

Sucrose or starch? The influence of tonoplast sucrose transporter perturbation on carbon partitioning for growth, defense, and winter protection in coppiced poplar

Trevor T. Tuma¹, Batbayar Nyamdari¹, Chen Hsieh², Yen-Ho Chen³, Scott A. Harding^{1,4}, & Chung-Jui Tsai^{1,2,3,4}

¹ Warnell School of Forestry and Natural Resources, University of Georgia, Athens, GA, 30602, USA.

² Institute of Bioinformatics, University of Georgia, Athens, GA, 30602, USA.

³ Department of Plant Biology, University of Georgia, Athens, GA, 30602, USA.

⁴ Department of Genetics, University of Georgia, Athens, GA, 30602, USA.

Key words: sucrose transporter, Populus, CRISPR, carbohydrates, metabolism, coppicing, remobilization

Running head: SUT4 and seasonal carbohydrate reserve partitioning

Corresponding author: Chung-Jui Tsai (cjtsai@uga.edu)

Abstract

Non-structural carbohydrate reserves of stems and roots underpin overall tree fitness as well as productivity under short-rotation management practices such as coppicing for bioenergy. While both sucrose and starch comprise the predominant carbohydrate reserves of *Populus*, utilization is understood primarily in terms of starch turnover. The tonoplast sucrose transport protein SUT4 modulates sucrose export to distant sinks, but the possibility of its involvement in sink tissue carbohydrate remobilization has not been explored. Here, we used *PtaSUT4*-knockout mutants of *Populus tremula* × *alba* (INRA 717-1B4) in winter and summer glasshouse coppicing experiments to strain carbon demand and test for SUT4 involvement in reserve utilization. We show that epicormic bud emergence was delayed and subsequent growth reduced in *sut4* mutants following winter but not summer coppicing. Reserve depletion during post-coppice regrowth was not impaired in the *sut4* mutants under winter or summer glasshouse conditions. Interestingly, xylem hexose increased during post-coppice growth exclusively in the winter when osmoprotection is critical, and the increase was attenuated in *sut4* mutants. Accrual of abundant defense metabolites, including salicinoids, chlorogenic acids, and flavonoid products was prioritized in the summer, but conspicuously lower in *sut4* mutants than controls. Together, our results point to shifting priorities for SUT4 function from support for osmoprotection in winter to chemical defense in summer. Delayed bud release and growth following winter but not summer coppicing in the *sut4* mutants demonstrate the importance of SUT4 in modulating trade-offs between growth and the other priorities during reserve utilization in *Populus*.

Introduction

Renewable energy production from feedstock like tree crops is expected to help reduce fossil fuel use and greenhouse gas emissions responsible for global warming (Ragauskas *et al.*, 2006, Dhillon & von Wuehlisch, 2013, Nieminen *et al.*, 2012). *Populus* species (e.g., poplar, aspen, cottonwood, and their respective hybrids) have proven useful for this purpose due to their fast growth, high biomass production, and ability to grow in marginal environments (Bryant *et al.*, 2020, Kauter *et al.*, 2003, Chudy *et al.*, 2019). Coppicing, where trees are harvested near ground level and new shoots regenerate from the stool for repeated harvesting without replanting, is a common practice in short-rotation woody biomass production (Tschaplinski & Blake, 1989). Feedstock biomass yields from coppicing can rival those of trees grown from seedlings or cuttings (Labrecque *et al.*, 2023, Proe *et al.*, 1999).

The success of coppicing is influenced by the composition and utilization of non-structural carbohydrate reserves available from sink organs for new growth (Luostarinen & Kauppi, 2005, Karp & Shield, 2008). The carbohydrate reserves facilitate not only bud emergence and new shoot growth in the spring before source organ photosynthesis becomes active, but also after defoliation or stem girdling events (Regier *et al.*, 2010, Falk *et al.*, 2020, Gleason & Ares, 2004). In general, it is considered that both soluble sugars and starch comprise storage reserves, with exchange between the constituent pools occurring on daily and seasonal time-scales in trees (Tixier *et al.*, 2018, Martínez-Vilalta *et al.*, 2016). It is widely held that reserve dynamics are important to trade-offs between storage, growth, defense, and protection, all of which influence tree fitness (Blumstein *et al.*, 2022). Genetics and plasticity both contribute to these trade-offs

(Herms & Mattson, 1992). Genetic variation in carbohydrate storage and utilization has been explored to greater depth for annual food crops since digestibility, storability, and flavor can depend on reserve carbohydrate transformations (Baxter *et al.*, 2005, Sekhon *et al.*, 2016, Li *et al.*, 2005). In the case of trees, however, the molecular mechanisms by which non-structural carbohydrate reserves contribute to new vegetative growth are relatively under-described.

Starch is regulated diurnally (Hartmann & Trumbore, 2016, Noronha *et al.*, 2018, Klein & Hoch, 2015), and therefore has been the focus of most studies on reserve carbohydrate utilization. However, significant diurnal fluctuations in leaf sucrose reserves occur in *Populus* (Wang *et al.*, 2022, Zhang *et al.*, 2014). Recent evidence suggests that in the absence of starch, other carbohydrates, presumably including sucrose, can provide the reserves needed to sustain new vegetative growth (Wang *et al.*, 2022). Under extreme carbon limitation, carbohydrate storage can supersede growth as a priority (Wang *et al.*, 2022, Wiley *et al.*, 2019, Sala *et al.*, 2012, Piper & Fajardo, 2016, Huang *et al.*, 2021). Under such conditions, trees may rely instead on alternative substrates such as lipids (Huang *et al.*, 2021, Fischer *et al.*, 2015) and hemicelluloses (Schädel *et al.*, 2009) to sustain respiration and growth. It is worth noting that phenolic glycoside salicinoids, which are abundant defense compounds in *Populus* species, are turnover-resistant during severe carbon deprivation (Hillabrand *et al.*, 2023). This highlights the importance of chemical defense in relation to growth and storage as a carbon destination in these species. Given the diverse metabolic and osmotic demands that potentially govern competition for sucrose, limited attention has been given to the physiological importance of its dynamic compartmentalization.

Transmembrane sucrose transporters (SUTs) facilitate the movement of sucrose in all plants (Kühn *et al.*, 1999, Ayre, 2011). Several phylogenetically and subcellularly distinct isoforms are known (Peng *et al.*, 2014), but in *Populus*, *SUT4* encoding the tonoplast isoform is expressed at a relatively high level compared to most other *SUTs* throughout the plant (Payyavula *et al.*, 2011). *SUT4* facilitates sucrose export from the vacuole to the cytosol, and its manipulation affects tissue sucrose abundance (Payyavula *et al.*, 2011, Eom *et al.*, 2011, Schneider *et al.*, 2012). In addition, *SUT4* is expressed in overwintering sink organs of several temperate tree species including *Populus* (Dobbelstein *et al.*, 2018, Ko *et al.*, 2011), and its expression varies in accordance with abiotic stress (Xu *et al.*, 2017, Payyavula *et al.*, 2011, Frost *et al.*, 2012, Harding *et al.*, 2022). Effects of *SUT4* silencing or knockout (KO) on source leaf turgor control and starch homeostasis have also been reported (Harding *et al.*, 2020, Harding *et al.*, 2022).

These observations are all consistent with the general notion that *SUT4* may be involved with the control of carbohydrate utilization in sink organs of *Populus*. Here, we used coppicing as a tool to investigate whether tonoplast sucrose trafficking is important for sink organ function under carbon-limiting conditions. A *SUT4*-KO effect on resprouting or defense metabolite investments would imply that physiological trade-offs are governed by the export of vacuolar sucrose *per se*, rather than export as its component hexoses or other modified forms. We compared epicormic bud growth in the contexts of stool metabolism, chemical defense, and osmolyte maintenance to test for *SUT4* importance to both summer and winter tradeoffs between growth, storage, defense, and winter protection.

Materials and Methods

Plant production and maintenance

PtaSUT4-KO mutants and the *Cas9* empty vector control lines used in this study were generated by CRISPR/Cas9 mutagenesis as reported previously (Zhou *et al.*, 2015, Harding *et al.*, 2022) and grown in a glasshouse located in the Whitehall Forest at the University of Georgia. Supplemental LED lighting was used to maintain a 16-hour photoperiod, with photosynthetic photon flux density of at least 600 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at mid canopy. Plants were propagated by single-node cuttings as described (Frost *et al.*, 2012) year-round for the experiments. Once established, rooted propagules were transferred to 1-gallon pots containing commercial soil mix (Fafard 3B) supplemented with Osmocote (15-9-12, NPK 6-month slow release) and maintained with daily watering until coppicing.

Coppicing experiments

For the winter coppicing experiment (January), twelve control (six wild-type [WT] and six *Cas9*) and twelve *sut4* mutants (four plants each of three independent events: KO-18, KO-25, and KO-51), were equally and randomly spaced on the floor bench of the glasshouse. Ten days prior to coppicing, the number of fully expanded leaves was reduced to forty for each plant to minimize differences in water uptake and photosynthetic capacity. Plants ~1.5 m in height were then coppiced (Julian Day 18) to ~25 cm stumps and any remaining leaves were removed. Watering was reduced after coppicing to avoid root anoxia. Outdoor temperatures during the duration of the winter coppicing experiment ranged from -5°C to 16°C, with an average of 6.5°C. Day

length ranged from 10.2 hours to 10.7 hours. Supplemental heating prevented indoor glasshouse temperatures from dropping below 10°C.

For the summer coppicing experiment (August), plants were managed as described above and were approximately the same height at coppicing as those in the winter experiment. Ten control plants (six WT and four *Cas9*) and twelve *sut4* mutants (four plants each of KO-18, KO-25, and KO-51 as above), were coppiced (Julian Day 228). Outdoor temperatures during the duration of the summer coppicing experiment ranged from 20°C to 33°C, with an average of 26°C. Day length ranged from 12.9 to 13.4 hours. Evaporative cooling prevented glasshouse temperatures from exceeding a high of 35°C during the day. The coppicing experiments were carried out twice to ensure reproducibility. Results from the most comprehensive of the two experiments are reported here.

Pre- and post-coppice sampling

Immediately before coppicing, deveined lamina of two adjacent fully-expanded source leaves, *i.e.*, Leaf Plastochron Index (LPI) 15-16, were flash frozen in liquid N and stored at -80°C for analyses. Immediately after coppicing ($t=0$), xylem sap was collected from the cut surfaces of the stools. Bark and xylem tissues were collected from the top of the stump immediately after sap collection ($t=0$; Supplemental Figure S1) and again when epicormic bud elongation was measured ($t=1$; Supplemental Figure S1). The third and final sampling ($t=2$) took place during shoot extension. Tissues were snap frozen in liquid N and stored at -80°C. At $t=2$, root samples (terminal 6 cm of elongating roots excluding root tips) were washed in deionized water, dried, and snap frozen in liquid N. The largest (dominant) epicormic bud that emerged after coppicing

of each tree was photographed and its length determined at $t=1$ using the ImageJ software package (<https://imagej.net/ij/index.html>). Due to seasonal growth differences, sampling at times $t=1$ and $t=2$ was carried out 10 and 16 days, respectively, after summer coppicing and 11 and 20 days, respectively, after winter coppicing (Supplemental Figure S1). All tissue sampling occurred under sunny conditions between 11AM – 2PM.

Biomass

A second coppicing experiment was carried out to determine biomass of shoots produced from stumps ~1 month after coppicing. For this, coppicing of ~1.0 m plants ($n = 6 - 12$) was carried out in winter (December) and summer (June). Leaves (including petioles) and shoot tissues were dried in a forced air oven for 48 hours at 60°C. Independently of the coppicing experiments, cohorts of mature (~2 m) control and *sut4* mutant plants ($n = 8 - 14$ plants) were harvested in mid-winter (January) and mid-summer (July-August) for whole plant dry biomass analysis. Plant height and stem diameter were measured at harvest, and leaves (including petiole), bark, and wood (de-barked stem) were collected to determine biomass allocation patterns. Root tissues were washed in deionized water and dried.

Xylem sap

Xylem sap was collected from the stumps immediately after coppicing following a previously published approach (Siebrecht & Tischner, 1999). Bark was removed from the top 4 cm of the stump to expose the xylem cylinder and to avoid mixing of phloem and xylem sap during collection. The razor-cut surface of the wood was tamped clean with a damp kimwipe. The first 400 μ l of xylem sap was discarded. Over the next 45 min, ~100-200 μ l of xylem sap was

collected with a pipette from each stump and snap frozen in liquid nitrogen. Frozen sap was stored at -80°C, thawed on ice just before analysis, mixed, and derivatized after drying in glass inserts for metabolite analysis as described below.

Metabolite analysis

Tissues were freeze-dried using a FreezeZone 2.5 (Labconco), Wiley milled through a 40-mesh sieve (Thomas Scientific), and further ball-milled at 1500 rpm in a MiniG tissue homogenizer (Spex SamplePrep) to a fine powder. An aliquot of tissue powder (10.0 ± 0.5 mg) was used for metabolite profiling by gas chromatography-mass spectrometry (GC-MS) as detailed in Harding et al. (2022). Adonitol (Sigma-Aldrich) was used as an internal standard. Samples were analyzed on an Agilent 7890A GC coupled to an Agilent 5975C single quadrupole MS detector (Agilent Technologies). Peaks were deconvoluted using AnalyzerPro (SpectralWorks) and putative identities were assigned based on the NIST08, the Agilent Fiehn metabolomics library, and an in-house library of authentic standards (Harding et al., 2022). A standard mix containing succinic acid, sucrose, glutamic acid, fructose, glucose, and ascorbic acid was loaded at the beginning and end of each sample set to monitor derivatization and instrument performance.

Another tissue powder aliquot (5.0 ± 0.5 mg) was used for profiling by ultra-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (UPLC-QTOF). Tissue extraction was performed as above (Harding et al., 2022) with the exception that $^{13}\text{C}_6$ -cinnamic acid and D₅-benzoic acid were included as internal standards. Extracts were filtered through a 0.2 μm PTFE (polytetrafluoroethylene) filter (Agilent Technologies) and resolved on an Agilent Zorbax Eclipse Plus C18 column (2.1×50 mm, 1.8 μm) using an Agilent 1290

Infinity II UPLC coupled to an Agilent 6546 LC/Q-TOF tandem MS. The mobile solvent A comprised water and 0.1% formic acid and mobile solvent B comprised acetonitrile with 0.1% formic acid at a flow rate of 0.5 mL min⁻¹. The elution gradient was 3% B from 0 – 0.5 min, linear gradient to 15% B over 3.5 min, isocratic at 30% B over 4.5 min, linear gradient to 50% B over 5.5 min, and then to 100% B over 7 min, followed by a 3-min post-column run. Column temperature was 45°C. MS data were acquired in negative mode with the following parameters: gas temperature, 250°C; nebulizer gas, 40 psi; nozzle voltage, 500 V; and capillary voltage, 400 V. Data were processed using the MassHunter software suite Version 11.0 (Agilent). Peak identities were confirmed using an in-house library of authentic standards, when available, or assigned by searching against an accurate-mass NIST20 library. The highest database match score (usually >85%) obtained was used to support the putative identity of the QTOF metabolites.

GC-MS and LC-QTOF peak intensities were normalized by their respective internal standards and sample dry weight, with the exception of tissue sucrose and hexose (fructose and glucose) levels which are reported as percent dry weight based on their respective calibration curves.

Starch

Starch content was determined by enzymatic digestion with α -amylase and amyloglucosidase (Chow & Landhäusser, 2004). In brief, 10 mg of lyophilized, Wiley- and ball-milled tissue powder was pre-extracted three times with 1 ml of methanol by vortexing and sonication. The tissue pellet was resuspended in 0.5 ml of buffer (0.1 M sodium acetate, pH 5.0, with 5 mM CaCl₂) containing 500 U of α -amylase (Sigma A4551) and incubated for 2 hr at 65°C. Samples

were cooled and 10 µl of sodium acetate buffer (pH 5.0) containing 5 U amyloglucosidase (Sigma A1602) was added for further digestion at 50°C for 48 hr with shaking. Digests were then diluted with 500 µl of H₂O, vortexed, centrifuged, and 20 µl of the diluted supernatant were dried and derivatized for GC-MS as described above. Chromatograms contained only glucose and adonitol internal standard peaks. Total starch content was calculated based on a glucose standard curve and expressed as µg starch per mg of dry tissue.

Condensed tannins (CTs)

Freeze dried tissue powder (10 mg) was extracted in 600 µl of methanol for 15 minutes in a sonicator bath to extract interfering chlorophyll. The depigmented pellet was dried and CT content was estimated using the butanol-HCl method (Porter *et al.*, 1986) as described (Harding *et al.*, 2022). CT concentration was determined based on absorbance at 550 nm (SpectraMax M2; Molecular Devices) using purified aspen leaf CTs as standards.

Chlorophyll content

Ten mg of freeze-dried powder was extracted twice in 750 µl of cold 90% acetone – 10% water, with sonication and centrifugation. Absorbances at 645 nm and 663 nm were used to determine chlorophyll *a*, chlorophyll *b*, and total chlorophyll content (µg g⁻¹ DW) as described by Lichtenthaler (1987).

Statistics

Repeated measures analysis of variance (ANOVA) was used to determine significant differences at each stage of coppicing re-growth over time, between genotypes, and their interactions using *R*

v4.1.0 in Rstudio (version 2022.12.353). Linear regression was used to test for the relationships between sucrose or starch depletion and bud elongation of WT or *sut4* mutants between t=0 and t=1 of the winter and summer coppicing experiments.

Results

Seasonal coppicing response of sut4 mutants

We conducted coppicing experiments to study sink organ carbohydrate utilization under both short-day, cool winter conditions and long-day, warm summer conditions in a glasshouse (Figure S1). The rate at which new epicormic buds appeared on stumps after coppicing was largely similar between genotypes under the summer glasshouse conditions (Figure 1A, C). The total biomass of new shoots at t=2 in the *sut4* mutants was also similar to the controls despite initially faster bud elongation in *sut4* mutants (t=1) during the summer (Figure 1E-F). By contrast, epicormic bud release was delayed by several days in *sut4* mutants compared to controls in the winter but attained the same number of sprouts by t=2 (Figure 1B, D). Bud elongation at t=1 was slower and total biomass at t=2 was reduced in the *sut4* mutants compared to control plants following winter coppicing (Figure 1E-F). A replicate experiment was conducted to examine biomass partitioning in fully-grown new shoots 32 and 40 days after summer and winter coppicing, respectively. Total leaf and stem dry mass were significantly higher in *sut4* than control sprouts under summer conditions (Table 1). In contrast, total biomass trended lower, but not significantly, in *sut4* than in control plants in the winter (Table 1), which translates to overall less robust coppice growth of *sut4* mutants in the winter than summer. The seasonal differences

in biomass allocation of coppices were not observed in mature trees (t=0) which showed slight shoot biomass reductions in both seasons (Table S1), similar to previously reported for *SUT4*-silenced trees (Frost et al., 2012, Harding et al., 2020, Payyavula et al., 2011). The results hint at a seasonal influence of *SUT4* on coppicing responses.

Sink carbohydrate reserves

Sucrose contents of mature tree organs were higher in the *sut4* mutants when compared to the controls (Figure S2), as reported previously (Harding et al., 2022). Following summer or winter coppicing, bark and xylem sucrose abundance generally decreased (Figure 2A). The decrease was less marked in *sut4* than control by t=2 of the summer experiment, but sucrose was depleted to approximately the same low abundance in bark and xylem of both genotypes under winter conditions (Figure 2A). Root sucrose was not as depleted by coppicing and remained higher in *sut4* than control lines at t=2, especially in the summer (Figure 2A).

Bark hexose (sum of fructose and glucose) levels differed little between genotypes and decreased by t=2 in both the summer and the winter (Figure 2B). In contrast, xylem hexose levels increased in both lines in the winter between t=0 and t=2, but decreased in the summer by t=2 (Figure 2B). In both seasons, but most notably in the winter, xylem hexose levels were higher in control than *sut4* mutants. Root hexose levels increased sharply in both genotypes after summer coppicing, but the increase was observed only in control plants after winter coppicing (Figure 2B).

Starch levels in the bark and xylem were higher in control than *sut4* plants in both the summer and winter, and remained so as starch decreased through t=1 (Figure 2C). Bark and xylem starch

was depleted to similarly low levels in both genotypes by t=2, suggesting that starch abundance there had reached a minimum. Root starch content was similar in both genotypes at t=2 but was much less abundant in the summer than the winter (Figure 2C). Together, the data suggest a depletion of non-structural carbohydrates to less than 25% of pre-coppice levels, in shoot organs. Root sucrose also decreased sharply, but starch was only measured at final harvest (t=2).

Sucrose and starch utilization for bud growth

Slower post-coppice sprout emergence and growth in *sut4* than control trees in the winter led us to test whether sucrose and/or starch depletion between t=0 and t=1 in the bark predicted bud length at t=1 (Figure 3). Bud size did not correlate with sucrose depletion in the summer for either genotype (*sut4*: $r^2 = 0.17$, $p = 0.21$, control: $r^2 = 0.06$, $p = 0.49$), but the slope trended positive for *sut4* and negative for control plants (Figure 3A). Under winter conditions, bud length correlated with sucrose depletion in the *sut4* mutant trees ($r^2 = 0.39$, $p = 0.03$) but not in the controls ($r^2 = 0.16$, $p = 0.19$) where in fact the slope trended negative as in winter (Figure 3A). Bud expansion did not correlate with changes in starch content under summer (*sut4*: $r^2 = 0.02$, $p = 0.68$, control: $r^2 = 0.12$, $p = 0.439$) or winter (*sut4*: $r^2 = 0.00$, $p = 0.99$, control: $r^2 = 0.04$, $p = 0.54$) conditions (Figure 3B). Overall, post-coppice bud elongation in *sut4* mutant, but not in control plants, tended to correlate positively with sucrose but not with starch depletion. While bud growth was slower in *sut4* than control plants in the winter, sucrose depletion in *sut4* reserve organs was normal. Other factors related to sucrose utilization may have led to the appearance suggested in the regressions (Figure 3) of a sucrose limitation to *sut4* bud growth.

Xylem sap and root metabolites

Xylem sap sucrose abundance at $t=0$ was greater in winter than summer, but differed little between genotypes during either season (Figure 4A). Sap hexose levels did not differ between genotypes in the summer but were reduced in *sut4* compared to control plants in the winter (Figure 4B). Xylem sap is also the route by which most root-assimilated nitrogen travels to the transpiring leaves (Siebrecht & Tischner, 1999). Given that nitrogen assimilation depends on carbon skeletons in the assimilating organ, a comparison of xylem sap Krebs cycle intermediate and amino acid contents could reveal seasonal and genotypic differences in systemically available carbon in the roots. Krebs cycle intermediates malate and citrate comprised most of the xylem sap metabolic load in both summer and winter, and were more abundant in *sut4* than control xylem saps (Figure 4C-D). Glutamine and glutamate were the most abundant amino acids we detected in xylem sap. Both trended higher in *sut4* than control xylem sap in the summer but were significantly lower in *sut4* than control sap in the winter (Figure 4C-D). These four metabolites were also among the most abundant in root tissues, but only malate mimicked the xylem sap patterns, being most abundant in *sut4* (Supplemental Figure S3).

Bark and xylem primary metabolites

Bark and xylem metabolite response trends at $t=0$ roughly paralleled those observed in xylem saps, with amino acids being lower and Krebs cycle intermediates higher in *sut4* mutants, most markedly in winter xylem (Figure 5). Malate and citrate were more abundant in *sut4* than control bark and xylem during the winter, with the differential increasing during bud expansion ($t=1$ and $t=2$) (Figure 5). Phenylpropanoid pathway precursors (e.g., quinic acid and shikimic acid) and intermediates (e.g., hydroxycinnamates and catechin) were detected at comparatively low levels in *sut4* bark and xylem throughout the course of both seasons. Finally, levels of stress

metabolites and sugar alcohols including raffinose, galactinol and myo-inositol were generally greater in the bark of mature *sut4* plants (t=0) but lower than in controls by t=2 (Figure 5).

Chemical defense

Provisioning of nonstructural sinks related to poplar defense has been associated with carbohydrate transport (Arnold *et al.*, 2004, Kleiner *et al.*, 1999). In *Populus* and *Salix* species, chlorogenic acids, salicinoids (e.g., salicortin and tremulacin), and CTs derived from quinate-shikimate-phenylpropanoid pathway are present in especially large abundance for fitness and stress tolerance (Harding *et al.*, 2005, Tsai *et al.*, 2006, Zhang *et al.*, 2018). Chlorogenic acids (caffeoyl-quinate isomers) were at lower levels in *sut4* relative to control bark at all time points in both the summer and winter coppicing experiments (Figure 6A). Salicortin, by far the most abundant defense metabolite, was detected at higher levels in the summer than the winter (Figure 6B). Bark salicortin abundance was substantially lower in the *sut4* mutants than control during the summer but did not differ between genotypes during the winter (Figure 6B). The closely related tremulacin was also significantly lower in *sut4* mutant bark in the summer, but not the winter (Figure 6C). Bark CT levels were lower in *sut4* than control plants, especially in the summer (Figure 6D).

Chlorophyll content

We examined bark chlorophyll contents as proxies for photosynthetic capacity after coppicing. Bark chlorophyll content differed little between genotypes and remained relatively stable during summer coppicing experiment, but showed a transient decrease at t=1 during winter (Figure 7).

Discussion

Trees produce nonstructural carbohydrate reserves to sustain respiration and osmoregulation for winter protection and spring bud flush during perennial growth (Furze *et al.*, 2019). The plasticity of reserve utilization across species is well documented and probably reflects multiple environmental and genetic influences over the process (Sevanto *et al.*, 2014, Blumstein *et al.*, 2022, Martínez-Vilalta *et al.*, 2016, Furze *et al.*, 2019). While it is generally assumed that starch comprises the bulk of metabolically available reserve carbohydrate, this is mainly inferred from the diurnal accrual and depletion of transient starch in annual species (Smith & Stitt, 2007). However, a process as fundamental as bud flush in aspen can occur in the absence of starch (Wang *et al.*, 2022). Therefore, reserve carbohydrate accrual and utilization exhibits plasticity and can proceed through multiple routes. An analysis of carbohydrate active gene expression in overwintering stem tissue of poplar supports involvement of starch degradation as well as of sucrose synthesis and trafficking (Figure S4) (Ko *et al.*, 2011). Several studies report sustained winter expression of sucrose transporters in tree sink organs, with gene transcript levels for the tonoplast sucrose transporter *SUT4* peaking in the winter (Figure S4) (Dobbelstein *et al.*, 2018, Ko *et al.*, 2011). Therefore, it is likely that sucrose trafficking and starch metabolism are important for winter survival.

Under winter post-coppice conditions in the present study, stump starch and sucrose were reduced to 14% and 17% of their initial levels in control and mutant lines, respectively, but epicormic bud growth was slow in *sut4* mutants compared to controls. It is probable that sucrose trafficking increases in importance during the winter in line with reports of higher *SUT4* gene

expression in winter (Figure S4) (Dobbelstein et al., 2018, Ko et al., 2011). Stump sucrose levels were higher in winter than summer as others have reported (Furze et al., 2019), but reduced *sut4* bud elongation only in winter could suggest growth-hindering injury or stress of some sort.

It is worth considering that cytosolic sucrose sustains critical winter processes such as hexose production for non-nutritive purposes. We observed that xylem hexose levels increased in coppiced stumps in the winter, but not in the summer in all lines, and that the levels were overall lower in *sut4* mutants (Figure 2B). Hexose osmolytes in the xylem could represent a high priority allocation of nonstructural carbohydrates for winter protection. This is consistent with the previously established roles of sugar osmolytes in reactive oxygen species scavenging, cold tolerance, and embolism refilling during winter (Charrier *et al.*, 2018, Tarkowski & Van den Ende, 2015), and with abundant expression of cell wall invertases in winter stems (Ko et al., 2011). Our finding that both xylem hexose and bud growth were compromised in *sut4* raises the possibility that reduced cytosolic sucrose metabolism may have been injurious to the winter stumps. Besides the overall reduction in hexose, reductions in other important sucrose-derived protective osmolytes like raffinose were also observed in *sut4* compared to control stumps (Figure 5). A similar effect on raffinose was reported in leaves of drought-stressed poplar lines with reduced *SUT4* gene expression (Frost et al., 2012). The data suggest active participation of SUT4 during utilization of winter carbohydrate reserves for both growth and injury avoidance.

As in xylem, root hexose was detected at lower levels in *sut4* than control coppices in the winter at t=2. In root and xylem cells alike, sucrose that is exported from the vacuole is converted to hexoses by cytosolic and apoplastic cell wall invertases (Koch, 2004, Ruan, 2014). Utilization of

sucrose that is not exported from the vacuole depends on the activity of vacuolar invertase and tonoplast hexose transporters (Koch, 2004, Ruan, 2014, Schulz *et al.*, 2011). Their expression is modulated by stresses, such as drought and cold (Wormit *et al.*, 2006, Roitsch & González, 2004, Chen *et al.*, 2015, Xue *et al.*, 2016). Post-coppicing invertase gene expression likely varied due to seasonal differences in soil moisture and temperatures, resulting in more harmful consequences of *sut4* limitation in winter than in summer. The finding that root hexose increased sharply and equally in both control and mutant lines after summer coppicing, but did not increase in *sut4* after winter coppicing supports this interpretation.

In summer, the metabolic priority of coppices seemed to shift toward increased accrual of resource-intensive poplar defense metabolites such as chlorogenic acids, salicortin, and CTs. This is consistent with a greater depletion of sucrose and starch in control (to 6% of initial levels) than in *sut4* (to below 23%) in the summer. While epicormic bud emergence was similar in all lines following summer coppicing, defense metabolite levels were substantially lower and new sprout biomass was significantly higher in *sut4* than control lines. *Populus* species maintain constitutive levels of salicinoids even under severe carbon limitation (Hillabrand *et al.*, 2023, Fabisch *et al.*, 2019), but our results suggest that growth can be prioritized over defense under sufficiently carbon limiting conditions, at least when trafficking of vacuolar sucrose is impaired.

In conclusion, PtaSUT4 was revealed through seasonal differences in the coppicing responses of KO mutant lines to have a role in nonstructural carbohydrate allocations for growth and winter protection. Future research may investigate the effects of *SUT4*-KO under field conditions to further explore seasonal influences on SUT4 function and perennial growth habit.

Data and Materials Availability: Data and material will be made available upon reasonable request.

Supplemental data

Table S1. Biomass allocation of mature control and *sut4* poplar.

Figure S1. Schematics of coppicing experiments.

Figure S2. Sucrose levels in mature tree organs.

Figure S3. Root metabolites.

Figure S4. Seasonal variation of *SUT* and representative carbohydrate gene expression.

Conflict of interest: None declared.

Funding: This work was supported in part by a National Science Foundation Graduate Research Fellowship (DGE – 1842396) to T.T.T., a Department of Energy, Office of Science, Biological and Environmental Research Program Award (DESC0023166) to C.J.T., and the Georgia Research Alliance Hank Haynes Forest Biotechnology endowment to C.J.T. Funding for the Agilent UPLC-QTOF was provided by the U.S. Department of Agriculture, National Institute of Food and Agriculture, Equipment Grant Program (Award 2021-70410-35297 to C.J.T. and S.A.H.).

Acknowledgements: The authors thank Gilles Pilate of INRA, France for providing poplar clone INRA 717-1B4, Suzanne Tate for greenhouse assistance, and Khadijeh Mozaffari for LC-QTOF assistance.

Authors' Contributions: T.T.T, S.A.H, & C-J.T. conceived the study and designed all experiments. T.T.T. performed all experiments and analyzed the data. T.T.T, B.N., & S.A.H, performed metabolic analyses. Y-H.C. and C.H. helped with expression analysis. T.T.T. prepared the draft manuscript, which was edited by S.A.H. and C-J. T.

References

- Arnold, T., Appel, H., Patel, V., Stocum, E., Kavalier, A. and Schultz, J. (2004) Carbohydrate translocation determines the phenolic content of *Populus* foliage: a test of the sink-source model of plant defense. *New Phytologist*, **164**, 157-164.
- Ayre, B. G. (2011) Membrane-transport systems for sucrose in relation to whole-plant carbon partitioning. *Molecular Plant*, **4**, 377-394.
- Baxter, C. J., Carrari, F., Bauke, A., Overy, S., Hill, S. A., Quick, P. W., *et al.* (2005) Fruit carbohydrate metabolism in an introgression line of tomato with increased fruit soluble solids. *Plant and Cell Physiology*, **46**, 425-437.
- Blumstein, M., Sala, A., Weston, D. J., Holbrook, N. M. and Hopkins, R. (2022) Plant carbohydrate storage: intra- and inter-specific trade-offs reveal a major life history trait. *New Phytologist*, **235**, 2211-2222.
- Bryant, N. D., Pu, Y., Tschaplinski, T. J., Tuskan, G. A., Muchero, W., Kalluri, U. C., *et al.* (2020) Transgenic poplar designed for biofuels. *Trends in Plant Science*, **25**, 881-896.
- Charrier, G., Lacoïnte, A. and Améglio, T. (2018) Dynamic modeling of carbon metabolism during the dormant period accurately predicts the changes in frost hardness in walnut trees *Juglans regia* L. *Front Plant Sci*, **9**, 1746.
- Chen, Z., Gao, K., Su, X., Rao, P. and An, X. (2015) Genome-wide identification of the invertase gene family in *Populus*. *PLoS One*, **10**, e0138540.
- Chow, P. S. and Landhäusser, S. M. (2004) A method for routine measurements of total sugar and starch content in woody plant tissues. *Tree Physiology*, **24**, 1129-1136.
- Chudy, R. P., Busby, G. M., Binkley, C. S. and Stanton, B. J. (2019) The economics of dedicated hybrid poplar biomass plantations in the western U.S. *Biomass and Bioenergy*, **124**, 114-124.
- Dhillon, R. S. and von Wuehlisch, G. (2013) Mitigation of global warming through renewable biomass. *Biomass and Bioenergy*, **48**, 75-89.

- Dobbelstein, E., Fink, D., Öner-Sieben, S., Czempik, L. and Lohaus, G. (2018) Seasonal changes of sucrose transporter expression and sugar partitioning in common European tree species. *Tree Physiology*, **39**, 284-299.
- Eom, J. S., Cho, J. I., Reinders, A., Lee, S. W., Yoo, Y., Tuan, P. Q., *et al.* (2011) Impaired function of the tonoplast-localized sucrose transporter in rice, OsSUT2, limits the transport of vacuolar reserve sucrose and affects plant growth. *Plant Physiology*, **157**, 109-119.
- Fabisch, T., Gershenzon, J. and Unsicker, S. B. (2019) Specificity of herbivore defense responses in a woody plant, black poplar (*Populus nigra*). *Journal of Chemical Ecology*, **45**, 162-177.
- Falk, M. A., Donaldson, J. R., Stevens, M. T., Raffa, K. F. and Lindroth, R. L. (2020) Phenological responses to prior-season defoliation and soil-nutrient availability vary among early- and late-flushing aspen (*Populus tremuloides* Michx.) genotypes. *Forest Ecology and Management*, **458**, 117771.
- Fischer, S., Hanf, S., Frosch, T., Gleixner, G., Popp, J., Trumbore, S., *et al.* (2015) *Pinus sylvestris* switches respiration substrates under shading but not during drought. *New Phytologist*, **207**, 542-550.
- Frost, C. J., Nyamdari, B., Tsai, C.-J. and Harding, S. A. (2012) The tonoplast-localized sucrose transporter in *Populus* (PtaSUT4) regulates whole-plant water relations, responses to water stress, and photosynthesis. *PLoS One*, **7**, e44467.
- Furze, M. E., Huggett, B. A., Aubrecht, D. M., Stolz, C. D., Carbone, M. S. and Richardson, A. D. (2019) Whole-tree nonstructural carbohydrate storage and seasonal dynamics in five temperate species. *New Phytologist*, **221**, 1466-1477.
- Gleason, S. M. and Ares, A. (2004) Photosynthesis, carbohydrate storage and survival of a native and an introduced tree species in relation to light and defoliation. *Tree Physiology*, **24**, 1087-1097.
- Harding, S. A., Frost, C. J. and Tsai, C.-J. (2020) Defoliation-induced compensatory transpiration is compromised in *SUT4*-RNAi *Populus*. *Plant Direct*, **4**, e00268.
- Harding, S. A., Jiang, H. Y., Jeong, M. L., Casado, F. L., Lin, H. W. and Tsai, C. J. (2005) Functional genomics analysis of foliar condensed tannin and phenolic glycoside regulation in natural cottonwood hybrids. *Tree Physiology*, **25**, 1475-1486.
- Harding, S. A., Tuma, T. T., Aulakh, K., Ortega, M. A., Ci, D., Ou, Y., *et al.* (2022) Tonoplast sucrose trafficking modulates starch utilization and water deficit behavior in poplar leaves. *Plant and Cell Physiology*, **63**, 1117-1129.
- Hartmann, H. and Trumbore, S. (2016) Understanding the roles of nonstructural carbohydrates in forest trees – from what we can measure to what we want to know. *New Phytologist*, **211**, 386-403.
- Hermes, D. A. and Mattson, W. J. (1992) The dilemma of plants: To grow or defend. *The Quarterly Review of Biology*, **67**, 283-335.
- Hillabrand, R. M., Gordon, H., Hynes, B., Constabel, C. P. and Landhäusser, S. M. (2023) *Populus* root salicinoid phenolic glycosides are not mobilized to support metabolism and regrowth under carbon limited conditions. *Tree Physiol.*
- Huang, J., Hammerbacher, A., Gershenzon, J., van Dam, N. M., Sala, A., McDowell, N. G., *et al.* (2021) Storage of carbon reserves in spruce trees is prioritized over growth in the face of carbon limitation. *Proceedings of the National Academy of Sciences*, **118**, e2023297118.

- Karp, A. and Shield, I. (2008) Bioenergy from plants and the sustainable yield challenge. *New Phytologist*, **179**, 15-32.
- Kauter, D., Lewandowski, I. and Claupein, W. (2003) Quantity and quality of harvestable biomass from *Populus* short rotation coppice for solid fuel use—a review of the physiological basis and management influences. *Biomass and Bioenergy*, **24**, 411-427.
- Klein, T. and Hoch, G. (2015) Tree carbon allocation dynamics determined using a carbon mass balance approach. *New Phytologist*, **205**, 147-159.
- Kleiner, K. W., Raffa, K. F. and Dickson, R. E. (1999) Partitioning of ¹⁴C-labeled photosynthate to allelochemicals and primary metabolites in source and sink leaves of aspen: evidence for secondary metabolite turnover. *Oecologia*, **119**, 408-418.
- Ko, J.-H., Prassinos, C., Keathley, D., Han, K.-H. and Li, C. (2011) Novel aspects of transcriptional regulation in the winter survival and maintenance mechanism of poplar. *Tree Physiology*, **31**, 208-225.
- Koch, K. (2004) Sucrose metabolism: regulatory mechanisms and pivotal roles in sugar sensing and plant development. *Current Opinion in Plant Biology*, **7**, 235-246.
- Kühn, C., Barker, L., Bürkle, L. and Frommer, W.-B. (1999) Update on sucrose transport in higher plants. *Journal of Experimental Botany*, **50**, 935-953.
- Labrecque, M., Daigle, S. and Olishchevska, S. (2023) Comparing biomass yields of various willow cultivars in short-rotation coppice over six growing seasons across a broad climatic gradient in Eastern Canada. *Canadian Journal of Forest Research*, **53**, 533-543.
- Li, L., Strahwald, J., Hofferbert, H. R., Lübeck, J., Tacke, E., Junghans, H., *et al.* (2005) DNA variation at the invertase locus *invGE/GF* is associated with tuber quality traits in populations of potato breeding clones. *Genetics*, **170**, 813-821.
- Lichtenthaler, H. K. (1987) Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. In: *Methods Enzymol.* Academic Press, pp. 350-382.
- Luostarinen, K. and Kauppi, A. (2005) Effects of coppicing on the root and stump carbohydrate dynamics in birches. *New Forests*, **29**, 289-303.
- Martínez-Vilalta, J., Sala, A., Asensio, D., Galiano, L., Hoch, G., Palacio, S., *et al.* (2016) Dynamics of non-structural carbohydrates in terrestrial plants: a global synthesis. *Ecological Monographs*, **86**, 495-516.
- Nieminen, K., Robischon, M., Immanen, J. and Helariutta, Y. (2012) Towards optimizing wood development in bioenergy trees. *New Phytologist*, **194**, 46-53.
- Noronha, H., Silva, A., Dai, Z., Gallusci, P., Rombolà, A. D., Delrot, S., *et al.* (2018) A molecular perspective on starch metabolism in woody tissues. *Planta*, **248**, 559-568.
- Payyavula, R. S., Tay, K. H. C., Tsai, C.-J. and Harding, S. A. (2011) The sucrose transporter family in *Populus*: The importance of a tonoplast PtaSUT4 to biomass and carbon partitioning. *Plant Journal*, **65**, 757-770.
- Peng, D., Xue, L.-J., Gu, X., Leebens-Mack, J. H. and Tsai, C.-J. (2014) Bayesian phylogeny of sucrose transporters: Ancient origins, differential expansion and convergent evolution in monocots and dicots. *Frontiers in Plant Science*, **5**, 615.
- Piper, F. I. and Fajardo, A. (2016) Carbon dynamics of *Acer pseudoplatanus* seedlings under drought and complete darkness. *Tree Physiology*, **36**, 1400-1408.
- Porter, L. J., Hrstich, L. N. and Chan, B. G. (1986) The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin. *Phytochemistry*, **25**, 223-230.

- Proe, M. F., Craig, J., Griffiths, J., Wilson, A. and Reid, E. (1999) Comparison of biomass production in coppice and single stem woodland management systems on an imperfectly drained gley soil in central Scotland. *Biomass and Bioenergy*, **17**, 141-151.
- Ragauskas, A. J., Williams, C. K., Davison, B. H., Britovsek, G., Cairney, J., Eckert, C. A., *et al.* (2006) The path forward for biofuels and biomaterials. *Science*, **311**, 484-489.
- Regier, N., Streb, S., Zeeman, S. C. and Frey, B. (2010) Seasonal changes in starch and sugar content of poplar (*Populus deltoides* × *nigra* cv. Dorskamp) and the impact of stem girdling on carbohydrate allocation to roots. *Tree Physiology*, **30**, 979-987.
- Roitsch, T. and González, M.-C. (2004) Function and regulation of plant invertases: sweet sensations. *Trends in Plant Science*, **9**, 606-613.
- Ruan, Y.-L. (2014) Sucrose metabolism: Gateway to diverse carbon use and sugar signaling. *Annual Review of Plant Biology*, **65**, 33-67.
- Sala, A., Woodruff, D. R. and Meinzer, F. C. (2012) Carbon dynamics in trees: feast or famine? *Tree Physiology*, **32**, 764-775.
- Schädel, C., Blöchl, A., Richter, A. and Hoch, G. (2009) Short-term dynamics of nonstructural carbohydrates and hemicelluloses in young branches of temperate forest trees during bud break. *Tree Physiology*, **29**, 901-911.
- Schneider, S., Hulpke, S., Schulz, A., Yaron, I., Holl, J., Imlau, A., *et al.* (2012) Vacuoles release sucrose via tonoplast-localised SUC4-type transporters. *Plant Biology*, **14**, 325-336.
- Schulz, A., Beyhl, D., Marten, I., Wormit, A., Neuhaus, E., Poschet, G., *et al.* (2011) Proton-driven sucrose symport and antiport are provided by the vacuolar transporters SUC4 and TMT1/2. *Plant Journal*, **68**, 129-136.
- Sekhon, R. S., Breitzman, M. W., Silva, R. R., Santoro, N., Rooney, W. L., de Leon, N., *et al.* (2016) Stover composition in maize and sorghum reveals remarkable genetic variation and plasticity for carbohydrate accumulation. *Frontiers in Plant Science*, **7**.
- Sevanto, S., McDowell, N. G., Dickman, L. T., Pangle, R. and Pockman, W. T. (2014) How do trees die? A test of the hydraulic failure and carbon starvation hypotheses. *Plant, Cell & Environment*, **37**, 153-161.
- Siebrecht, S. and Tischner, R. (1999) Changes in the xylem exudate composition of poplar (*Populus tremula* × *P. alba*)—dependent on the nitrogen and potassium supply. *Journal of Experimental Botany*, **50**, 1797-1806.
- Smith, A. M. and Stitt, M. (2007) Coordination of carbon supply and plant growth. *Plant Cell and Environment*, **30**, 1126-1149.
- Tarkowski, Ł., P. and Van den Ende, W. (2015) Cold tolerance triggered by soluble sugars: a multifaceted countermeasure. *Front Plant Sci*, **6**, 203.
- Tixier, A., Orozco, J., Roxas, A. A., Earles, J. M. and Zwieniecki, M. A. (2018) Diurnal variation in nonstructural carbohydrate storage in trees: Remobilization and vertical mixing. *Plant Physiology*, **178**, 1602-1613.
- Tsai, C.-J., Harding, S. A., Tschaplinski, T. J., Lindroth, R. L. and Yuan, Y. (2006) Genome-wide analysis of the structural genes regulating defense phenylpropanoid metabolism in *Populus*. *New Phytologist*, **172**, 47-62.
- Tschaplinski, T. J. and Blake, T. J. (1989) Photosynthetic reinvigoration of leaves following shoot decapitation and accelerated growth of coppice shoots. *Physiologia Plantarum*, **75**, 157-165.
- Wang, W., Talide, L., Viljamaa, S. and Niittylä, T. (2022) Aspen growth is not limited by starch reserves. *Current Biology*, **32**, 3619-3627.e3614.

- Wiley, E., King, C. M. and Landhäusser, S. M. (2019) Identifying the relevant carbohydrate storage pools available for remobilization in aspen roots. *Tree Physiology*, **39**, 1109-1120.
- Wormit, A., Trentmann, O., Feifer, I., Lohr, C., Tjaden, J., Meyer, S., *et al.* (2006) Molecular identification and physiological characterization of a novel monosaccharide transporter from *Arabidopsis* involved in vacuolar sugar transport. *Plant Cell*, **18**, 3476-3490.
- Xu, Q., Chen, S., Yunjuan, R., Chen, S. and Liesche, J. (2017) Regulation of Sucrose Transporters and Phloem Loading in Response to Environmental Cues. *Plant Physiology*, **176**, 930-945.
- Xue, L.-J., Frost, C. J., Tsai, C.-J. and Harding, S. A. (2016) Drought response transcriptomics are altered in poplar with reduced tonoplast sucrose transporter expression. *Scientific Reports*, **6**, 33655.
- Zhang, C., Han, L., Slewinski, T. L., Sun, J., Zhang, J., Wang, Z.-Y., *et al.* (2014) Symplastic phloem loading in poplar. *Plant Physiology*, **166**, 306-313.
- Zhang, J., Yang, Y., Zheng, K., Xie, M., Feng, K., Jawdy, S. S., *et al.* (2018) Genome-wide association studies and expression-based quantitative trait loci analyses reveal roles of HCT2 in caffeoylquinic acid biosynthesis and its regulation by defense-responsive transcription factors in *Populus*. *New Phytologist*, **220**, 502-516.
- Zhou, X., Jacobs, T. B., Xue, L.-J., Harding, S. A. and Tsai, C.-J. (2015) Exploiting SNPs for biallelic CRISPR mutations in the outcrossing woody perennial *Populus* reveals 4-coumarate:CoA ligase specificity and redundancy. *New Phytologist*, **208**, 298-301.

Figure Legends

FIGURE 1: Epicormic bud emergence and growth following coppicing.

(A-B) Representative images of *sut4* and control poplar stools under summer (A) and winter (B) conditions immediately after coppicing (t=0), during epicormic bud expansion (t=1), and during sprout growth (t=2). (C-D) Bud emergence after summer (C) and winter (D) coppicing. Black and grey represent control and *sut4* plants, respectively. Values are means $\pm$ SD of $n = 8$ biological replicates. The effects of time (t), genotype (g), or their interaction (t $\times$ g) were assessed by a repeated measures two-way ANOVA. (E) Length of largest (dominant) epicormic bud at t=1. Data are mean $\pm$ SD of $n = 8 - 12$ biological replicates. Statistical significance was determined by Student's *t* test between control and *sut4* plants. (F) Sprout biomass at t=2. Data are mean $\pm$ SD of $n = 8 - 12$ biological replicates. Statistical significance was determined by

Student's *t* test between control and *sut4* plants. ns, nonsignificant; *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$.

FIGURE 2: Non-structural carbohydrate abundance dynamics following coppicing.

(A) Sucrose, (B) Hexose (sum of fructose and glucose), and (C) Starch. Black and grey are for control and *sut4* plants, respectively. Values are mean $\pm$ SD of $n = 10$ biological replicates. The effects of time (t), genotype (g) or their interaction ($t \times g$) were assessed by repeated measures two-way ANOVA. Statistical significance for root starch levels at $t=2$ was determined by Student's *t* test between control and *sut4* plants. ns, nonsignificant; *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$.

FIGURE 3: Epicormic bud length as a function of bark carbohydrate depletion under summer and winter conditions.

(A-B) Bud length at $t=1$ versus sucrose (A) or starch (B) depletion between $t=0$ and $t=1$. Linear regression trendlines are depicted in blue and red for control and *sut4*, respectively. Shading highlights the 95% confidence intervals.

FIGURE 4: Xylem sap metabolites.

(A) Sucrose, (B) Hexoses, (C-D) Abundance ranking of the most prevalent metabolites after sucrose and hexose in control and *sut4* sap in summer (C) and winter (D). Krebs cycle intermediates are in green and amino acids are in brown colors, with control and *sut4* in darker and lighter shading, respectively. Values represent the mean $\pm$ SE ($n = 8-10$). Statistical

significance between genotypes was determined by Student's *t* test. *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$.

FIGURE 5: Post coppice metabolic reprogramming in bark and xylem.

Heatmap depiction of log₂-transformed metabolite response ratio (*sut4*/control) in bark (A) and xylem (B). Blue and red colors indicate decreased or increased metabolite abundance in mutants, while grey indicates not detected. Significance between genotypes was obtained by Student's *t*-test and is indicated in bold underlined ($P \leq 0.01$) or bold font ($P \leq 0.05$).

FIGURE 6: Bark secondary metabolites

(A) Chlorogenic acids (sum of caffeoyl-quinic acid isomers), (B) salicortin, (C) tremulacin, and (D) condensed tannins. Values represent the mean $\pm$ SD of $n = 8$ biological replicates. The effects of time (t), genotype (g) or their interaction were assessed by repeated measures two-way ANOVA. *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$.

FIGURE 7: Bark chlorophyll content.

Changes in bark chlorophyll following summer (A) and winter (B) coppicing. Black and grey are for control and *sut4* plants, respectively. Data are presented as the mean $\pm$ SD ($n = 10$). The effects of time (t), genotype (g) or their interaction were assessed by repeated measures two-way ANOVA. *, $P \leq 0.05$; ***, $P \leq 0.001$.

Table 1. Total biomass of new sprout growth following coppicing in control and *sut4* poplar.

	Summer		Winter	
	Control	<i>sut4</i>	Control	<i>sut4</i>
Tissue dry mass (g)				
Leaf	10.97 ± 2.80	16.15 ± 3.40**	10.35 ± 1.55	9.55 ± 1.74
Stem	5.37 ± 1.35	7.55 ± 1.76**	5.22 ± 0.96	4.89 ± 0.78
Root ¹	11.47 ± 2.37	11.55 ± 2.76	10.88 ± 1.17	9.42 ± 1.42

Values represent mean and SD of $n = 6 - 11$ biological replicates. Student's *t*-test was used to determine statistical significance between genotypes. **, $P \leq 0.01$; ***, $P \leq 0.001$.

¹Root biomass represent total growth pre- and post-coppicing.

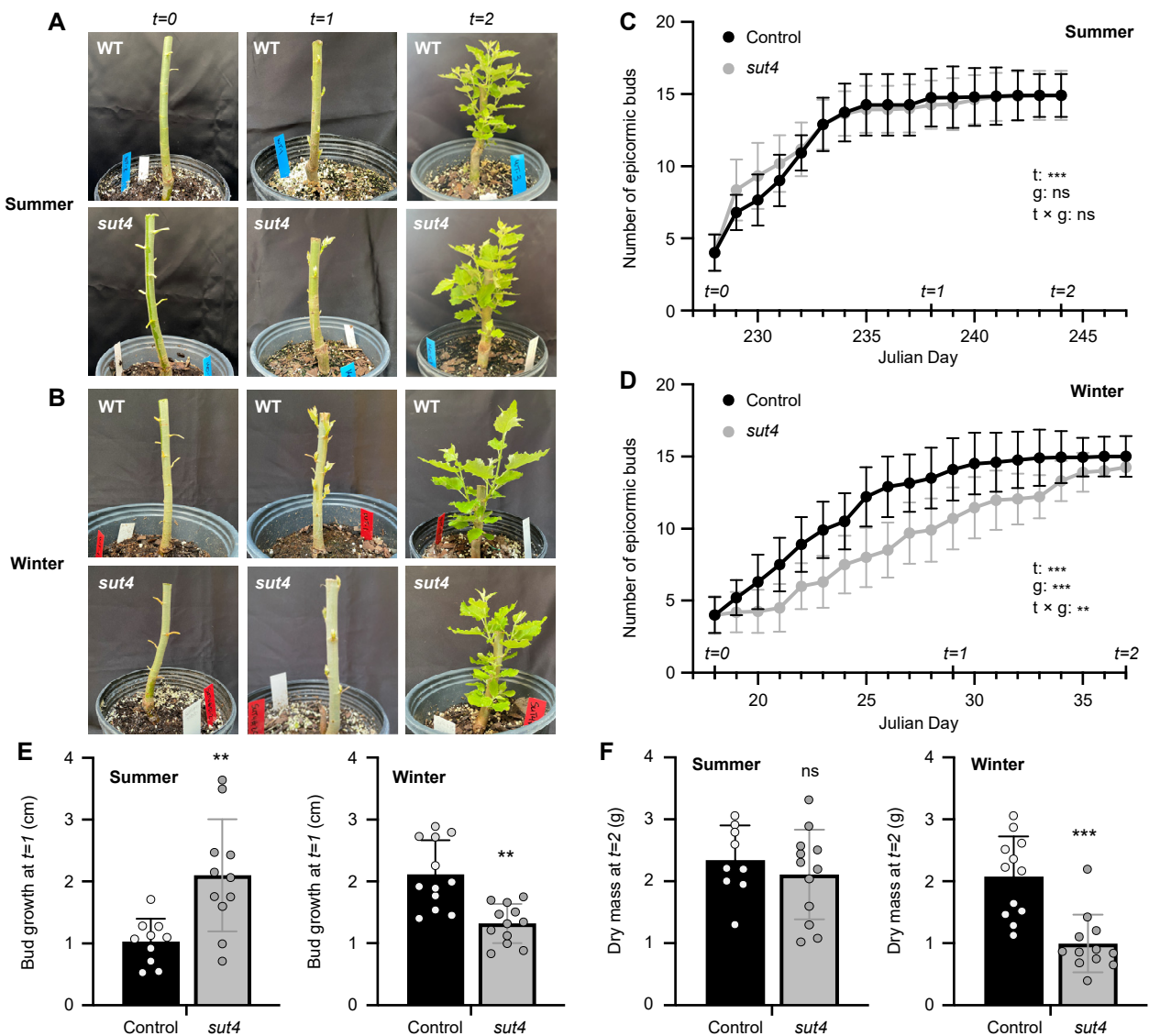


FIGURE 1. Epicormic bud emergence and growth following coppicing.

(A-B) Representative images of *sut4* and control poplar stools under summer (A) and winter (B) conditions immediately after coppicing (*t=0*), during epicormic bud expansion (*t=1*), and during sprout growth (*t=2*). (C-D) Bud emergence after summer (C) and winter (D) coppicing. Black and grey represent control and *sut4* plants, respectively. Values are means $\pm$ SD of *n* = 8 biological replicates. The effects of genotype or time were assessed by a repeated measures two-way ANOVA and indicated by *P* values. *t*, time; *g*, genotype; *t* × *g*, time × genotype interaction. (E) Length of largest (dominant) epicormic bud at *t=1*. Data are mean $\pm$ SD of *n* = 8-12 biological replicates. Statistical significance was determined by Student's *t* test between control and *sut4* plants. (F) Sprout biomass at *t=2*. Data are mean $\pm$ SD of *n* = 8-12 biological replicates. Statistical significance was determined by Student's *t* test between control and *sut4* plants. ns, nonsignificant; *, *P* $\leq$ 0.05; **, *P* $\leq$ 0.01; ***, *P* $\leq$ 0.001.

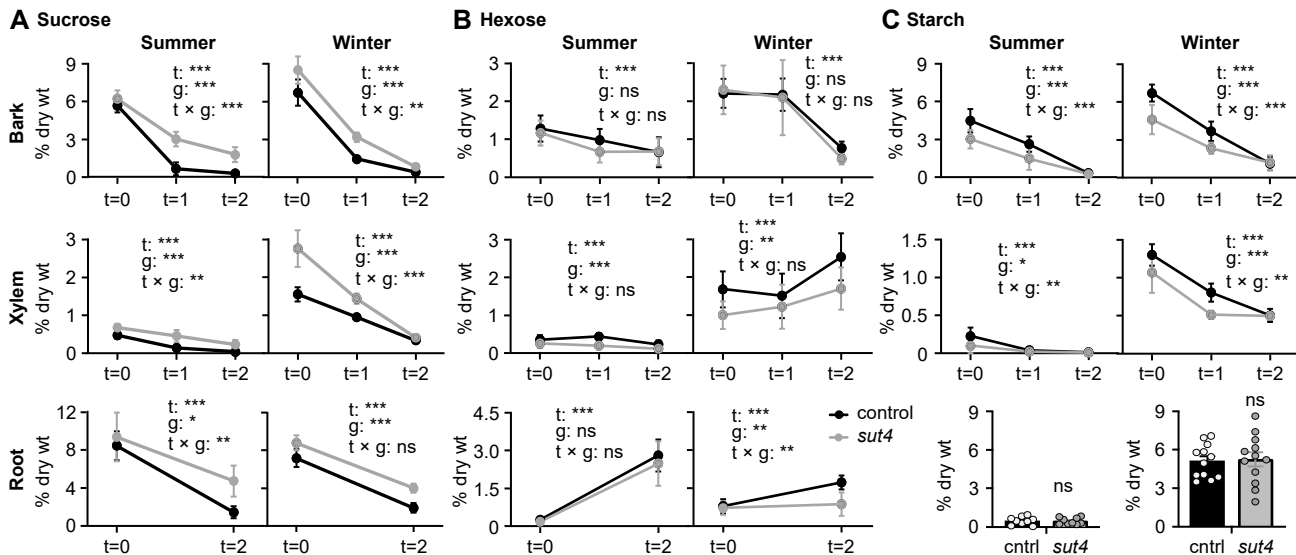


FIGURE 2: Non-structural carbohydrate abundance dynamics following coppicing.

(A) Sucrose, (B) Hexose (sum of fructose and glucose), and (C) Starch. Black and grey are for control and *sut4* plants, respectively. Values are mean $\pm$ SD of $n = 10$ biological replicates. The effects of time (t), genotype (g) or their interaction (t $\times$ g) were assessed by repeated measures two-way ANOVA. Statistical significance for root starch levels at t=2 was determined by Student's *t* test between control and *sut4* plants. ns, nonsignificant; *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$.

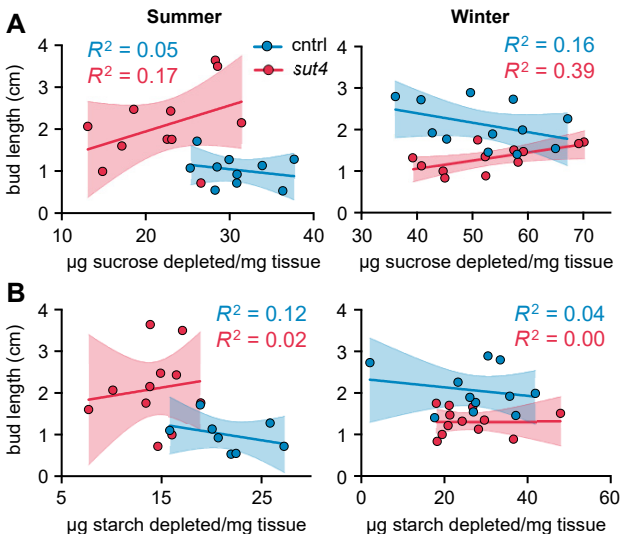


FIGURE 3: Epicormic bud length as a function of bark carbohydrate depletion under summer and winter conditions.

(A-B) Bud length at $t=1$ versus sucrose (A) or starch (B) depletion between $t=0$ and $t=1$. Linear regression trendlines are depicted in blue and red for control and *sut4*, respectively. Shading highlights the 95% confidence intervals.

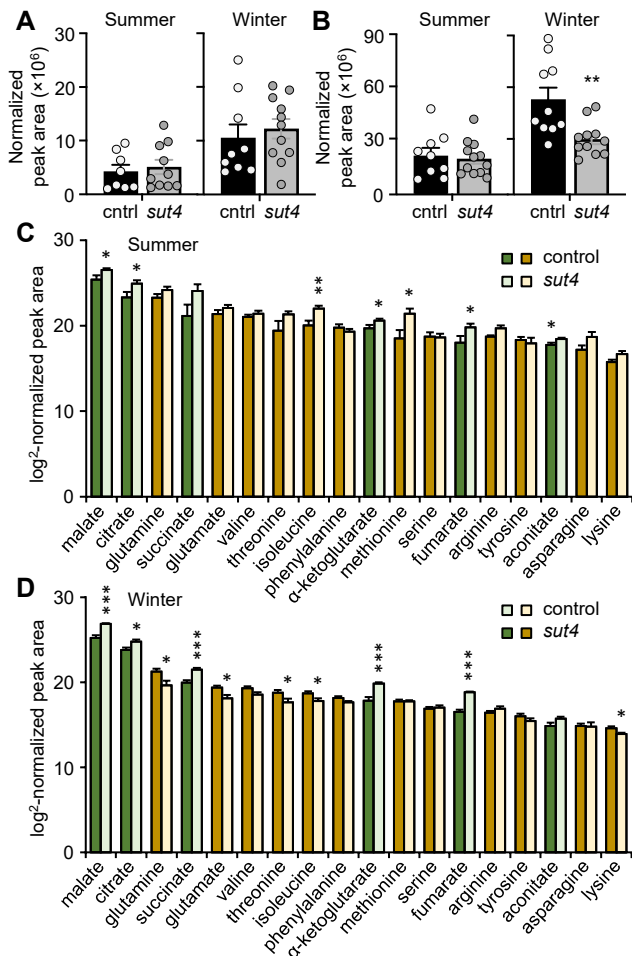
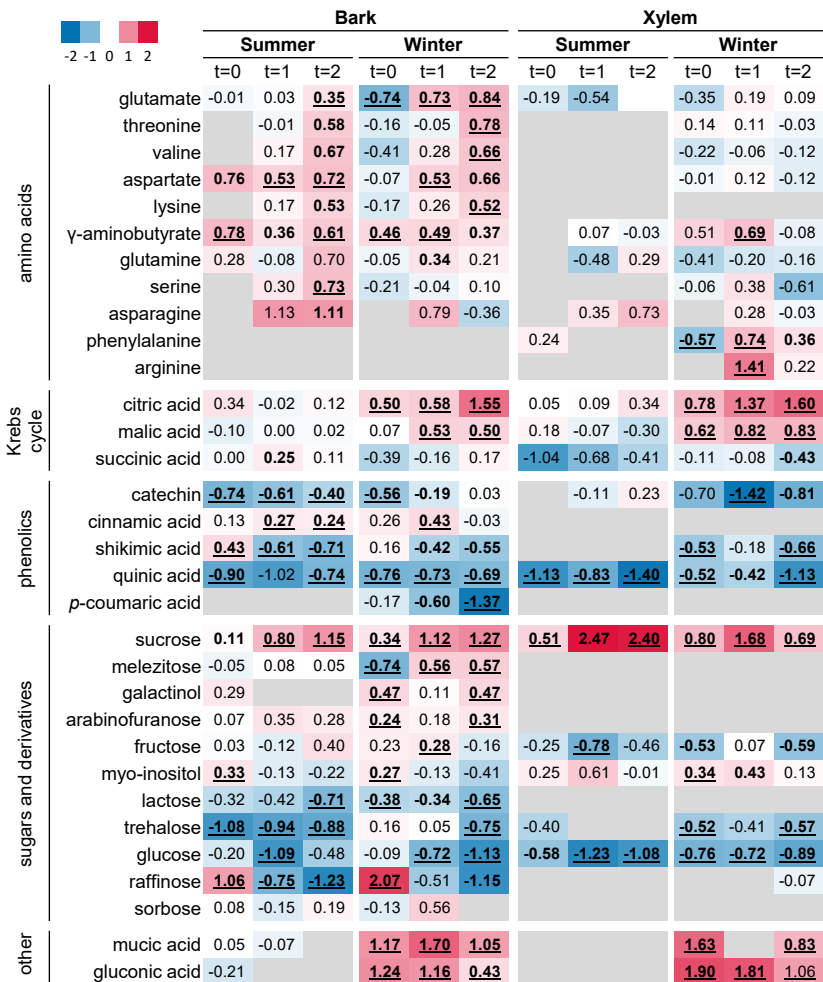


FIGURE 4: Xylem sap metabolites.

(A) Sucrose, (B) hexoses, (C-D) abundance ranking of the most prevalent metabolites after sucrose and hexose in control and *sut4* sap in summer (C) and winter (D). Krebs cycle intermediates are in green and amino acids are in brown colors, with control and *sut4* in darker and lighter shading, respectively. Values represent the mean $\pm$ SE ($n = 8-10$). Statistical significance between genotypes was determined by Student's *t* test. *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$.



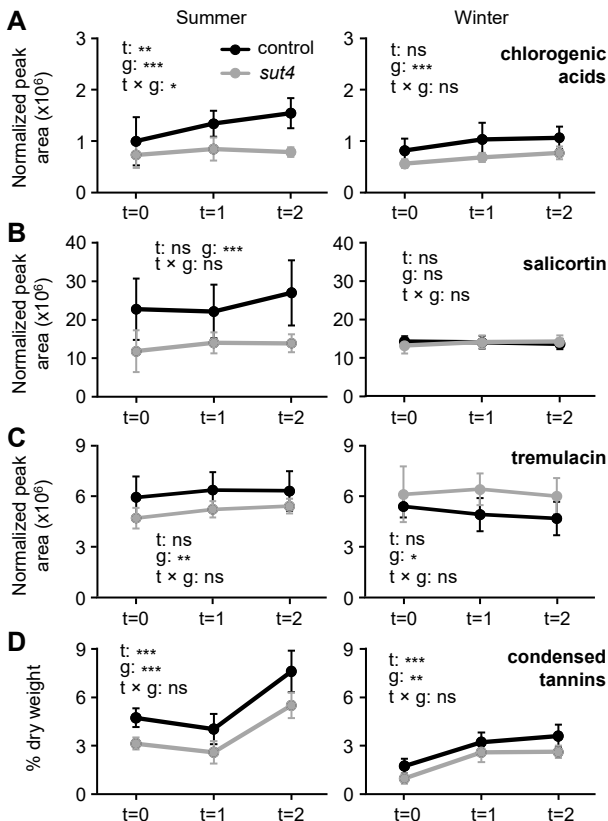


FIGURE 6: Bark secondary metabolites

(A) Chlorogenic acids (sum of caffeoyl-quinate isomers), (B) salicortin, (C) tremulacin, and (D), condensed tannins. Values represent the mean $\pm$ SD of $n = 8$ biological replicates. The effects of time (t), genotype (g) or their interaction were assessed by repeated measures two-way ANOVA. *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$.

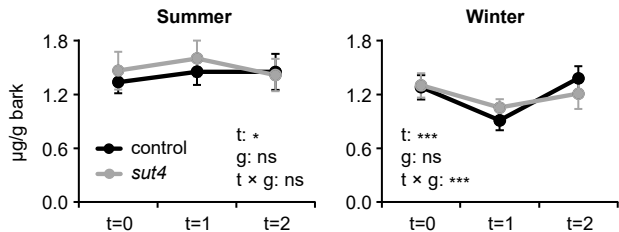


FIGURE 7: Bark chlorophyll content.

Changes in bark chlorophyll following summer (A) and winter (B) coppicing. Black and grey are for control and *sut4* plants, respectively. Data are presented as the mean $\pm$ SD ($n = 10$). The effects of time (t), genotype (g) or their interaction were assessed by repeated measures two-way ANOVA. *, $P \leq 0.05$; ***, $P \leq 0.001$.