after an
204 extraction procedure: the residual pellet was suspended in 1.5 ml acetate
205 buffer (pH 5), brought to boil at 100°C for 1h in a sand bath, and then cooled
206 at room temperature. After incubation at 55°C for 16h with 150 µl
207 amyloglucosidase from *Aspergillus niger* (Fluka), samples were diluted with
208 distilled water to 5 ml and three 0.25 ml aliquots of each sample were assayed
209 colorimetrically using glucose oxydase (Sigma-Aldrich, Italy).

210 Seasonal changes in soluble NSCs and starch content were compared
211 between elevation and species using repeated-measures analysis of variance
212 (ANOVAr) (Gumpertz and Brownie 1993; von Ende 1993). For the within-

subject analysis, a Huynh-Feldt corrected probability was used to overcome the sphericity assumption in the case of univariate repeated-measures analysis (von Ende 1993). Differences were considered significant at $P<0.05$. When significant effects were found, mean comparisons by sampling date using ANOVA were performed to identify when the differences occurred. All ANOVA analyses were performed using the JMP® 8.0 software (SAS Institute Inc.).

Monitoring of foliar and wood formation

Foliar and cambial phenology were monitored on a weekly basis over the entire growing season 2010. For this purpose, and to prevent any potential influence of the sugar sampling on growth, 12 additional trees were selected: four spruce and larch trees at N13 and four larch trees at S22. The date of budburst for each tree was defined when 50% of its buds were broken (Brügger and Vassella 2003). Exact dates were estimated by linear interpolation, with these values averaged per site and species for a site date.

Cambial phenology was quantified weekly on the same trees. The forming annual ring was monitored for each tree by analysing tracheid formation from microcores collected weekly between April and November after (Moser et al. 2010). Microcores were collected from the stem at 1-2 m height using a Trephor (Rossi et al. 2006a) preferentially perpendicular to the slope direction to avoid reaction wood. Sampling was conducted along an oblique line and 3–5 cm apart to minimize wound reactions caused by earlier samplings (Forster et al. 2000). Microcores were placed for 24 h in a dilution of acetic acid and ethanol to preserve forming cells from degradation, and then stored in a 70%

237 alcohol solution. Samples were prepared for cellular analysis by cutting 20–30
238 µm thick transversal microsections using a sliding microtome. Microsections
239 were stained with safranin and astrablue and fixed to microscope slides with
240 Canada balsam. Ring formation was analysed at a magnification of 400–600x
241 and the number of tracheids in the different phases of cell development (i.e.
242 enlargement, wall thickening and maturity) assessed by averaging the
243 counting along three radial files of each microsection. Enlarging cells were
244 characterized by thin primary cell walls with radial diameter roughly two or
245 more times larger than that of dividing cambial cells. Polarized light was used
246 to discriminate between enlarging and wall thickening tracheids. Mature cells
247 were recognized by completely lignified secondary walls and empty cell
248 bodies (Rossi et al. 2006b; Rossi et al. 2007). Dates representing critical
249 phenological stages of wood formation were calculated for each site and
250 species based upon the cell counts.

251

252 **RESULTS**

253 *Growth dynamics and timing of NSC sampling*

254 At the valley bottom, larch budburst occurred on DOY 135 ± 3.5 (mean ±
255 standard deviation) and xylem formation (defined in this work as the timing of
256 the first observed enlarging xylem cells) on DOY 138 ± 3. Budburst and xylem
257 formation in spruce occurred later, i.e. on DOY 149 ± 3.5 and DOY 143 ± 0,
258 respectively. Budburst in larch occurred before or close to the first
259 observations of xylem cells entering the enlargement phase. In contrast,
260 spruce initiated growth of new xylem tracheids before the emergence of

current-year needles. These observations indicate that spruce and larch adopt different sequential arrangements in the timing of foliar and xylem growth resumption. At S22L, in comparison to larch growing 900m lower or at a 3.5°C warmer site, budburst was delayed by about two weeks (DOY 149 ± 3.5) and xylem formation onset by about three weeks (DOY 159 ± 3.5; (Figure1,Table 2). The timing of growth resumption significantly differed (Table 3) between species (~6 days; P=0.01) and elevation (~22 days; P<.0001).

The onset of wall thickening, the formation of fully mature cells, as well as the maximum number of cells observed in the phases of enlargement and wall thickening occurred earlier at N13S than N13L (Figure 1). The first wall thickening and mature cells were both observed 8 days later for spruce. Despite their delay in the onset of wall thickening (9 days) and mature cells (12 days), S22L soon reached similar levels of cell production as found at N13L. The total number of xylem cells in the 2010 ring varied among larch (ranging from 10 to 30) and spruce (from 25 to 70), but was in general higher for spruce.

The differences between both species and elevation decreased toward the end of the growing season. Significant differences in the end of xylogenesis (DOY 308 in average) between N13L and N13S disappeared, although larch at the treeline stopped xylogenesis a few days earlier. Thus, the shorter total duration of xylogenesis at S22L compared to N13L (18 days, P=0.0004) and for N13S compared to N13L (8 days, P=0.02) was mainly due to a difference in the onset of xylem differentiation (Table 3).

NSC concentrations

285 Significant seasonal variations of NSCs in the cambial zone were observed
286 (Figure 2). In general, the soluble fraction peaked between July and August,
287 corresponding to the times of high rates of cell division and enlargement
288 (period 2) and when many cells were in the wall thickening phase (period 3;
289 Table 1). Total soluble NSC concentrations increased by more than 50%
290 between the onset of the growing season and the period of maximum cell
291 division. In September, during latewood cell wall thickening (period 4) the
292 soluble carbohydrate concentrations decreased, and increased again during
293 the subsequent dormant season (period 5). Larch and spruce displayed
294 similar seasonal variations, with particularly high NSC concentrations during
295 periods 2 and 3, but the NSC concentrations in spruce peaked during the
296 dormant season. S22L and N13L showed similar seasonal patterns.

297 The starch concentrations for all sites and species were high towards the end
298 of the growing season (periods 3 and 4) and decreased dramatically during
299 dormancy. However, lower starch concentrations were measured at N13L
300 during the early growing season (period 1) .

301 On average, cambium sugars consist of around 40% glucose, 35% fructose,
302 10% starch, 10% pinitol, 5% sucrose and less than 1% raffinose (Figure 2).
303 However, these proportions slightly vary in time, between species and
304 elevations. Total NSC concentrations closely follow those of glucose and
305 fructose together, showing similar patterns and accounting for nearly up to
306 80% of the growing season soluble NSCs (Figure 2) and leading to high
307 hexose (glucose + fructose) to sucrose ratios (in average from 15 to 30 during

the growing season). A fructose to glucose ratio of approximately 1 was also observed throughout the whole growing season.

Glucose, fructose and sucrose concentrations for spruce tend to be lower during the growing season and higher during the dormant season, however fructose concentration was not found to be as species dependant as the other sugars (Table 4). Notably, both raffinose and pinitol displayed unique intra-seasonal changes in concentrations. For all species and elevations raffinose was only found during the dormant season (period 5), whereas pinitol, in contrast to all other sugars did not show an increase during the dormant season. In addition, pinitol was the only sugar with higher concentrations in spruce during the active growing season (periods 2-3).

Highly significant relationships between species and the phases of cambial phenology were observed for starch, glucose, sucrose, and raffinose, while elevation was not a significant factor contributing NSC concentration variations in the cambial zone (Table 4).

DISCUSSION AND CONCLUSIONS

Our intra-annual NSC measurements sampled directly in the stem cambial zone of mature trees growing in the subalpine zone improve the understanding of carbohydrate variation during a complete annual cycle. The dynamics of the different mobile carbohydrates observed at both elevations and for the two species reflect the changing requirements for storage, mobilisation and use of C resources needed to sustain growth as well as

protecting vital tissue from harsh environmental conditions, e.g., during the winter.

The annual cycle

In temperate regions with a distinct seasonal cycle and a dormant period for vegetation in winter, the onset of wood formation is usually temperature driven (Moser et al. 2010). Photoperiod may also provide secondary control for the growth onset, at least for the primary meristem (Chuine et al. 2010; Körner and Basler 2010). Wood formation is terminated in late fall when the chain of maturation processes is completed (Rossi et al. 2012). These notions of the annual cycle are broadly reflected in the cellular developmental stages (**Figure 1**) and concentrations of the different types of NSCs (**Figure 3**) for both larch and spruce

The general paralleling of the NSC compounds in the cambium and the annual dynamics of wood formation has been also observed in the other few studies with comparable approaches, i.e. for poplars (Deslauriers et al. 2009; Giovannelli et al. 2011), Scots pines (Sundberg et al. 1993; Uggla et al. 2001) and eucalyptus (Stewart et al. 1973). Collectively, these studies suggest that the dynamics of NSC concentrations in the cambium of different species, habitats, and angiosperm versus gymnosperm lineages follow a similar seasonal pattern

During the growing season, NSCs sustain all metabolic processes involved in the formation of new cells within the cambium. We observed very high

amounts of soluble sugars, (primarily glucose and fructose, collectively named hexose) the cambial zone of both larch and spruce. While lower at the beginning and the end of the growing season, soluble sugar concentration increased rapidly and peaked when the resource demand was higher, i.e. when a greater number of cells were both in the enlargement and cell wall thickening phases. Such high levels of glucose and fructose are unusual compared to what is normally observed in other tissues such as stem wood (Damesin and Lelarge (2003), Gruber et al. (2011), Streit et al. (2013)). In our study of larch and spruce, we found similar concentrations of fructose and glucose within the cambial zones. Glucose to fructose ratios approximately equal to unity were similarly reported for Scots pine by Uggla et al. (2001), who also observed strongly decreasing sucrose concentration gradients (yet, strongly increasing glucose and fructose levels) from functional phloem to developing xylem. Relative to sucrose, high levels of hexose within the cambial zone are consistent with the high metabolic activity of the dividing and rapidly growing cells.

In addition to serving as the building blocks for growth itself, sugars play an important role as signalling molecules and/or as global regulators of gene expression (Eveland and Jackson (2011), Koch (2004)). Glucose and fructose in spruce and larch likely originated from the cleavage of sucrose as suggested by high levels of sucrose cleaving enzymes such as AI (acid invertase), and to a lesser extent Susy (sucrose synthase), in the cambial zone of Scots pine (Uggla et al. 2001). The relative ratio of hexose to sucrose concentrations are maintained by these various enzymes which collectively coordinate and fine-tune growth during key phases of development (Eveland

379 and Jackson 2011; Koch 2004). Hexose is regarded to have a greater
380 signalling potential in promoting growth and cell proliferation whereas sucrose
381 is typically associated with differentiation and maturation (Eveland and
382 Jackson 2011; Koch 2004). While we did not measure enzyme
383 concentrations, we found that particularly the hexose to sucrose ratio in the
384 cambium zone of spruce and larch generally decreased during the growing
385 season, as the cambial phenology shifted from rapidly dividing cells peaking
386 around DOY 165 (10.06.2010) to the wall-thickening phases peaking
387 approximately 2 months later (**Figure 1**).

388 Starch concentration commonly shows considerable seasonal variation in the
389 stem and branches of temperate zone trees. Lower reserves at budburst, a
390 late summer maximum as growth slows down, and starch hydrolysis to sugar
391 in autumn when days are short and nights cold are all patterns previously
392 reported (Gruber et al. 2011; Kozlowski 1992). Decreasing levels of starch
393 during the summer in tree stem, branches and cambial zone have also been
394 observed (Deslauriers et al. 2009; Hoch et al. 2003; Sundberg et al. 1993). In
395 comparison to reports from poplar (Deslauriers et al. 2009) or Scots pine
396 (Sundberg et al. 1993) cambial zones, we found starch levels in the cambial
397 zone of spruce and larch at all three sites remained relatively constant during
398 the growing season (**Figure 3**). Similarly, Geisler-Lee et al. (2006) found little
399 expression of genes related to starch metabolism in comparison to
400 carbohydrate-related enzymes (e.g. Susy, cellulose synthase) in the cambium
401 of poplar during xylogenesis.

Large amounts of starch are consumed during cambial reactivation, with reserves replenished only sometime after the onset of xylem differentiation (Begum et al. 2013). The concentrations we observed tended to be lower than those reported for Scots pine (Sundberg et al. 1993) and higher than those from poplar (Deslauriers et al. 2009). Our first sampling campaign (DOY 155 - N13, DOY 178 - S22) was possibly too late to catch the minimum starch level as cambium reactivation, xylem differentiation, and budburst had already occurred. During reactivation of the cambium, starch is used as the main source of energy, but later on, the continuation of cambial activity seems to require a continuous supply of sucrose (Oribe et al. 2003) for cell wall biosynthesis. The low variation of starch during the growing season suggests a constant supply of fresh assimilates to the cambium of larch and spruce at our sites.

For all of the tree groups investigated in our study, we found starch breakdown during the cold season, and synthesis from soluble sugars in late winter-springtime (**Figure 3**). Our findings support previous observations of α-amylase activation and starch synthase genes in dormant poplar tissues during cold periods supporting starch breakdown for cryoprotection purposes (Geisler-Lee et al. 2006). Similarly, resynthesis of starch in late winter (Kozlowski 1992), was documented in needles (Bansal and Germino 2009; Chen et al. 2012; Hansen and Beck 1994; Hoch et al. 2003) and in the trunk (Fischer and Holl 1992; Hansen and Beck 1994; Hoch et al. 2003; Michelot et al. 2012) of various tree species. Our results demonstrate that these starch 425 dynamics also apply to the cambial region.

426 Raffinose, and pinitol are both compatible solutes (i.e. osmotically active
427 compounds) that help cells survive osmotic stress (Bachmann et al. 1994;
428 Bohnert and Shen 1998). While increased concentrations of raffinose and
429 pinitol can decrease the osmotic potential of cells to maintain the water
430 balance, their main function might be to stabilize proteins, protein complexes,
431 or membranes by scavenging radical oxygen species (ROS) that build up
432 during environmental stress (e.g. cold, drought, high salinity) (Bohnert and
433 Shen 1998; Orthen et al. 1994). Levels of raffinose increased during winter
434 (**Figure 3**), thereby presumably protecting cell membranes from damage
435 during frost-induced dehydration by detoxifying ROS that accumulate at low
436 temperatures (Nishizawa et al. 2008). Pinitol was present year-round in both
437 species, however, concentrations peaked in the cambial zone of spruce when
438 growth processes were most active Streit et al. (2013) observed higher pinitol
439 concentrations in branch bark and branch wood of larch growing at another
440 tree line location in Switzerland in comparison to larch from lowland (500 m
441 asl) and interpreted this difference in terms of long-term adaptation to high
442 levels of ROS in response to low temperature. Similar pinitol concentrations to
443 Streit et al. (2013) in larch growing both at the valley bottom (1300 m asl) and
444 the tree line (2200 m asl) suggest that long-term adaptation responses to high
445 levels of ROS already occur at 1300 m asl and that pinitol concentrations may
446 not increase linearly with elevation. The conclusions for greater environmental
447 stress towards the upper elevations and the valley bottom are similarly
448 supported by analyses of the climatic sensitivity of radial growth variations 449
449 along an ~900 meter elevational transect (King et al. 2013b).

450

Species and climatic controls on NSC

The general trends in the dynamics of NSC concentrations in this study were similar between species and elevations. Differences relate primarily to the absolute concentrations: the protective sugars raffinose (during dormancy) and pinitol (during the warmest period corresponding to July/August), as well as glucose, fructose and sucrose (during dormancy) differed in spruce and larch. The species-specific concentrations are potentially explained by differing climatic sensitivities of larch and spruce, also observed in an Europe-wide multi-species tree-ring network (Babst et al. 2013) to cold (in winter) and drought (in summer). Hinesley et al. (1992) observed links between the raffionse content in the foliage of different conifers with their level of cold hardiness. A similar relationship was also found for the presence of other NSCs (glucose, fructose, sucrose and raffinose) in the shoots of shrubs and in tree stem (Lee et al. 2012; Morin et al. 2007). Trees with thinner bark like spruce are more sensitive to frost damage in the cambium zone (Gurskaya and Shiyatov 2006) and might thus need a different strategy for additional protection. Higher levels of soluble NSCs in the cambial zone of spruce, in comparison to larch, in a period where the risk of freezing injuries is high would meet this requirement. The lower level of raffinose in particular, and total soluble NSCs in general, observed in larch might also reflect a higher degree of spring de-hardening compared to spruce in early March (sampling period 5). This would further be supported by the earlier bud break and onset of xylem differentiation of larch.

474 During June and July, pinitol concentrations were found to be higher in spruce
475 compared to larch (in average 1.5- to 2.5-fold) at the valley bottom, while
476 glucose levels were lower. Gluocse has been described as one of the
477 precursors of pinitol synthesis (Obendorf et al. 2008) and therefore the lower
478 levels of glucose relative to pinitol in in spruce suggest that some glucose was
479 directed towards pinitol synthesis. However, why the pinitol levels significantly
480 increased in the cambial zone of spruce during the summer months, while
481 remaining constant (and at similar levels) in larch at both the valley and tree
482 line sites, is uncertain. These patterns might reflect a greater need to maintain
483 turgor potential (Aranjuelo et al. 2011) within the cambial zone of spruce to
484 sustain cell enlargement, or may result from a higher sensitivity of spruce to
485 environmental stress leading to enhanced generation of ROS (Orthen et al.
486 1994). A recent investigation showed that spruce growing at our study site
487 exploit their internal stem water reserves more quickly during drier conditions
488 compared to larch (King et al. 2013).

489 While total NSC concentrations remained similar throughout the growing
490 season in our study, higher concentrations of the soluble sugar fraction
491 (except pinitol) during dormancy were observed in spruce compared to larch.
492 While winter respiration of living tissue of the dormant deciduous trees depend
493 exclusively on reserves (starch + soluble sugars), the higher levels in our
494 evergreen species might reflect photosynthesis (Kozlowski 1992) and phloem
495 transport (Blechschmidt-Schneider 1990) during mild winter days.

496 Despite a temperature difference of 3.5 °C and difference in the length of the
497 growing season of 30 days between the treeline and valley bottom larch sites,

498 we found similar seasonal variations in the NSC concentrations. This similarity
499 likely has to do with our sampling keyed into expected cambial activity rather
500 than only calendar dates. Notable differences between elevations occured
501 only for starch concentrations in the early growing season (period 1) although
502 no statistical significant differences were found (Table 4). These
503 concentrations were already near their maximum in early summer at the
504 treeline whereas at the valley bottom they only peaked at the beginning of
505 July. Attributing these differences to particular mechanisms remains
506 speculative. However, higher respiration losses due to warmer temperatures
507 at the valley bottom in addition to active growth of competing sinks while
508 needles are still not fully photosynthetically functional (Kozlowski 1992) would
509 be consistent with our intra-seasonal NSC data.

510

511 *Is secondary growth allocation carbon or sink limited?*

512 Our data, although focused to one growing cycle and limited to only the
513 cambial zone, contribute to a better understanding of the potential functions
514 on NSC allocation and storage within trees. Notable for our investigation are
515 the direct observations of NSC concentration at the sites of secondary
516 growth, and the ability to link these changes with cambial zone phenology.
517 The reduced growth (i.e. number of tracheids produced) observed for larch in
518 comparison to spruce growing at the same site and for S22L compared to
519 N13L, cannot be explained by temporary limitation in NSC supply in the
520 cambium. The high similarities in the NSC concentrations during the growing
521 season of trees at different elevations, together with the delayed start of

522 cambial activity and xylem cell production at the tree line, support the
523 hypothesis of Körner (2003) that tree line trees are not carbon limited. Factors
524 affecting carbohydrate conversion to new tissues, rather than carbohydrate
525 availability, seem to control cambial activity (Kozlowski 1992; Sundberg et al.
526 1993). Similarly, Hoch et al. (2002) observed higher levels of mobile carbon
527 pools in needles, branches, stems and roots of trees with increasing elevation
528 and conclude that sink activity of trees growing at high elevations was limited
529 by low temperature rather than carbon availability. Similar conclusions were
530 reached by Fajardo et al. (2013) who compared deciduous and evergreen
531 treeline species across elevational gradients. Although no significant increase
532 of NSC concentration in the cambium zone was observed with elevation in our
533 study, we did observe a higher contribution of starch to the total NSC pool in
534 larch at the tree line (P=0.03, data not shown). Our data thus support growth
535 limitation at the tree line in response to sink activity rather than inability of
536 trees to supply growing tissues with carbohydrates.

537 In conclusion, the first study of intra-seasonal NSC dynamics at the cambial
538 level in subalpine forests indicated that carbohydrate fluctuations at the sink
539 level are closely linked to cambial activity (carbon sink demand) and the
540 metabolic needs associated with cell formation, rather than to species or
541 climate (elevation). Variation in the concentration of NSC with more specific
542 functions such as raffinose or pinitol seemed to be, however, species
543 dependant. Observations on larch growing across an elevational gradient of
544 nearly 1000 m suggest that temperature limits the cambial activity of tree and
545 not the availability of carbohydrates within the cambium.

546

547 **ACKNOWLEDGEMENTS**

548 The authors thank Daniel Nievergelt and Bastian Ullrich for fieldwork
549 assistance and Maria Burger for processing the tree microcore samples. S.
550 Simard was funded by a postdoctoral fellowship from the Fond de recherche
551 Nature et Technologies (FQRNT). This work was funded by a Swiss National
552 Science Foundation (projects INTEGRAL no. 121859 and ISOPATH no
553 130112) and NCCR Climate (DE-TREE). We thank two anonymous reviewers
554 for comments that helped to improve the manuscript

555

556 **REFERENCES**

557 Aranjuelo, I., G. Molero, G. Erice, J.C. Avice and S. Nogués. 2011. Plant
558 physiology and proteomics reveals the leaf response to drought in
559 alfalfa (Medicago sativa L.). Journal of Experimental Botany. 62:111-
560 123.
561 Babst, F., B. Poulter, V. Trouet, K. Tan, B. Neuwirth, R. Wilson, M. Carrer, M.
562 Grabner, W. Tegel, T. Levanic, M. Panayotov, C. Urbinati, O. Bouriaud,
563 P. Ciais and D. Frank. 2013. Site- and species-specific responses of
564 forest growth to climate across the European continent. Global Ecology
565 and Biogeography:n/a-n/a.
566 Bachmann, M., P. Matile and F. Keller. 1994. Metabolism of the Raffinose
567 Family Oligosaccharides in Leaves of Ajuga reptans L. (Cold
568 Acclimation, Translocation, and Sink to Source Transition: Discovery of
569 Chain Elongation Enzyme). Plant Physiology. 105:1335-1345.
570 Barbaroux, C. and N. Bréda. 2002. Contrasting distribution and seasonal
571 dynamics of carbohydrate reserves in stem wood of adult ring-porous
572 sessile oak and diffuse-porous beech trees. Tree Physiology. 22:1201-
573 1210.
574 Begum, S., S. Nakaba, Y. Yamagishi, Y. Oribe and R. Funada. 2013.
575 Regulation of cambial activity in relation to environmental conditions:
576 understanding the role of temperature in wood formation of trees.
577 Physiologia Plantarum. 147:46-54.
578 BhupinderpalSingh, A. Nordgren, M.O. Lofvenius, M.N. Hogberg, P.E.
579 Mellander and P. Hogberg. 2003. Tree root and soil heterotrophic
580 respiration as revealed by girdling of boreal Scots pine forest:

581 extending observations beyond the first year. Plant Cell and
582 Environment. 26:1287-1296.
583 Blechschmidt-Schneider, S. 1990. Phloem transport in Picea abies (L.) Karst.
584 in mid-winter. Trees. 4:179-186.
585 Bohnert, H.J. and B. Shen. 1998. Transformation and compatible solutes.
586 Scientia Horticulturae. 78:237-260.
587 Breda, N., R. Huc, A. Granier and E. Dreyer. 2006. Temperate forest trees
588 and stands under severe drought: a review of ecophysiological
589 responses, adaptation processes and long-term consequences. Annals
590 of Forest Science. 63:625-644.
591 Brügger, R. and A. Vassella. 2003. Pflanzen im Wandel der Jahreszeiten -
592 Anleitung für pnänologische Beobachtungen., Geographica Bernensia,
593 Bern Edn.
594 Chapin, F.S., E.D. Schulze and H.A. Mooney. 1990. The ecology and
595 economics of storage in plants. Annual Review of Ecology and
596 Systematics. 21:423-447.
597 Chuine, I., X. Morin and H. Bugmann. 2010. Warming, Photoperiods, and
598 Tree Phenology. Science. 329:277-278.
599 Damesin, C. and C. Lelarge. 2003. Carbon isotope composition of current-
600 year shoots from *Fagus sylvatica* in relation to growth, respiration and
601 use of reserves. Plant Cell and Environment. 26:207-219.
602 Deslauriers, A., A. Giovannelli, S. Rossi, G. Castro, G. Fragnelli and L.
603 Traversi. 2009. Intra-annual cambial activity and carbon availability in
604 stem of poplar. Tree Physiology. 29:1223-1235.
605 Epron, D., Y. Nouvellon and M.G. Ryan. 2012. Introduction to the invited issue
606 on carbon allocation of trees and forests. Tree Physiology. 32:639-643.
607 Fajardo, A., F.I. Piper and G. Hoch. 2013. Similar variation in carbon storage
608 between deciduous and evergreen treeline species across elevational
609 gradients. Annals of Botany. *in press*
610 Fischer, C. and W. Holl. 1992. Food reserves of Scots Pine (Pinus sylvestris
611 L) .2. Seasonal-changes and radial-distribution of carbohydrate and fat
612 reserves in pine wood. Trees-Structure and Function. 6:147-155.
613 Forster, T., F.H. Schweingruber and B. Denneler. 2000. Increment puncher -
614 A tool for extracting small cores of wood and bark from living trees.
615 Iawa Journal. 21:169-180.
616 Genet, H., N. Bréda and E. Dufrêne. 2010. Age-related variation in carbon
617 allocation at tree and stand scales in beech (Fagus sylvatica L.) and
618 sessile oak (Quercus petraea (Matt.) Liebl.) using a chronosequence
619 approach. Tree Physiology. 30:177-192.
620 Giovannelli, A., G. Emiliani, M.L. Traversi, A. Deslauriers and S. Rossi. 2011.
621 Sampling cambial region and mature xylem for non structural
622 carbohydrates and starch analyses. Dendrochronologia. 29:177-182.
623 Gruber, A., D. Pirkebner, C. Florian and W. Oberhuber. 2012. No evidence for
624 depletion of carbohydrate pools in Scots pine (Pinus sylvestris L.)
625 under drought stress. Plant Biology. 14:142-148.
626 Gruber, A., D. Pirkebner, W. Oberhuber and G. Wieser. 2011. Spatial and
627 seasonal variations in mobile carbohydrates in Pinus cembra; in the
628 timberline ecotone of the Central Austrian Alps. European Journal of
629 Forest Research. 130:173-179.

630 Gumpertz, M.L. and C. Brownie. 1993. Repeated measures in a randomized
631 block and split-plot experiment. Canadian Journal of Forest Research.
632 23:625-639.
633 Gurskaya, M.A. and S.G. Shiyatov. 2006. Distribution of frost injuries in the
634 wood of conifers. Russian Journal of Ecology. 37:7-12.
635 Hartmann, H., W. Ziegler, O. Kolle and S. Trumbore. 2013. Thirst beats
636 hunger – declining hydration during drought prevents carbon starvation
637 in Norway spruce saplings. New Phytologist. *in press*
638 Hinesley, L.E., D.M. Pharr, L.K. Snelling and S.R. Funderburk. 1992. Foliar
639 raffinose and sucrose in four conifer species : relationship to seasonal
640 temperature. Journal of the American Society for Horticultural Science.
641 117:852-855.
642 Hoch, G. 2005. Fruit-bearing branchlets are carbon autonomous in mature
643 broad-leaved temperate forest trees. Plant Cell and Environment.
644 28:651-659.
645 Hoch, G. and C. Körner. 2003. The carbon charging of pines at the climatic
646 treeline: a global comparison. Oecologia. 135:10-21.
647 Hoch, G. and C. Körner. 2012. Global patterns of mobile carbon stores in
648 trees at the high-elevation tree line. Global Ecology and Biogeography.
649 21:861-871.
650 Hoch, G., M. Popp and C. Korner. 2002. Altitudinal Increase of Mobile Carbon
651 Pools in Pinus cembra Suggests Sink Limitation of Growth at the Swiss
652 Treeline. Oikos. 98:361-374.
653 Hoch, G., A. Richter and C. Körner. 2003. Non-structural carbon compounds
654 in temperate forest trees. Plant Cell and Environment. 26:1067-1081.
655 King, G., P. Fonti, U. Büntgen, D. Nievergelt and D. Frank. 2013. Climatic
656 drivers of hourly to yearly tree radius variations along a 6°C natural
657 warming gradient. Agricultural and Forest Meteorology. 168:36-46.
658 King, G., P. Fonti, S. Simard, C. Bigler, C.B.K. Rathgeber and D. Frank. in.
659 prep. Impacts of climate change on wood formation
660 Körner, C. 2003. Carbon limitation in trees. Journal of Ecology. 91:4-17.
661 Körner, C. and D. Basler. 2010. Warming, Photoperiods, and Tree Phenology
662 Response. Science. 329:278-278.
663 Kozlowski, T.T. 1992. Carbohydrate sources and sinks in woody plants.
664 Botanical Review. 58:107-222.
665 Lee, J.H., D.J. Yu, S.J. Kim, D. Choi and H.J. Lee. 2012. Intraspecies
666 differences in cold hardiness, carbohydrate content and β-amylase
667 gene expression of Vaccinium corymbosum during cold acclimation
668 and deacclimation. Tree Physiology. 32:1533-1540.
669 McDowell, N.G. and S. Sevanto. 2010. The mechanisms of carbon starvation:
670 how, when, or does it even occur at all? New Phytologist. 186:264-266.
671 Michelot, A., S. Simard, C. Rathgeber, E. Dufrêne and C. Damesin. 2012.
672 Comparing the intra-annual wood formation of three European species
673 (Fagus sylvatica, Quercus petraea and Pinus sylvestris) as related to
674 leaf phenology and non-structural carbohydrate dynamics. Tree
675 Physiology
676 Millard, P. and G.A. Grelet. 2010. Nitrogen storage and remobilization by
677 trees: ecophysiological relevance in a changing world. Tree Physiology.
678 30:1083-1095.

679 Millard, P., M. Sommerkorn and G.-A. Grelet. 2007. Environmental change
680 and carbon limitation in trees: a biochemical, ecophysiological and
681 ecosystem appraisal. New Phytologist. 175:11-28.
682 Morin, X., T. Améglio, R. Ahas, C. Kurz-Besson, V. Lanta, F. Lebourgeois, F.
683 Miglietta and I. Chuine. 2007. Variation in cold hardiness and
684 carbohydrate concentration from dormancy induction to bud burst
685 among provenances of three European oak species. Tree Physiology.
686 27:817-825.
687 Moser, L., P. Fonti, U. Büntgen, J. Esper, J. Luterbacher, J. Franzen and D.
688 Frank. 2010. Timing and duration of European larch growing season
689 along altitudinal gradients in the Swiss Alps. Tree Physiology. 30:225-
690 233.
691 Nishizawa, A., Y. Yabuta and S. Shigeoka. 2008. Galactinol and Raffinose
692 Constitute a Novel Function to Protect Plants from Oxidative Damage.
693 Plant Physiology. 147:1251-1263.
694 Obendorf, R.L., E.M. Sensenig, J. Wu, M. Ohashi, T.E. O'Sullivan, S.M.
695 Kosina and S.R. Schnebly. 2008. Soluble carbohydrates in mature
696 soybean seed after feeding d-chiro-inositol, myo-inositol, or d-pinitol to
697 stem-leaf-pod explants of low-raffinose, low-stachyose lines. Plant
698 Science. 175:650-655.
699 Oberhuber, W., I. Swidrak, D. Pirkebner and A. Gruber. 2011. Temporal
700 dynamics of nonstructural carbohydrates and xylem growth in Pinus
701 sylvestris exposed to drought. Canadian Journal of Forest Research-
702 Revue Canadienne De Recherche Forestiere. 41:1590-1597.
703 Orthen, B., M. Popp and N. Smirnoff. 1994. Hydroxyl radical scavenging
704 properties of cyclitols. Proceedings of the Royal Society of Edinburgh
705 Section B: Biology. 102:269-272.
706 Palacio, S., A.J. Hester, M. Maestro and P. Millard. 2008. Browsed Betula
707 pubescens trees are not carbon-limited. Functional Ecology. 22:808-
708 815.
709 Rossi, S., T. Anfodillo and R. Menardi. 2006a. Trephor: A new tool for
710 sampling microcores from tree stems. Iawa Journal. 27:89-97.
711 Rossi, S., A. Deslauriers and T. Anfodillo. 2006b. Assessment of cambial
712 activity and xylogenesis by microsampling tree species: An example at
713 the alpine timberline. Iawa Journal. 27:383-394.
714 Rossi, S., A. Deslauriers, T. Anfodillo and V. Carraro. 2007. Evidence of
715 threshold temperatures for xylogenesis in conifers at high altitudes.
716 Oecologia. 152:1-12.
717 Ryan, M.G. 2011. Tree responses to drought. Tree Physiology. 31:237-239.
718 Sala, A., F. Piper and G. Hoch. 2010. Physiological mechanisms of drought-
719 induced tree mortality are far from being resolved. New Phytologist.
720 186:274-281.
721 Sala, A., D.R. Woodruff and F.C. Meinzer. 2012. Carbon dynamics in trees:
722 feast or famine? Tree Physiology
723 Silpi, U., A. Lacointe, P. Kasempsap, S. Thanysawanyangkura, P. Chantuma,
724 E. Gohet, N. Musigamart, A. Clément, T. Améglio and P. Thaler. 2007.
725 Carbohydrate reserves as a competing sink: evidence from tapping
726 rubber trees. Tree Physiology. 27:881-889.

727 Stewart, C.M., J.F. Melvin, N. Ditchburne and S.H. Tham. 1973. The effect of
728 season of growth on the chemical composition of cambial saps of
729 Eucalyptus regnans trees. Oecologia. 12:349-372.
730 Streit, K., K.T. Rinne, F. Hagedorn, M.A. Dawes, M. Saurer, G. Hoch, R.A.
731 Werner, N. Buchmann and R.T.W. Siegwolf. 2013. Tracing fresh
732 assimilates through Larix decidua exposed to elevated CO2 and soil
733 warming at the alpine treeline using compound-specific stable isotope
734 analysis. New Phytologist. 197:838-849.
735 Sundberg, B., A. Ericsson, C.H.A. Little, T. Nasholm and R. Gref. 1993. The
736 relationship between crown size and ring width in Pinus sylvestris L.
737 stems: dependance on indole-3-acetic acid, carbohydrates and
738 nitrogen in the cambial region. Tree Physiology. 12:347-362.
739 Uggla, C., E. Magel, T. Moritz and B. Sundberg. 2001. Function and dynamics
740 of auxin and carbohydrates during earlywood/latewood transition in
741 scots pine. Plant Physiology. 125:2029-2039.
742 von Ende, C.N. 1993. Repeated-measures analysis: growth and other time-
743 dependant measures. *In* Design and analysis of ecological experiments
744 Eds. S.M. Scheiner and J. Gurevitch. Chapman and Hall, Inc., New
745 York, pp 113-137.
746 Wiley, E. and B. Helliker. 2012. A re-evaluation of carbon storage in trees
747 lends greater support for carbon limitation to growth. New Phytologist.
748 195:285-289.
749 Woodruff, D.R. and F.C. Meinzer. 2011. Water stress, shoot growth and
750 storage of non-structural carbohydrates along a tree height gradient in
751 a tall conifer. Plant Cell and Environment. 34:1920-1930.

752

753

754 **TABLES AND FIGURES**

755

756 **Table 1:** Days of the year (DOY) and corresponding dates of NSC sampling
757 for each related phenological stages and sites

758

759
760 **Table 2:** Onset and duration of the growing season, and budburst for larch
761 growing at the valley bottom and the treeline (1300 asl and 2200 m asl,
762 respectively) and spruce growing at the valley bottom. Numbers in
763 parenthesis are the standard deviation of the mean (n=4).

764
765 **Table 3:** ANOVA (*F*- and *P*-values) of the onset, end and duration of xylem
766 differentiation, and budburst, for differences between species and elevation.
767 Significance at ($P < 0.05$).

768

769 **Table 4:** ANOVA (*F*- and *P*-values) for testing the differences in the
770 carbohydrates compounds. Tests include differences between species (S),
771 period (P) and their interaction (S*P); and for elevation (E), period (P) and
772 their interaction (E*P). *P*-values for repeated-measures ANOVA are presented
773 with Huynh-Feldt corrected probabilities. Significance at ($P < 0.05$).

774

775 **Figure 1:** Number of cells in the cambial zone (CZ), enlarging phase (E),
776 secondary wall formation and lignification phase (W) and mature xylem cells
777 (M) of individual *Larix decidua* (blue -1300m; grey -2200m; n=4) and *Picea*
778 *abies* (orange -1300m; n=4) at both elevations in the Lötschental as counted
779 on a weekly basis during the growing season 2010. The average number of
780 cells during the season is shown. The date of budburst (Bb) for both species
781 at both sites is also indicated.

782

783 **Figure 2:** Concentration of non-structural carbohydrates (Fru=fructose,
784 Glc=glucose, Suc=sucrose, Pin=pinitol, Raff=raffinose, Starch; mg g^{-1} DW) in
785 the cambial zone of larch and spruce at both elevation in the Lötschental
786 during the growing season 2010. Mean values are presented (n=3; each
787 representing 5 pooled trees). DW = dry weight. Stars indicate significant
788 differences between *Larix decidua* and *Picea abies* at 1300 m.

789

790 **Figure 3:** Individual non-structural carbohydrates (glucose, fructose, sucrose,
791 pinitol, raffinose: µmol g^{-1} DW; starch: mg g^{-1} DW) concentrations in the
792 cambial zone of larch (blue circles-full line - 1300m; grey circles-dotted line -
793 2200m) and spruce (orange triangles-full line) at both elevations in the
794 Lötschental during the growing season 2010. Values are mean ± 1 SE (n=3;
795 each representing 5 pooled trees). Note the scale break for glucose and
796 fructose. DW = dry weight. Stars indicate significant differences between *Larix*
797 *decidua* and *Picea abies* at 1300 m.

Table 1: DOY (dates) of NSC sampling for each corresponding phenological stages and sites.

Stage	Period	N13	S22
		DOY (Date)	DOY (Date)
Highly active cambial division/EW cell enlargement	1	155 (31.05.2010)	178 (23.06.2010)
EW cell enlargement and wall thickening	2	191 (05.07.2010)	201 (15.07.2010)
No cell division but active wall thickening processes	3	222 (04.08.2010)	222 (04.08.2010)
LW cell wall thickening	4	265 (15.09.2010)	265 (15.09.2010)
Dormancy	5	69 (09.03.2011)	39 (08.02.2011)

EW = earlywood, LW = latewood

Table 2: Onset, duration of the growing season and budburst for *Larix decidua* growing at the valley bottom and the treeline (1300 m asl and 2200 m asl, respectively) and *Picea abies* growing at the valley bottom. Numbers in parenthesis are the standard deviation of the mean.

Elevation (m)	Species	Onset of xylem production (DOY)	End of xylem differentiation (DOY)	Duration of xylogenesis (days)	Budburst (DOY)
1300	***Larix***	138 (3.0)	311 (0.0)	173 (3.0)	135 (3.5)
1300	***Picea***	143 (0.0)	305 (6.9)	162 (6.9)	149 (3.5)
2200	***Larix***	159 (3.5)	302 (6.0)	143 (7.9)	149 (3.5)

Table 3: ANOVA (*F*- and *P*-values) of the onset, end and duration of xylem differentiation, and budburst, for differences between species and elevation. Significance at ($P < 0.05$).

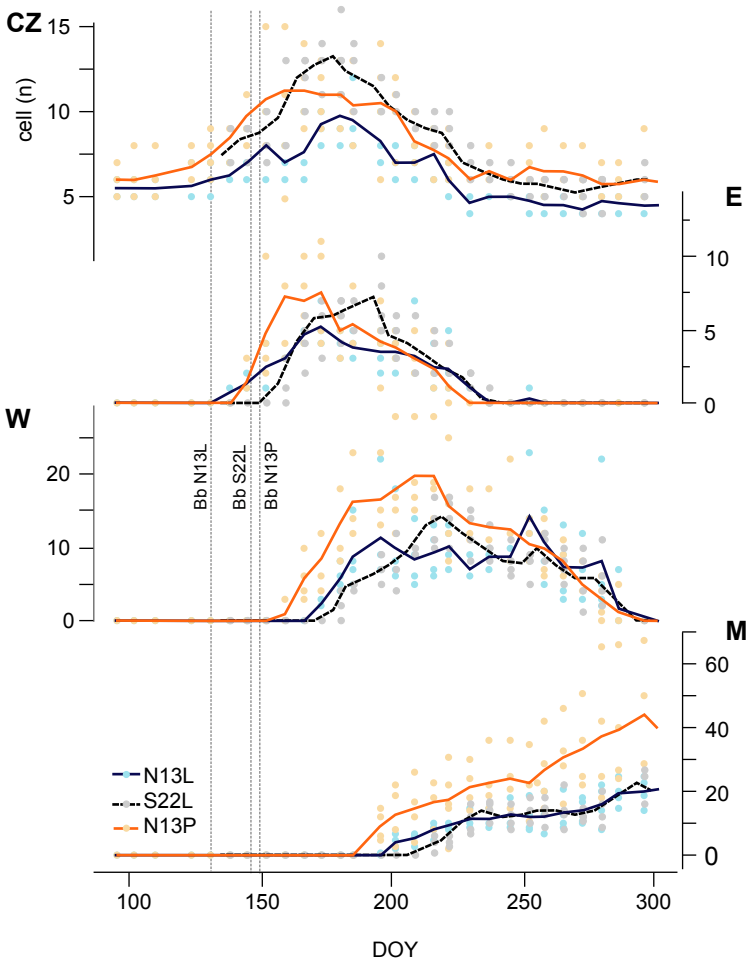
	Onset of xylem differentiation		**End of xylem differentiation**		**Duration of xylogenesis**		**Budburst**	
Source	***F***	***P-value***	***F***	***P*-value**	***F***	***P*-value**	***F***	***P-value***
Species	13.44	*	3.00	ns	9.28	*	409.9	***
Elevation	85.00	***	9.00	*	51.37	**	630.1	***

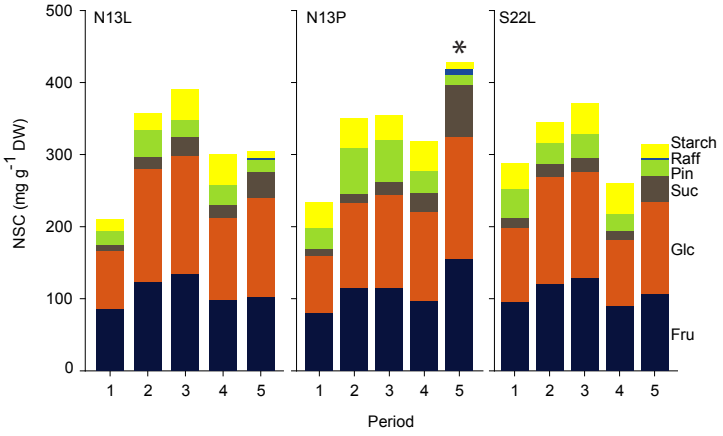
** P<.05, ** P<.001, *** P<.0001, ns = non significant*

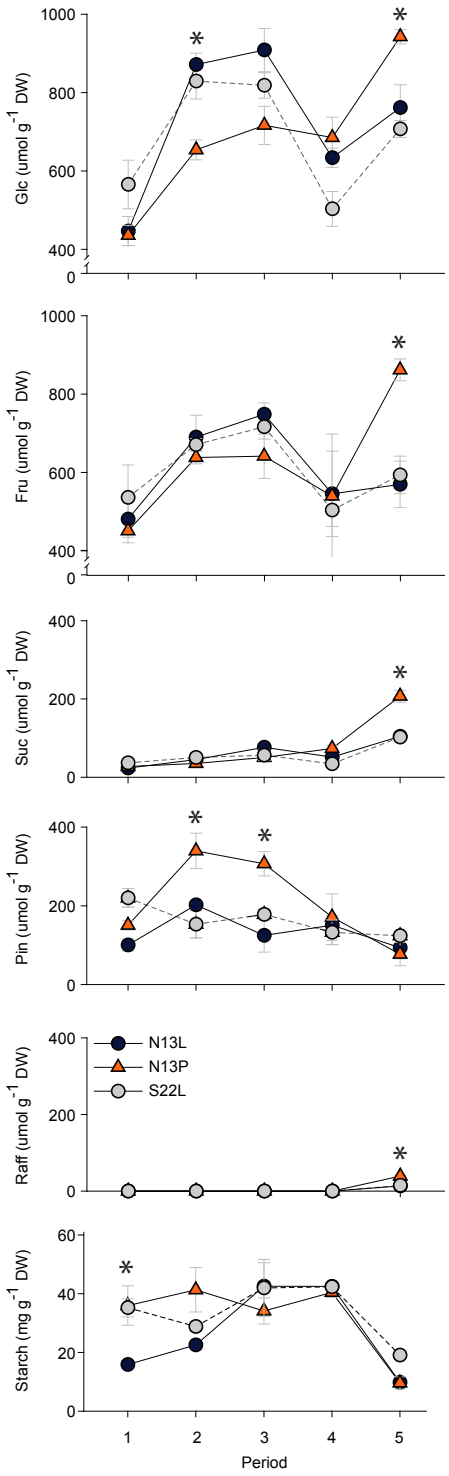
Table 4: ANOVA (*F*- and *P*-values) for testing the differences in the carbohydrates compounds. Tests include differences between species (S), period (P) and their interaction (S*P); and for elevation (E), period (P) and their interaction (E*P). *P*-values for repeated-measures ANOVA are presented with Huynh-Feldt corrected probabilities. Significance at ($P < 0.05$).

Carbohydrates	Source	*F*	*P*-value	Source	*F*	*P*-value
Glucose	S	5.85	ns	E	9.27	ns
	P	29.03	***	P	17.33	***
	S*P	7.64	**	E*P	2.81	ns
Fructose	S	0.24	ns	E	0.04	ns
	P	4.25	*	P	3.57	*
	S*P	2.57	ns	E*P	3.56	ns
Sucrose	S	2.76	ns	E	0.28	ns
	P	60.53	***	P	11.38	**
	S*P	14.71	***	E*P	1.38	ns
Raffinose	S	100.76	**	E	0.24	ns
	P	412.71	***	P	59.73	**
	S*P	100.76	***	E*P	0.24	ns
Pinitol	S	40.82	*	E	7.48	ns
	P	8.37	**	P	1.78	ns
	S*P	2.64	ns	E*P	2.9	ns
NSC total	S	4.39	ns	E	0.53	ns
	P	15.40	***	P	9.74	**
	S*P	3.47	*	E*P	2.49	ns
Starch	S	2.11	ns	E	6.4	ns
	P	20.37	***	P	15.22	**
	S*P	5.41	**	E*P	0.77	ns

** P<.05, ** P<.001, *** P<.0001, ns = non significant*


CZ
E
W
M
cell (n)
15
10
5
0
20
60
40
Bb N13L
Bb S22L
Bb N13P
N13L
S22L
N13P
100
150
200
250
300
DOY


N13L
N13P
S22L
*
NSC (mg g-1 DW)
500
400
300
200
100
0
1
2
3
4
5
Period
Starch
Raff
Pin
Suc
Glc
Fru


Glc (umol g-1 DW)
Fru (umol g-1 DW)
Suc (umol g-1 DW)
Pin (umol g-1 DW)
Raff (umol g-1 DW)
Starch (mg g-1 DW)
N13L
N13P
S22L
Period