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# Sugar loading is not required for phloem sap flow in maize plants

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**Title: Sugar loading is not required for phloem sap flow in maize plants**

Short Title: Sugar loading is not required for phloem transport

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**Abstract:**

Phloem transport of photoassimilates from leaves to non-photosynthetic organs, such as the root and shoot apices and reproductive organs, is crucial to plant growth and yields. For nearly 90 years, evidence generally has been consistent with the theory of a pressure-flow mechanism of phloem transport. Central to this hypothesis is the loading of osmolytes, principally sugars, into the phloem to generate the osmotic pressure that propels bulk flow. Here we used genetic and light manipulations to test whether sugar import into the phloem is required as the driving force for phloem sap flow. Using carbon-11 radiotracer, we show that a maize *sucrose transporter1* (*sut1*) loss-of-function mutant has severely reduced export of carbon from photosynthetic leaves (only ~4% the level of wild type). Yet, remarkably the mutant maintains phloem pressure at ~100 % and sap flow speeds at ~50-75% of wild type. Potassium ($K^+$) abundance in the phloem was elevated in *sut1* mutant leaves. Fluid dynamic modeling supports the conclusion that increased $K^+$ loading compensated for decreased sucrose loading to maintain phloem pressure, and thereby maintained phloem transport via the pressure-flow mechanism. Furthermore, these results suggest that sap flow and transport of other phloem-mobile nutrients and signaling molecules could be regulated independently from sugar loading into the phloem, potentially influencing carbon-nutrient homeostasis and the distribution of signaling molecules in plants encountering different environmental conditions.

**Key words:** phloem, sucrose transporter, pressure-flow hypothesis, osmotic potential

## Introduction

In plants, the phloem serves to move carbon and energy captured by photosynthetic leaves (i.e., source tissues) to non-photosynthetic regions (i.e., sink tissues) that need the photoassimilates to grow and develop. Transfer of carbon as sugars from leaves is a major determinant of growth rate, is essential for food and biomass production [1-6], and contributes to shaping the architecture of plants [7].

It is widely accepted that loading of sugars into the phloem in source leaves and unloading of sugars in sink tissues (e.g., stems, roots, young developing leaves) together are the major drivers of phloem sap flow [8]. According to the pressure-flow hypothesis first proposed by Ernst Münch [9], solutes, primarily sugars, are loaded into the phloem in leaves, providing a gradient in water potential across the sieve element membrane [2,10,11]. This gradient causes water to enter the phloem by osmosis, thereby generating a region of high hydrostatic pressure in source phloem. In the majority of plants tested, sucrose is the most abundant solute in phloem by at least an order of magnitude, although some species translocate sugar alcohols or raffinose series sugars [12-15]. Evidence has accumulated over decades that is almost uniformly consistent with aspects of the pressure-flow hypothesis, and the importance of sugar loading in phloem sap flow [8]. However, robust tests of the pressure-flow hypothesis and its components have been elusive (e.g., [16,17]; for reviews see [8,11,18]), partly due to the very limited availability of technology that allows not only observation, but quantification of phloem loading, pressure, sap flow and conductivity, and partly due to the challenge of targeted experimental manipulation of sugar loading.

Mutant lines with altered sugar loading into the phloem present the opportunity to test the importance of sugar loading to phloem transport. In multiple species, sucrose transporters

(SUTs) have been identified that function in sucrose loading into the phloem, as indicated qualitatively by carbohydrate accumulation in source leaves of mutants, or semi-quantitatively by feeding $^{14}CO_2$ or $^{14}$C-sucrose and estimating $^{14}$C export from source leaves [19-23]. Sugars may be loaded into the phloem passively via symplastic pathways, through plasmodesmata (PD) complexes that connect phloem parenchyma cells with the sieve element-companion cell (SE-CC) complex, or actively via apoplastic (i.e., extracellular) pathways requiring transmembrane transporters [3,14,15,24-27]. Each species has its own characteristic loading regime, which is often a spatial or temporal blending of both loading types [3,14,28]. Employing a genetic approach to eliminate or substantially reduce sugar loading into the phloem can be difficult to accomplish in plants with a blended phloem loading type, but can be achieved where loading is primarily apoplastic. Many of the most important agronomic crops (e.g., maize, barley, wheat, potato) and bioenergy crops (e.g., sugarcane, sorghum, switchgrass) are primarily apoplastic phloem loaders [1,29]. For example, in maize, PDs between the SE-CC complex and vascular parenchyma cells are rare in leaf veins [1,30]. Also in maize, the principle form of carbon in the phloem is sucrose [31-34], and SUCROSE TRANSPORTER 1 (SUT1) is of prime importance to phloem loading in maize, although there are 7 *SUT* genes in its genome [1,29]. Maize loss-of-function *sut1* mutants exhibit carbohydrate accumulation in leaves, and severely reduced growth of sink tissues [22], both of which suggest a strong reduction of sucrose loading into the phloem.

The pressure-flow mechanism can be modeled by fluid dynamic equations, involving multiple variables, each of which is difficult to study and even more difficult to quantify. Several authors who have studied SUTs reported evidence that SUTs function in the sucrose leakage-retrieval cycle of transport phloem in the stem and roots, in addition to loading sucrose into the collection phloem in leaves [23,35,36], but no studies have been designed to comprehensively

investigate the impact of phloem loading on phloem sap flow. Here, we quantified four key variables in a maize *sut1* loss-of-function mutant; (1) the magnitude of decreased carbon export from the leaf; (2) the resulting pressure inside of the phloem sieve elements; (3) the phloem sap flow velocity; and (4) phloem conductance calculated from anatomical measurements; which we then used to parameterize a computational model of solute transport by bulk flow to test the assumption that sucrose is the major osmolyte of the pressure-flow mechanism that causes phloem sap to flow. In order to avoid the potentially confounding effects of carbohydrate accumulation in *sut1* mutant leaves, experiments were conducted with and without a dark treatment period long enough to deplete carbohydrates to wild type (WT) levels immediately prior to measurements. Additionally, we quantified potassium ($K^+$), which is the most abundant inorganic osmolyte in phloem, and tested the influence of $K^+$ on pressure and sap flow in the model. Quantification allowed comparison of our actual results with predicted values based on modeling of phloem sap flow. Results of our experiments, utilizing both genetic and environmental manipulations and modeling, demonstrate that, contrary to the current paradigm, sugar loading is not required as the driving force for phloem sap flow.

## Results

**Drastic reduction of carbon export from maize *sut1* mutant leaves.** Using carbon-11 ($^{11}C$) administered to a leaf as $^{11}CO_2$ (Extended Data Fig. 1), we quantified the amount of recently fixed carbon exported from leaves of WT and *sut1* mutant maize plants. Export was reduced to 4%, and thus almost eliminated in *sut1* mutants when compared to WT leaves (Fig. 1a, b, and c). Specific activity, the amount of radioactivity per unit mass, must be accounted for when using radiotracer as an indicator of transport of the labeled biochemical. As the specific activity

decreases, the isotopic dilution may reduce the amount of radioactivity transported even if the overall transport rate of labeled plus unlabeled molecules stays the same. The specific activity of sucrose was low in *sut1* mutant leaves due to high sucrose concentrations (Fig. 1f-h; Extended Data Fig. 2). However, dark treatment to reduce leaf sucrose concentrations and increase the sucrose specific activity to levels comparable to WT (Fig. 2a) demonstrated that the reduced $^{11}$C-export by *sut1* mutant leaves was not an artifact of isotopic dilution (Fig. 2b; Extended Data Fig. 3). Thus, SUT1 is responsible for nearly all of the sucrose loading into the phloem, and the *sut1* mutant in maize provides an ideal system to test the role of sucrose loading in driving phloem sap flow.

**Maize *sut1* mutants defective in sucrose loading maintain high phloem pressures and sap flow rates.** If loading of sugars is required to generate the osmotic pressure to drive bulk phloem flow, then the drastic decrease of phloem sugar export in *sut1* mutant plants is therefore expected to be associated with a strong reduction in phloem pressure and sap flow speed. To test this prediction, we measured phloem pressure in sieve elements of WT and *sut1* leaves using a pico gauge pressure probe [37] and phloem translocation speed by tracking pulses of [$^{11}$C]-photoassimilate (see Methods) [31,38]. Despite the drastic reduction in carbon export in the *sut1* mutant, a small amount of [$^{11}$C]-photoassimilate still entered the phloem, allowing observation of phloem transport (Fig 1a). We found strikingly similar phloem pressures in WT and *sut1* mutant leaves (Fig. 1e, $P_{t\text{-}test} = 0.41$), which was highly unexpected given the large difference in carbon export rates. Moreover, we found only modest differences (mutant was 50-75% of WT) between WT and *sut1* mutant phloem flow velocities (Fig. 1d, 2c), which is in accordance with a previous study that used a sucrose analog tracer [39]. Although we would expect velocity to change directly

proportional to pressure, there was a slight disagreement between the pressure (unchanged) and flow velocities (slightly decreased). This could be due to slightly different experimental conditions in the two separate facilities where phloem pressure and flow velocity were measured.

**The *sut1* mutation did not alter phloem anatomy or conductivity.** Since phloem pressure and sap flow velocity could be affected by changes in phloem conductivity [40], we tested for alterations to phloem anatomy or phloem cross-sectional area in *sut1* mutant plants. However, we found no major changes in the size, shape, or lignification patterns of vascular bundles between *sut1* and WT leaves (Fig. 3a, b, c, and e). Also, the number of conductive major and minor veins in *sut1* mutant leaves was comparable to that in WT leaves, and the *sut1* mutant veins were equally competent as WT to transport the phloem-mobile dye carboxyfluorescein (CF), indicating there were no obstructions to prevent phloem transport in the mutants (Fig. 3d, f, and g-j). Phloem conductivity of leaves, based on independent anatomical measurements (Fig. 3k and l), was similar in the *sut1* mutant ($0.19 \pm 0.11$ μm$^2$) and WT ($0.23 \pm 0.10$ μm$^2$; $P_{t\text{-}test} = 0.78$, $n > 20$). Likewise in stems, phloem conductivity in the *sut1* mutant ($1.18 \pm 0.38$ μm$^2$) was not significantly different than WT ($0.91 \pm 0.38$ μm$^2$; $P_{t\text{-}test} = 0.61$, $n > 20$). Thus, the anatomy and functional capacity of the phloem to transport solutes were not compromised in the *sut1* mutants.

**How are phloem pressure and sap flow maintained in *sut1* mutants?** The generation of pressure in the phloem is thought to be driven mainly by soluble carbohydrate loading into the phloem [2,10,11]. Decreased sucrose loading in the maize *sut1* mutants had a relatively minor impact on the velocity of sap flow, which seems opposed to the pressure-flow hypothesis. However, in theory, osmosis could be driven by osmolytes other than sucrose. Therefore, the observed

similarity in pressure between the *sut1* mutant and WT phloem suggests that a Münch pressure-flow mechanism may maintain sap flow in *sut1* mutant plants, but some other osmotic substance must be loaded into the phloem in *sut1* leaves to generate the high pressure needed for phloem transport. Thus, the observed minor reduction of sap flow velocity in *sut1* mutants could reflect an ability to mostly compensate for the drastically diminished sucrose loading.

Since other organic molecules, such as hexoses (Figs. S2 and S3) or amino acids [41], would have been labeled with $^{11}C$ within the time-frame of our experiments, increased loading of these organic molecules into the phloem would have been included in our measurement of $^{11}C$ export from leaves. Thus, it is unlikely that loading of other organic molecules was increased to compensate for reduced sucrose loading, although we cannot completely rule out a small contribution.

Alternatively, loading of inorganic solutes such as $K^+$ or chloride, both abundant osmolytes in maize phloem sap [32,33], could potentially compensate for reduced sucrose loading in *sut1* mutant plants. Indeed, phloem $K^+$ was substantially elevated in *sut1* mutants, consistent with this hypothesis. But the picture is more complex. Analysis of maize phloem sap that was collected by aphid stylectomy indicated no correlation between sucrose and $K^+$ or any other common osmolyte (Table 1; Extended Data Fig. 4). However, sucrose in the phloem sap of *sut1* mutants collected by aphid stylectomy was generally not as low as expected (Extended Data Table 1). Since selection of sieve tubes by aphids depends on neutral pH, high sucrose content and high pressure [42-44], it is possible that aphids in our experiment were selecting the sieve tubes with the highest sucrose content in *sut1* mutants, rather than selecting sieve tubes that were truly representative of the plant. Furthermore, it is possible that aphid salivation into the sieve tubes prior to stylectomy could have altered the carbohydrate or ion composition of the sap, since

200 aphids may elicit defensive responses from plants [45], and since aphid saliva contains ion-binding
201 proteins and enzymes that degrade carbohydrates [46-48].

202 To avoid these concerns and to measure plants actually used in $^{11}C$ sap flow studies, we
203 used an alternative method to measure ions, x-ray fluorescence (XRF) microscopy of leaf cross-
204 sections [49-52], which indicated that $K^+$ content in *sut1* mutant phloem (564 ± 78 mol $m^{-3}$) was
205 about twice as high as in WT phloem (297 ± 74 mol $m^{-3}$; Fig. 4B). The resolution of XRF was
206 not fine enough to resolve individual sieve elements, but we could measure $K^+$ as signal emitted
207 by the entire phloem cell consortium. The phloem region includes phloem parenchyma and
208 apoplast, and so would tend to slightly underestimate the $K^+$ concentration inside of the sieve
209 elements, where $K^+$ is expected to be highest. Using an independent technique, impaling $K^+$-
210 selective microelectrodes into individual sieve elements, we confirmed that the $K^+$ concentration
211 inside the phloem of *sut1* mutants was higher than inside of WT phloem (Extended Data Fig. 5).
212 A similar trend for $K^+$ was observed for phloem sap collected by aphid stylectomy, although the
213 difference was not statistically significant in this case (Extended Data Table 1). Concentrations
214 of $K^+$ measured by aphid stylectomy and by $K^+$-selective intracellular microelectrodes were
215 lower than the XRF measurements, which we attribute to differences in growth conditions
216 between the three experiments. The phloem $K^+$ concentrations measured by XRF and
217 microelectrodes are near the upper and lower limits, respectively, of previous measurements of
218 phloem sap $K^+$ by aphid stylectomy, which ranged from 40 – 500 mol $m^{-3}$ [32,33]. From XRF,
219 calcium in *sut1* mutant phloem was not significantly different than in WT phloem, and the
220 abundance of calcium in the phloem was much lower than $K^+$ (Fig. 4D). Xylem $K^+$ and calcium
221 levels were not significantly different between WT and *sut1* mutants (Fig. 4C, E). Increased $K^+$
222 availability (as KCl supplementation) in the soil did not impact phloem sap flow velocity in

either WT or *sut1* mutant plants (Extended Data Fig. 6), suggesting that soil $K^+$ availability was not limiting for phloem transport in either the WT or *sut1* mutant plants.

**The pressure-flow hypothesis predicts pressure and sap flow velocity in *sut1* mutants when both sucrose and $K^+$ are included as osmolytes, but not sucrose alone.** Eliminating sucrose loading is expected to significantly reduce phloem pressure and consequently reduce phloem sap flow velocity [17,53,54]. By contrast, our experiments show only modest changes in pressure and sap flow velocity (Figs. 1 and 2). Although the changes in sap pressure and flow velocity appear to be incongruent with the drastic decrease in photoassimilate export, phloem $K^+$ concentration was higher in *sut1* leaves (Figs. 4B and Extended Data Fig. 5, but also see Extended Data Table 1), which may have offset the decrease in sucrose concentration. Therefore, since phloem anatomy was not altered by the *sut1* mutation (Fig. 3), we used a mathematical model of phloem transport to determine whether the Münch hypothesis would predict that phloem pressure would be maintained or sap flow velocity only moderately decreased, given the drastic reduction in photoassimilate export and the increase in phloem $K^+$ concentration.

Based on the differences in flow velocity and $^{11}C$-sugar export, we estimated that the phloem sugar concentration in *sut1* mutants was 6.4 ± 1.4 % that of the WT (eq. 3 in Appendix A). We then estimated the overall solute concentrations (which could include $K^+$) that would be required to maintain the phloem pressure of WT phloem at 1.39 ± 0.44 MPa, to be 571 mol $m^{-3}$ ($c_s^{WT}$ in Appendix A). Assuming the WT phloem ion concentration was between 0% and 50% of the phloem osmotic potential, and assuming *sut1* sucrose concentration was 6.4% of WT, *sut1* phloem ion concentration would need to be approximately 414 – 765 mol $m^{-3}$ ($c_i^M$ in Appendix A). The actual phloem $K^+$ concentration measured in the $^{11}C$ experimental plants was very close

to the center of that range, 564 mol $m^{-3}$ for *sut1* mutants elevated from 297 mol $m^{-3}$ in WT phloem (Fig. 4).

Furthermore, decreased loading of sucrose into the phloem affects both the osmotic pressure of the source phloem and the viscosity of the phloem sap, which would have opposing effects on phloem sap flow velocities: A decrease in sap sucrose content would lead to a lower osmotic pressure, but also a reduction in sap viscosity. In contrast, changes in $K^+$ concentration would have little effect on sap viscosity. Specifically, sap velocity would follow Darcy's Law and change directly proportional with an increase in pressure differential, and inversely proportional to variations in sap viscosity, assuming there are no differences in phloem anatomy or phloem cross-sectional area [40]. Since $K^+$ was measured in the same experimental plants as sap flow velocity, but phloem pressure was measured in separate plants, we inserted the measured $K^+$ concentrations into the model to compare predicted and measured values of sap flow velocity in *sut1* mutants (Appendix A). Darcy's Law predicted the sap flow velocity of the *sut1* mutant to be 0.95 ± 0.13 m/hr, which is close to the measured values in three independent $^{11}C$ experiments (0.81 ± 0.09 m/hr in Fig. 1; 1.16 ± 0.15 m/hr in Fig. 2; and 0.95 ± 0.09 m/hr in Extended Data Fig. 6).

When $K^+$ was excluded as a solute or remained at WT levels in the low-Reynold's number equations, the predicted sap flow velocities (0.03 m $hr^{-1}$, and 0.51 m $hr^{-1}$, respectively) were well below experimental mean values and outside of the 95% confidence intervals in both cases (Appendix A). Furthermore, apoplastic concentrations of sucrose were likely higher in *sut1* mutant leaves than in the WT, since we observed sugars exuded to the *sut1* mutant leaf surfaces, as previously reported [55], and substantial amounts of $^{11}C$-labeled photoassimilates leaked into the xylem (see $^{11}C$ transport toward the tip of the *sut1* mutant leaf in Fig. 1a). Abnormally high

apoplastic sugar concentrations could further challenge the ability to generate pressure inside the phloem sieve tubes, since the generation of osmotic pressure is dependent on an osmotic gradient across the sieve element membranes. Thus, the observed pressure and sap flow velocity in *sut1* mutants were substantially greater than predicted by the pressure-flow hypothesis if sucrose is assumed to be the main osmolyte responsible for generating osmotic pressure. However, these data overall support the pressure-flow hypothesis, and suggest that loading of ions such as $K^+$ into the phloem may compensate when sucrose loading is extremely low.

## Discussion

Although sugar loading is thought to be the major driver of phloem sap flow [2,10,11], these experiments demonstrate that substantial sugar loading into the phloem is not necessary to drive phloem sap flow. The rates of movement of $^{11}C$ through the maize *sut1* mutant plants that we measured were much too fast, 0.75 - 1 m/hr, to be explained by diffusion, but instead are indicative of an energy-dependent process driving the moderately fast laminar flow of phloem sap. Further, the sap flow velocities in maize *sut1* mutants are within the range of WT sap flow velocities previously measured in non-mutant plants of other crop species and trees (Extended Data Table 2) [36,38,39,56-62].

Loading of inorganic solutes such as $K^+$, which is abundant in maize phloem sap [32,33], could potentially compensate for reduced sucrose loading in maize *sut1* mutant plants. The observed high pressures in sieve tubes of *sut1* mutant leaves are consistent with pressure-driven flow of the phloem sap, and x-ray fluorescence imaging of vascular bundles suggests that phloem loading of ions such as $K^+$ may increase in *sut1* mutants to maintain osmotic pressure. This is consistent with a previous report of $K^+$ abundance increasing when sucrose decreased by

50 – 80% in the phloem of castor bean plants maintained under continuous darkness, whereas phloem $K^+$ levels were relatively stable in castor bean plants kept in 16:8 hr photoperiod, which maintained high phloem sucrose concentrations [63]. However, $K^+$ loading into the phloem is not generally discussed as a major driver of phloem sap flow in contemporary literature. Rather, $K^+$ has been ascribed other functions in the phloem, such as regulating sucrose loading [64], maintaining $K^+$ homeostasis and phloem electrical potential [65], and acting as a mobile energy source to drive sucrose loading or retrieval into the phloem [66,67]. For example, $K^+$ may regulate sucrose loading into the phloem by stabilizing the membrane potential as it depolarizes during sucrose loading through active SUTs. In this respect it was shown that the loss of *akt2/3* $K^+$ channel function resulted in a 50% reduction of sucrose in the *Arabidopsis* mutant sieve tubes [64]. Besides AKT2/3, phloem expresses KAT1/2-type hyperpolarization-activated K+ uptake channels, and likely HAK-type $K^+$ transporters, which together may control the phloem K+ content [64]. In addition to the previously identified roles, our results provide evidence that $K^+$ in the phloem can be a major contributor to phloem pressure, and thus to sap flow.

A shift to passive phloem loading cannot be invoked as the motivational force that maintains phloem sap flow in maize *sut1* mutants. Many plants rely on or can shift to passive phloem loading (i.e., diffusion through plasmodesmata) [3,14,15,24-28], but maize appears to be incapable of transitioning to diffusion of sugars into the phloem sufficiently to meet the needs of the growing sink tissues [22]. Whereas sugars move into the phloem through symplastic pathways in leaves of passive phloem loaders, the scarcity of PDs connecting the phloem parenchyma cells to the SE-CC complex in maize minor veins suggests that the symplastic pathway can make only a minor contribution in maize leaves [29,30]. The considerably reduced $^{11}C$ export rate in the *sut1* mutants confirms the inability of maize to shift to substantial rates of passive loading. It has been

315 postulated that the selective advantage of active phloem loading of sugars may not be accelerated
316 sap flow velocity, *per se*, as sap flow velocity may be similar or only slightly slower in passive
317 loading species than active loaders [11,25,68]. Rather, the primary adaptive value of active phloem
318 loading is the enhanced amount of photoassimilates exported to growing tissues, as well as
319 relieving photosynthesis from down-regulation by high foliar sugar concentrations [11,25,68].
320 Implicit in this model is the idea that passive movement of sugars into the phloem drives sap
321 flow in the absence of active loading. However, our results go much further, suggesting that
322 phloem sap flow does not require either active or passive movement of sugar into the phloem as
323 a substantial osmolyte, but that plants may load some other osmolyte to drive phloem sap flow,
324 such as $K^+$.

325 Importantly, phloem has dual functions. In addition to transporting sugars and a host of
326 other nutrients (e.g., amino acids and $K^+$), the phloem also serves in long-distance
327 communication by transporting signaling molecules, such as phytohormones, RNAs, and
328 proteins from source to sink tissues [69-76]. The presence of vascular tissue in multicellular algae, in
329 which photosynthesis is distributed broadly across the body of the organism [77-80], is suggestive
330 that a primitive vascular system may have evolved to serve the communication function initially,
331 or at the same time as the need for sugar and nutrient translocation, which became more
332 important when early plants began to colonize land and required greater specialization of tissue
333 function. This primitive vascular system may have been less reliant on sugars as the osmotic
334 drivers, and our results suggest that aspects of this system have been retained to the present day
335 in vascular plants. Furthermore, our results suggest that, even when there is reduced need or
336 ability for carbon transport (e.g., under low light conditions, at night), delivery of other nutrients
337 and signaling molecules throughout the plant could be maintained independently of sugar

loading via loading of other osmolytes, such as $K^+$, which are loaded by discrete mechanisms into the phloem (e.g., $K^+$ transporters, amino acid transporters, phytohormone transporters, etc.). Maintaining long-distance communication and coordination between roots and shoots without massive sugar loading may be crucial in many ecological contexts, particularly where there may be low or no photosynthesis, such as drought, or when flooding inhibits sugar unloading in sink tissues.

## Materials and Methods

### Plant material, growth conditions and treatments

Seed of self-fertilized *sut1-m1* heterozygote plants backcrossed five or more times into the B73 inbred line was sown to generate homozygous WT, heterozygous, and homozygous *sut1* mutant plants in an approximately 1:2:1 ratio [22], since homozygous *sut1* mutants do not typically produce fertile reproductive structures and are often seedling lethal when grown in the field. Because of the greater introgression, the maize *sut1* mutant plants characterized for the current research exhibited stronger mutant phenotypes than those initially reported for the F2 generation [22], which had a large amount of genetic heterozygosity and hybrid vigor. A small piece of the third leaf was taken to verify the genotype of each plant by PCR using primers IDP8570F: 5'-CGCTAAGCTCGTCCTTCTCC-3' and IDP8570R: 5'-GGTTTGTGATCTTTGTGTCACC-3', or UMC2225F: 5'-TCGGCTGACATAATAAAACCATAGC-3' and UMC2225R: 5'-ATGCGAATTTTACCGGGTTTTT-3' [81]. Plants were grown in 75% Promix BX, 25% medium coarse sand, supplemented with 3.4 g/L slow release fertilizer pellets (Scotts Osmocote® Plus) in 3.8 L round plastic pots (8" Regal Standard, Kord, Twinsburg, OH). Plants were watered, as needed. Water was supplemented with 19.7 mg/L iron chelate (Sprint® 330). Except where

described otherwise, plants used in this study were grown for 3 weeks prior to $^{11}C$ labeling experiments in a growth chamber (Conviron) at 22°C during the light period and 19°C during the dark period, with 600 µmol $m^{-2}$ $s^{-1}$ incident photosynthetic photon flux density (PPFD) and a 14:10 hrs light:dark photoperiod.

For $K^+$ supplementation studies (Extended Data Fig. 6), approximately 2 weeks after sowing, plants were treated with either 200 mL of 20 mol $m^{-3}$ KCl, or 200 mL of distilled water to each pot once per week.

**Measurement of Carbon-11 export and phloem sap flow velocity**

The positron-emitting isotope carbon-11 ($^{11}C$; $t_{1/2}$ = 20.4 mins), was generated as $^{11}CO_2$ from the $^{14}N(p,\alpha)^{11}C$ nuclear transformation, by irradiating a $N_2$ gas target with a 17 MeV proton beam using an EBCO T19 cyclotron, as previously described [31], and was administered to the youngest fully expanded leaf of 3 week old plants as a 30 s pulse in continuously streaming air in a leaf cuvette with PAR 600 µmol $m^{-2}$ $s^{-1}$, as previously described [31,57] (Extended Data Fig. 1). Leaf fixation and export of $^{11}C$ from the leaf was monitored in real-time using a detector built into the leaf cuvette. Flow velocity of $^{11}C$-labeled photoassimilates through the phloem was determined using two detectors shielded with collimated lead and positioned to detect radioactivity moving down through the base of the leaf blade [38]. The time taken for the $^{11}C$ photoassimilates in the phloem to move between the upper and lower stem detectors was used to calculate phloem sap flow velocities (distance ÷ time).

**Measurement of unlabeled and $^{11}C$-labeled carbohydrates**

Both $^{11}C$ and $^{12}C$ (unlabeled) soluble sugars were measured using high-performance thin-layer chromatography (HPTLC), due to the need to rapidly analyze multiple samples and the high sensitivity of the $^{11}C$ detection on TLC plates [31]. After monitoring $^{11}C$-photoassimilate transport for 1 hr, the labeled leaf area was excised, frozen in liquid nitrogen, and ground to a fine powder using a ball mill (MM400, Retsch). Soluble sugars were extracted with 4 µL 75% methanol per mg leaf tissue at 4°C in an ultrasonicator bath. After centrifuging, the supernatant was spotted onto $NH_2$-bonded HPTLC plates using a Linomat 5 sample applicator (Camag Scientific, Inc.), and separated using 75:25 acetonitrile:water mobile phase. Radioactivity on TLC plates was immediately scanned using phosphor plate imaging, and quantified using Fuji Image Gauge (ver 3.46). TLC plates were heated to 200°C for 10 mins, and photographed under long-wave UV radiation. Unlabeled sucrose, glucose and fructose in plant samples were quantified based on fluorescence, using NIH ImageJ V1.53a for image analysis and known standards that were run on each TLC plate to generate a standard curve. For starch measurement, first soluble sugars were thoroughly extracted from a separate aliquot of plant tissue with 80% ethanol, and then remaining insoluble starch was digested to glucose using a heat tolerant α-amylase, followed by amyloglucosidase [31]. The glucose released from starch was then analyzed by HPTLC, as described above for soluble sugars.

**Observing functionality of veins using CFDA dye**

CFDA was fed to a mature source leaf tip by gently abrading the adaxial epidermal surface with fine grain sandpaper as described [82]. After 3 hrs, leaf segments 20-25 cm proximal to the fed site were sectioned and used for fluorescence microscopy [83]. CF in the phloem in this region of the leaf indicates that the dye was transported through the phloem.

406

407 **Determination of phloem conductivity**

408 Conductivity measurements were done on intact 3-4-week-old plants. Pieces of the leaves, stem,
409 and roots were frozen in liquid nitrogen to prevent damage response (e.g. callose formation), and
410 then placed into 100% ethanol at -20° C overnight. Digestion of the cytoplasm was carried out as
411 previously detailed [84]. Samples were freeze dried, mounted on pegs, sputter coated using
412 platinum palladium and imaged using a FEI Quanta 200 F scanning electron microscope. ImageJ
413 was used to measure all applicable parameters on sieve plates, which were used to calculate
414 conductivity [84].

415

416 **Pico-gauge pressure probes to measure phloem pressure**

417 Plants at the same stage of development were used for pressure measurements. Pico gauges were
418 fabricated using the method detailed in [37]. Cortical cells covering the midrib of the leaf were
419 carefully removed by hand section using a razor blade to expose the phloem. Phloem recovery
420 medium was immediately added [17]. For a discussion on the impact of this procedure on phloem
421 pressure measurements, see [17]. Sieve elements, at least one cell layer below the lowest point of
422 the hand section were targeted for pressure measurements.

423

424 **Analysis of phloem sap contents by aphid stylectomy.**

425 Sieve tube sap was obtained from severed stylets of the oat-bird cherry aphid, *Rhopalosiphum*
426 *padi* (L.). Ten aphids were caged for about 5 h on each leaf. The stylets of feeding aphids were
427 cut by a laser beam [32], and the exuding phloem sap (100–500 nl) was collected in micro-
428 capillaries (total volume 0.5 µl). Evaporation of the phloem sap was prevented by bringing the

front edge of the capillary in close contact with the leaf surface and surrounding the end with a plastic cap. The humidity around the capillary was about 80%. Under these conditions no evaporation from reference capillaries was detectable. The samples were ejected into 100 µl of distilled sterile water and stored at -80°C until analysis. The phloem sap was analyzed as previously described for sugar content by HPLC with pulse amperometric detector [85], for amino acids by HPLC with *o*-pthaldialdehyde precolumn derivatization and fluorescence detection, and for inorganic cations, anions, and malate by ion exchange chromatography [32].

**Synchrotron-based X-ray fluorescence imaging of maize veins and quantification of ions.** For X-ray fluorescence (XRF) imaging, pieces were cut from leaves of three week old plants, grown as described above, and were placed in O.C.T. compound and flash-frozen in liquid nitrogen. O.C.T.-embedded leaf pieces were cryo-sectioned using a cryostat (Leica Biosystems) set to 50 µm thickness. At least 1 mm of the cut end of the leaf piece was removed, and only intact sections were used for XRF imaging. Sample slides were attached to a cold stage using O.C.T., keeping samples frozen during the entire XRF imaging process.

Synchrotron-based XRF analysis of frozen hydrated tissue sections was performed at Beamline X27A of the National Synchrotron Light Source (NSLS) at Brookhaven National Laboratory (Upton, NY). Briefly, this bend-magnet beamline used Kirkpatrick-Baez (KB) mirrors to produce a focused spot (ca. 7 µm) of hard X-rays with tunable energy. For XRF imaging of leaf tissue, the incident beam energy was fixed at 7.2 keV. Samples were oriented 45° to the incident beam and rastered in the path of the beam by an XY stage while X-ray fluorescence was detected by a 4-element Vortex silicon drift detector positioned 90° to the incident beam. Elemental maps were typically collected from a 1 $mm^2$ sample area that included

the phloem and xylem tissue of the midrib using a step size of 7 μm (fine map) and a dwell time of 0.5 seconds. Fluorescence yields were normalized to the changes in intensity of the X-ray beam (I0) and the dwell time. Data acquisition and processing were performed using IDL-based beamline software designed by CARS (U. Chicago, Consortium for Advanced Radiation Sources). Standards-based quantification as described in [50] was used for abundance calculation (conversion of fluorescence yields to ion concentration on a per weight basis). National Institute of Standards and Technology (NIST) SRM 1831 and SRM 1832 standard reference materials were used to ascertain the relationship between concentration and intensity and to resolve spectral overlaps (e.g., Ar Kb with K Ka). Mass of $K^+$ in the xylem and phloem regions were determined by extracting the sum total counts of $K^+$ in the region of interest, then multiplying by a scaling factor that is based on NIST 1832 and 1833 standard reference materials with known $K^+$ mass per unit area measured under the same experimental conditions. We used the Center for X-ray Optics (CXRO) attenuation length calculator (https://henke.lbl.gov/optical_constants/) to calculate the attenuation length of K Ka X-rays in water (50 microns) and cellulose (74 microns). Since the attenuation length was greater than the sample thickness, we did not apply a correction. Potassium mass was divided by volume of the tissue (area of the selected region of interest multiplied by the tissue section thickness, 50 microns) to convert from mass to concentration on a volume basis, which was used for fluid dynamic modeling.

**Analysis of phloem $K^+$ sap contents using $K^+$-selective intracellular microelectrodes.**

Plants were prepared similar as described in the 'pico-gauge pressure probes section'. The external phloem recovery medium was grounded using an agar bridge ground electrode backfilled with 100 mol $m^{-3}$ NaCl. For the internal reference electrodes, borosilicate glass

capillaries with filament (Hilgenberg, Malsfeld, Germany) were pulled on a horizontal laser puller (P2000, Sutter Instruments, Novato, CA). They were backfilled with 100 mol $m^{-3}$ NaCl and connected via an Ag/AgCl half-cell to a headstage (1 GΩ, HS-2A, Axon, Union City, CA). The tip-resistance was about 60 MΩ. The same glass capillaries were used as $K^+$-selective electrodes. For this purpose they were dried overnight at 220 °C after pulling and silanized with N, N-Dimethyltrimethylsilylamine (Sigma-Aldrich) for 1 hr. The $K^+$-selective cocktail contained 2% w/v Potassium tetrakis(4-chlorophenyl)borate, 5 % w/v Potassium ionophore I, 5 % w/v 1,2-Dimethyl-3-nitrobenzene, 10 % w/v 2-Nitrophenyl octyl ether, 20 % w/v Poly(vinyl chloride) high molecular weight, and 58 % w/v Tetrahydrofuran. All Chemicals were purchased from Sigma-Aldrich (Germany). The liquid cocktail was filled from the back, free of air bubbles, into the electrode tip. This was dried for 24 h at 60 °C to harden the cocktail. The electrodes were connected via Ag/AgCl half-cells to head stages (Applicable Electronics, USA) of an Ion/ Polarographic Amplifier (Applicable Electronics, USA). Calibration of selective electrodes was performed subtracting the reference electrode potential and using KCl concentrations ranging from 1000 mol $m^{-3}$ to 1 mol $m^{-3}$. Only electrodes that recorded a shift in voltage of around 59 mV per 10x change in $K^+$ concentration were used for impalement. The cells were impaled by both electrodes (selective and reference) using two electronic micromanipulators (NC-30, Kleindiek Nanotechnik, Germany). After both electrodes were inserted into the same phloem cell and had stabilized, the differential voltage was measured (Patchmaster, HEKA Elektronik, Germany). This voltage was then converted into the $K^+$ concentration using the calibration data.

**Statistics**

All data were analyzed with SAS v9.4 software (SAS Institute Inc., Cary, NC) using the PROC GLM ANOVA procedure. Each measurement contributing to sample size was taken from a

distinct sample (i.e., not from the same sample measured repeatedly), and data were checked for normality. Data are presented as means ± standard error (SEM), unless otherwise indicated. Statistical significance was tested using ANOVA, and is indicated in figures by *($P<0.05$), **($P<0.01$), ***($P<0.001$), or NS (not significant). Where heterozygotes were included and ANOVA indicated a statistical significance, Tukey's post hoc test was performed to determine which genotypes were statistically different.

**Data availability:**

The authors declare that to the best of our ability we have included all of the data supporting the findings of this study within the article and the accompanying supplementary material.

**Availability of materials:**

*Zea mays sut1* loss-of-function mutant seed may be obtained from the Maize Genetics Stock Center (http://maizecoop.cropsci.uiuc.edu/).

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**Author contributions:** BAB, AK, DK, and JR performed $^{11}C$ radiotracer experiments. RFB and TMT performed genotyping by PCR, vein counts and carboxyfluorescein experiments. JK performed pico gauge measurements of sieve tube pressures and determination of phloem conductivity. BAB and DMB designed initial $^{11}C$ experiments. BAB, DMB, JK, MK, KHJ, RH, and SS designed subsequent experiments. BAB and RVT performed synchrotron X-ray fluorescence measurements on frozen hydrated tissue and RVT performed image analysis and quantification. GL performed aphid stylectomy and analysis of phloem sap. SS and RH conducted $K^+$ microelectrode experiment. KHJ completed model of sugar transport, with input from BAB, JK, and MK. BAB drafted the manuscript with input and editing from all coauthors.

**Competing Interests:**

The authors declare no competing interests.

**Fig. 1**. **Export of carbon-11 labeled photoassimilate from maize leaves decreased drastically, but phloem pressure did not decrease and sap flow velocity was only moderately decreased.** (*a*) Autoradiograph showing [$^{11}$C]-photoassimilate distribution in young leaves of 3-week-old maize plants after 1 hr incubation. The dark regions are where $^{11}CO_2$ was administered to the leaves. (*b*) $^{11}$C radioactivity declined in the labeled leaf over time, indicating export (arrow) from wild type (WT) and heterozygous (het) leaves. There was very little export from *sut1* homozygous mutant leaves. The higher radioactivity at time 0 is due to the pulse of $^{11}CO_2$ as it passed through the cuvette, after which only the $^{11}$C that was assimilated by photosynthesis remained in the leaf. (*c*) $^{11}$C export rates were very low in *sut1* homozygous mutant leaves compared to WT ($P_{\text{overall model}} < 0.0001$; $P_{\text{wt-vs-}sut1} < 0.0001$; $P_{\text{het-vs-}sut1} < 0.0001$; $P_{\text{wt-vs-het}} = 0.96$). (*d*) Moderately reduced phloem sap flow velocity of $^{11}$C-photoassimilates in *sut1* homozygous mutants ($P_{\text{overall model}} = 0.0003$; $P_{\text{wt-vs-}sut1} = 0.0012$; $P_{\text{het-vs-}sut1} = 0.0008$; $P_{\text{wt-vs-het}} = 1.0$) (WT n = 5 biologically independent samples, het n = 6, and *sut1* n = 9 in panels *b*, *c* and *d*). (*e*) Phloem pressure was not affected by *sut1* loss-of- function ($P < 0.41$) (WT n = 16, *sut1* mutant n = 15). Analysis of leaf extracts for (*f*) unlabeled ($^{12}$C) sucrose concentrations ($P_{\text{overall model}} = 0.0011$; $P_{\text{wt-vs-}sut1} = 0.0023$; $P_{\text{het-vs-}sut1} = 0.0023$; $P_{\text{wt-vs-het}} = 1.0$), (*g*) partitioning of $^{11}$C to sucrose as a percentage of the total fixed $^{11}$C ($P_{\text{overall model}} = 0.0023$; $P_{\text{wt-vs-}sut1} < 0.0001$; $P_{\text{het-vs-}sut1} = 0.0036$; $P_{\text{wt-vs-het}} = 0.92$), and (*h*) specific activity of sucrose shown as GBq of $^{11}$C radioactivity per gram of sucrose ($P_{\text{overall model}} = 0.018$; $P_{\text{wt-vs-}sut1} = 0.049$; $P_{\text{het-vs-}sut1} = 0.023$; $P_{\text{wt-vs-het}} = 0.87$)

766 (WT n = 3, het n = 3, *sut1* n = 4 in panels *f*, *g* and *h*). In panels c – h, the central line in boxplots
767 is the median, the box indicates the first and third quartiles, and the whiskers indicate the
768 minimum and maximum values. Statistical significance according to a two-sided ANOVA is
769 indicated by *(P<0.05), **(P<0.01), ***(P<0.001), or NS (not significant). Different letters
770 above bars indicate which genotypes were statistically different based on a Tukey's post hoc test.
771
772 **Fig. 2**. **Reduced $^{11}$C export is due to *sut1* loss-of-function, not an artifact of isotopic dilution.**
773 To eliminate isotopic dilution of $^{11}$C in homozygous *sut1* mutant leaves, *sut1* plants were kept in
774 the dark for 42 hrs until sucrose specific activity (*a*), which indicates the proportion of $^{11}$C to $^{12}$C,
775 was similar in wild type (WT) and *sut1* mutant plants ($P_{\text{overall model}} = 0.43$). (*b*) Severely reduced
776 $^{11}$C export rates from *sut1* homozygous mutant leaves ($P_{\text{overall model}} < 0.0001$; $P_{\text{wt-vs-}sut1} < 0.0001$;
777 $P_{\text{het-vs-}sut1} = 0.0001$; $P_{\text{wt-vs-het}} = 0.96$). (*c*) Marginally reduced transport speed of $^{11}$C-
778 photoassimilates in *sut1* leaves ($P_{\text{overall model}} = 0.022$; $P_{\text{wt-vs-}sut1} = 0.14$; $P_{\text{het-vs-}sut1} < 0.022$; $P_{\text{wt-vs-het}} =$
779 0.54). The central line, box, and whiskers indicate the median, the first and third quartiles, and
780 the minimum and maximum values, respectively. Statistical significance according to a two-
781 sided ANOVA is indicated by *(P<0.05), **(P<0.01), ***(P<0.001). Different letters above bars
782 in *b* – *c* indicate which genotypes were statistically different based on a Tukey's post hoc test
783 (WT n = 5, het n = 5, and *sut1* n = 7, except for transport speed het n = 4).
784
785 **Fig. 3**. **Wild type (WT; *a, c, d, h, k*) and *sut1* mutant leaves (*sut1*; *b, e, f, i, l*) do not differ in**
786 **leaf vein numbers, lignification patterns, anatomy, or transport of carboxyfluorescein (CF)**
787 **fluorescent dye through the phloem.** Phloroglucinol staining (*a, b*) reveals that the pattern of
788 lignification does not differ between WT and mutant veins. CF localization (*c-f*) does not differ
789 between WT and mutant phloem. CF was introduced to the phloem at the tip of the leaf, and

transported towards the base of the leaf before taking these sections near the leaf base. *c* and *e* shows the UV autofluorescence of *d* and *f*. For *a-f*, scale bar = 50 µm, and n = 5 independent plants. The number of major and minor veins in *sut1* and WT leaves were counted under a microscope (two-sided ANOVA: $P_{major} = 1.0$; $P_{minor} = 0.86$) (*g*; box and whiskers indicate median, 1st and 3rd quartiles, and minimum and maximum, n=10). Number of major veins appears as a single line in panel *g* because variability was low. Functional major veins were determined as the number of major veins containing $^{11}C$ in autoradiographs of WT (*h*) and *sut1* (*i*) plants. To aid analysis by reducing background, leaves were cut to physically separate regions where $^{11}CO_2$ was administered directly to the leaf (red arrows) from the regions where $^{11}C$ presence was due to vascular transport. Brightness and contrast of WT and *sut1* images were scaled independently here for ease of viewing veins. All major veins basipetal of the load zone that were labeled with $^{11}C$ were counted as functional ($P_{ANOVA\ two\text{-}sided} = 0.33$) (*j*; box and whiskers indicate median, 1st and 3rd quartiles, and minimum and maximum, n=3). Representative scanning electron micrographs of sieve plates used to calculate phloem conductivity in WT (*k*) and *sut1* (*l*) plants. Scale bar for *k-l* = 10 µm. There were 35 micrographs taken from 4 *sut1* plants, and 22 micrographs taken from 2 WT plants.

**Fig. 4**. **Imaging of ions in major veins by x-ray fluorescence microscopy show increased levels of potassium in the phloem.** Image of $K^+$ distribution in vascular bundle of maize leaf (*a*). Red arrow indicates phloem. Scale bar indicates 50 microns. Potassium (*b-c*) and calcium (*d-e*) contents of phloem and xylem were extracted from calibrated XRF images, concentrations determined based on known standards and the volume of the region of interest ($P_{phloem\ K} = 0.043$; $P_{xylem\ K} = 0.23$; $P_{phloem\ Ca} = 0.08$; $P_{xylem\ Ca} = 0.38$). Box and whiskers indicate median, 1st and 3rd

quartiles, and minimum and maximum (WT n = 6, *sut1* n = 4). * P<0.05 for two-sided t-test comparing WT to *sut1*.

**Table 1. Phloem amino acid and inorganic ion contents did not significantly correlate with phloem sucrose content.** Statistics for linear regression between sucrose and other phloem sap constituents. P value is from two-sided ANOVA. Independent biological replicates are indicated by n.

| | slope | intercept | F | n | P value |
|---|---|---|---|---|---|
| potassium | 0.022 | 72.9 | 0.52 | 19 | 0.48 |
| chloride | -0.028 | 67.7 | 2.17 | 18 | 0.16 |
| sodium | < 0.001 | 8.48 | 0.01 | 19 | 0.93 |
| magnesium | < 0.001 | 5.6 | 0.01 | 9 | 0.94 |
| calcium | -0.002 | 4.2 | 1.16 | 9 | 0.32 |
| amino acids | 0.013 | 15.5 | 0.99 | 12 | 0.34 |
| all cations | -0.029 | 152.8 | 0.73 | 9 | 0.42 |
| all ions | -0.090 | 251.1 | 2.52 | 8 | 0.16 |

## Extended Data

**Extended Data Figure 1. Feeding of $^{11}CO_2$ to a leaf.** Photograph showing Carbon-11 ($^{11}C$) being administered as $^{11}CO_2$ to a single leaf of a maize plant, clamped in a leaf cuvette. There was a single radiation detector built into the leaf cuvette, and two other detectors positioned along the leaf, basipetal to the leaf cuvette.

**Extended Data Figure 2. Homozygous *sut1* mutant leaves had high carbohydrate concentrations, and low carbohydrate specific activity.** Analysis of leaf extracts for (***a***) unlabeled ($^{12}C$) soluble sucrose ($P_{overall\ model} = 0.0011$; $P_{wt\text{-}vs\text{-}sut1} = 0.0023$; $P_{het\text{-}vs\text{-}sut1} = 0.0023$; $P_{wt\text{-}vs\text{-}het} = 1.0$), glucose ($P_{overall\ model} = 0.007$; $P_{wt\text{-}vs\text{-}sut1} = 0.013$; $P_{het\text{-}vs\text{-}sut1} = 0.014$; $P_{wt\text{-}vs\text{-}het} = 0.99$), fructose ($P_{overall\ model} = 0.007$; $P_{wt\text{-}vs\text{-}sut1} = 0.013$; $P_{het\text{-}vs\text{-}sut1} = 0.013$; $P_{wt\text{-}vs\text{-}het} = 1.0$), and starch concentrations ($P_{overall\ model} = 0.003$; $P_{wt\text{-}vs\text{-}sut1} = 0.008$; $P_{het\text{-}vs\text{-}sut1} = 0.005$; $P_{wt\text{-}vs\text{-}het} = 0.92$), (***b***) partitioning of $^{11}C$ to soluble sucrose ($P_{overall\ model} = 0.0014$; $P_{wt\text{-}vs\text{-}sut1} = 0.002$; $P_{het\text{-}vs\text{-}sut1} = 0.004$; $P_{wt\text{-}vs\text{-}het} = 0.92$), glucose ($P_{overall\ model} = 0.009$; $P_{wt\text{-}vs\text{-}sut1} = 0.015$; $P_{het\text{-}vs\text{-}sut1} = 0.02$; $P_{wt\text{-}vs\text{-}het} = 0.97$), fructose ($P_{overall\ model} = 0.054$), and starch ($P_{overall\ model} = 0.16$) as a percentage of the total fixed $^{11}C$, and (***c***) specific activity of sucrose ($P_{overall\ model} = 0.018$; $P_{wt\text{-}vs\text{-}sut1} = 0.049$; $P_{het\text{-}vs\text{-}sut1} = 0.023$; $P_{wt\text{-}vs\text{-}het} = 0.87$), glucose ($P_{overall\ model} = 0.003$; $P_{wt\text{-}vs\text{-}sut1} = 0.007$; $P_{het\text{-}vs\text{-}sut1} = 0.006$; $P_{wt\text{-}vs\text{-}het} = 0.99$), fructose ($P_{overall\ model} = 0.008$; $P_{wt\text{-}vs\text{-}sut1} = 0.008$; $P_{het\text{-}vs\text{-}sut1} = 0.052$; $P_{wt\text{-}vs\text{-}het} = 0.40$), and starch ($P_{overall\ model} = 0.022$; $P_{wt\text{-}vs\text{-}sut1} = 0.66$; $P_{het\text{-}vs\text{-}sut1} = 0.02$; $P_{wt\text{-}vs\text{-}het} = 0.08$) shown as GBq of $^{11}C$ radioactivity per gram of the sugar or starch, respectively (WT n = 3, het n = 3, *sut1* n = 4). Specific activity indicates the proportion of $^{11}C$ to $^{12}C$. Biochemical partitioning of $^{11}C$ to soluble sugars but not starch was elevated in *sut1* mutant leaves. The central line is the median, the box

indicates the first and third quartiles, and the whiskers indicate the minimum and maximum values. Statistical significance according to a two-sided ANOVA is indicated by *($P<0.05$), **($P<0.01$), ***($P<0.001$), or NS (not significant). Different letters above bars indicate which genotypes were statistically different based on a Tukey's post hoc test.

**Extended Data Figure 3. When $^{11}C$ dilution was mitigated by reducing sugar concentrations in *sut1* mutant leaves by dark treatment, $^{11}C$ export remained extremely low in *sut1* mutants, but sap flow velocity remained high by comparison.** Because the concentration of [$^{12}C$]-sucrose was extremely high in *sut1* leaves, and the specific activity was low (Fig. 1*F* and *H*, respectively; Extended Data Fig. 2), one could speculate that the reduced $^{11}C$ export in *sut1* might have appeared more drastic than overall $^{12}C$ export due to isotopic dilution. However, it is possible that much of the sucrose was unavailable for phloem loading in *sut1* mutants, for example if much of it is stored in vacuoles or exuded from hydathodes [44,74,75]. To measure export under more uniform cellular sugar status, we moved *sut1* mutant plants into constant darkness for 42 hrs immediately prior to $^{11}C$ assays, reducing the unlabeled ($^{12}C$) leaf sucrose, glucose, fructose and starch concentrations (***a***) to near WT levels. Note the 30 fold difference in Y-axis scaling for sugar concentrations compared to Extended Data Fig. 2a (6 fold for starch). (***b***) $^{11}C$-labeled sucrose ($P_{\text{overall model}} < 0.0001$; $P_{\text{wt-vs-}sut1} < 0.0001$; $P_{\text{het-vs-}sut1} = 0.0002$; $P_{\text{wt-vs-het}} = 0.89$), glucose ($P_{\text{overall model}} = 0.0051$; $P_{\text{wt-vs-}sut1} = 0.0056$; $P_{\text{het-vs-}sut1} = 0.042$; $P_{\text{wt-vs-het}} = 0.61$), fructose ($P_{\text{overall model}} = 0.017$; $P_{\text{wt-vs-}sut1} = 0.018$; $P_{\text{het-vs-}sut1} = 0.091$; $P_{\text{wt-vs-het}} = 0.71$), and starch. (***c***) Specific activities of sucrose, glucose, fructose, and starch were similar in WT and *sut1* mutant plants after dark treatment. For all panels, the central line is the median, the box indicates the first and third quartiles, and the whiskers indicate the minimum and maximum

values (WT n = 5, het n = 4, and *sut1* n = 7). Statistical significance according to a two-sided ANOVA is indicated by *(P<0.05), **(P<0.01), ***(P<0.001). Different letters above bars in indicate which genotypes were statistically different based on a Tukey's post hoc test. In these conditions, we confirmed that the greatly reduced $^{11}$C-export by *sut1* mutant leaves (Figs. 1*c* and 2*b*) was not an artifact of isotopic dilution by high [$^{12}$C]-sucrose concentrations. The reduction in phloem sap flow velocities in the low-carbohydrate, dark-treated *sut1* mutant leaves relative to WT remained modest in this experiment (Fig. 2*c*), compared to the large reduction of $^{11}$C-photoassimilate export (Fig. 2*b*), similar to the experiment with no dark treatment (Fig. 1*c*-*d*).

**Extended Data Figure 4. Maize phloem sap contents did not significantly correlate with phloem sucrose content.** Phloem sap was collected from wild type (blue diamonds) and *sut1* mutant (yellow circles) leaves by aphid stylectomy. We tested for a negative correlation between sucrose and (***a***) potassium, (***b***) chloride, (***c***) sodium, (***d***) total amino acids, (***e***) magnesium, (***f***) calcium, (***g***) total cations, and (***h***) potassium, sodium, and chloride combined (Table 1).

**Extended Data Figure 5. Phloem sap potassium ($K^+$) concentrations were higher in *sut1* mutant leaves than in wild type leaves.** (***a***) Comparison of wild type (WT) and mutant (*sut1*) leaf phloem. The central line is the median, and the box and whiskers indicate the first and third quartiles, and the the minimum and maximum values, respectively (n = 13, *** indicates P = 0.0003 for a two-sided Student's T-test). (***b***) $K^+$-selective microprobes were calibrated using $K^+$ standard solutions of known concentration. The reference electrode (blue) was hardly affected by changing external $K^+$ concentrations (concentration shown below the graph ranging from 1M to 1 mM), while the $K^+$- selective electrode (red) shows a near Nernstian behavior. Thus, the

reference subtracted black line shifts with the given values of $K^+$ indicating a fast and functional $K^+$-selective setup (values used for calibration are given below the black line). (***c***) Microscopic image of $K^+$-selective electrode (upper) and reference electrode (lower) impaled into a phloem cell. Both electrodes were controlled by micromanipulators under a microscope for impalement, and both electrodes were calibrated before and after impalement. (***d***) Raw trace of an example measurement before and after impalement (blue arrow indicates time of reference electrode impalement; red arrow indicates timing of $K^+$-selective electrode impalement). Black trace shows the membrane potential (blue trace) subtracted from the $K^+$-selective potential (red trace). We measured the stable selective potential (grey area) for all plants before removing the selective electrode at the end of the measurement.

**Extended Data Figure 6. No effect of potassium supplementation on $^{11}$C-photoassimilate transport dynamics.** Potassium supplementation of the soil medium did not affect $^{11}$C export or transport dynamics in either wild type (WT) or *sut1* mutant leaves. (***a***) Severely reduced $^{11}$C export rates from *sut1* homozygous mutant leaves. (***b***) Marginally reduced sap flow velocity of $^{11}$C-photoassimilates in *sut1* mutant leaves was not alleviated by adding 200 mL of 20 mM KCl to each pot once per week, starting 2 weeks after sowing. HK, high potassium; LK, low potassium. For both panels, center line is median, boxes indicate first and third quartiles, and whiskers are minimum and maximum (n = 4 for all bars, except for sap flow velocity for *sut1* low $K^+$, which was n = 3). For export (***a***), the overall model was significant for a two-sided ANOVA ($P < 0.0001$), and effects of genotype were significant ($P < 0.0001$), but there were no statistically significant effects of $K^+$ treatment ($P = 0.40$) or interactions between genotype and potassium treatment ($P = 0.53$). For sap flow velocity (***b***), the overall model was not significant

for a two-sided ANOVA (P = 0.16), the effects of genotype were significant (P = 0.034), but there were no statistically significant effects of $K^+$ treatment (P = 0.84) or interactions between genotype and potassium treatment (P = 0.55). Statistical significance of genotype effects are indicated by *(P<0.05), **(P<0.01), ***(P<0.001).

## Appendix A. Model of sugar transport

**Table A1: Measured sugar export rate, flow speed, source pressure, and geometric flow parameters**

| Symbol | Parameter | wild type (WT) | *sut1* mutant (M) |
|---|---|---|---|
| $I$ | sugar export rate | 100 % | 4 ± 0.4% |
| $v$ | flow speed | 100 % | 62.5 ± 12.5 % |
| $\Delta p$ | phloem source pressure | 1.39 ± 0.44 MPa | 1.51 ± 0.38 MPa |
| $k$ | Phloem conductivity - stem | 0.91 μm$^2$ | 1.18 μm$^2$ |
| $k$ | Phloem conductivity - leaf | 0.23 μm$^2$ | 0.19 μm$^2$ |
| $N$ | number of veins | 100 % | 100 % |
| $A$ | Conductive area | 100 % | 100 % |

Photosynthesis in leaves converts light energy into chemical energy which is stored in sugar molecules for later use in metabolism and growth. Sugars are exported from the leaf by bulk liquid flow through the phloem which form a continuous network of cellular conduits. Measured values of the phloem sugar current $I$, flow speed $v$ and source pressure $\Delta p$ are given in Table A1, along with relevant geometric parameters.

In this section, we develop a model of phloem transport as a framework to understand our experiments. In particular, we seek to rationalize the surprising observation that sugar export is strongly diminished in *sut1* mutants, while the phloem flow speed and turgor pressure remains relatively constant (Table A1). Our working hypothesis is that a supplementary osmolyte, for

instance, one or more small ions, is present in higher concentrations in the mutant plants to compensate for the loss in osmotic potential.

We base our analysis on the simple observation that the sugar transport rate $I$ (mol/s) can be expressed as the product of three factors:

$$I = Avc_s, \qquad (1)$$

where $A$ ($m^2$) is the conductive area, $v$ the average flow speed and $c_s$ (mol/$m^3$) is the sap sugar concentration. Based on our transport experiments, we can deduce the relative sugar concentration in the wild type and *sut1* mutants. Taking the ratio of mutant to wild type values in Eq. (1) leads to

$$\frac{I^M}{I^{WT}} = \frac{A^M}{A^{WT}}\frac{v^M}{v^{WT}}\frac{c_s^M}{c_s^{WT}} \approx \frac{v^M}{v^{WT}}\frac{c_s^M}{c_s^{WT}}, \qquad (2a)$$

where the last equality holds because the variation in conductive area is relatively small.

To compute the expected sugar concentrations, we first rewrite Eq. (2a) as

$$\tilde{I} = \tilde{v}\tilde{c_s}, \qquad (2b)$$

where the tilde superscript indicates the mutant-to-wild type ratios, i.e., $\tilde{c_s} = c_s^M/c_s^{WT}$ and $\tilde{v} = v^M/v^{WT}$. Using the values in Table A1, we are now able to evaluate the relative concentration

$$\tilde{c_s} = \frac{\tilde{I}}{\tilde{v}} = \frac{0.04}{0.625} = 0.064 \pm 0.014, \qquad (3a)$$

which implies that the mutant concentration $c_s^M = \tilde{c_s}c_s^{WT} = (0.064 \pm 0.014)c_s^{WT}$ is expected to be more that ten-fold reduced in the mutant. In Eq. (3a) we estimated the uncertainty $\delta(\tilde{c_s})$ in the concentration ratio $\tilde{c_s}$ by propagating the measurement errors, i.e.,

$$\delta(\tilde{c_s}) \approx \frac{\tilde{I}}{\tilde{v}}\sqrt{\left(\frac{\delta\tilde{I}}{\tilde{I}}\right)^2 + \left(\frac{\delta\tilde{v}}{\tilde{v}}\right)^2} = 0.064\sqrt{0.1^2 + 0.2^2} = 0.014. \qquad (3b)$$

It is reasonable to assume that the strong decrease (Eq. (3a)) in the sap carbohydrate concentration also has an effect on the osmotic conditions in the phloem. In particular, we expect an effect on the source turgor pressure. Surprisingly, however, the source pressure in the mutants is slightly larger than in the wild type (Table A1). This suggests that a supplementary osmolyte could be present in the mutant phloem sap.

To quantify the magnitude of the auxiliary osmolyte concentration required to maintain approximately constant phloem pressure, we briefly summarize the core principle of the phloem pressure-flow mechanism: that loading of solutes generates the osmotic pressure that drives bulk flow. The osmolytes under consideration here are sucrose and one, or more, small ion(s). The pressure difference $\Delta p$ between source and sink in terms of the solute concentration $c$ and apoplast pressure $p$ is

$$\Delta p = RT\left[\left(c_s^{\text{source}} - c_s^{\text{sink}}\right) + \left(c_i^{\text{source}} - c_i^{\text{sink}}\right)\right] + \; p^{\text{source}} - p^{\text{sink}} \qquad (4)$$

where $R$ is the gas constant and $T$ is temperature. The subscripts $s$ and $i$ indicate sugar and ion concentrations, respectively, while the superscripts denote the source or sink region. Sugars removed by phloem unloading in the sink tissue lowers the concentration relative to the source region, i.e. $c_s^{\text{source}} \gg c_s^{\text{sink}}$ and we assume that similar conditions exist for the ions, i.e. $c_i^{\text{source}} \gg c_i^{\text{sink}}$. Moreover, the external pressure gradient $p^{\text{source}} - p^{\text{sink}}$ is expected to be relatively minor in small, well-watered plants [86], i.e., $RT(c_s^{\text{source}} + c_i^{\text{source}}) \gg p^{\text{source}} - p^{\text{sink}}$. This leads to a simplified expression for the pressure difference $\Delta p \simeq RT(c_s^{\text{source}} + c_i^{\text{source}})$, which, for the wild type and mutant plants can be expressed as

$$\Delta p^{WT} \simeq RT(c_s^{\text{WT}} + c_i^{\text{WT}}), \qquad (4b)$$

and

$$\Delta p^M \simeq RT\left(c_s^{\mathrm{M}} + c_i^{\mathrm{M}}\right) = RT(\widetilde{c_s} c_s^{WT} + c_i^{\mathrm{M}}), \qquad (4c)$$

where we used the relation in Eq. (3a) to link the mutant wild type sugar concentrations.

By combining the observed phloem pressures (Table A1) with the osmotic relations in Eq. (4), we can now deduce the ion concentration required to maintain phloem turgor. The only remaining question is the absolute composition of phloem sap in the wild type plants, i.e., the relative magnitude of $c_s^{\mathrm{WT}}$ and $c_i^{\mathrm{WT}}$. We denote this quantity $x$, i.e.,

$$x = \frac{c_s^{\mathrm{WT}}}{c_s^{\mathrm{WT}} + c_i^{\mathrm{WT}}} \qquad (4d)$$

Combining (4d) with (4b) and (4c) finally allows us to express the required sugar and ion concentrations in terms of known parameters

$$c_s^{WT} = \frac{\Delta p^{WT}}{RT} x, \qquad (5a)$$

$$c_i^{WT} = \frac{\Delta p^{WT}}{RT} (1 - x), \qquad (5b)$$

$$c_s^M = \widetilde{c_s} c_s^{WT} = \widetilde{c_s} \frac{\Delta p^{WT}}{RT} x, \quad \text{and} \qquad (5c)$$

$$c_i^M = \frac{\Delta p^M}{RT} - c_s^M. \qquad (5d)$$

Based on variability in the source pressure $\Delta p$ and concentration ratio $\widetilde{c_s}$, we can compute the associated errors

$$\delta(c_s^{WT}) = \frac{\delta(\Delta p^{WT})}{RT} x, \qquad (5e)$$

$$\delta(c_i^{WT}) = \frac{\delta(\Delta p^{WT})}{RT} (1 - x), \qquad (5f)$$

$$\delta(c_s^M) = c_s^M \times \left( \left( \frac{\delta(\widetilde{c_s})}{\widetilde{c_s}} \right)^2 + \left( \frac{\delta(\Delta p^{WT})}{\Delta p^{WT}} \right)^2 \right)^{1/2}, \text{ and} \qquad (5g)$$

$$\delta(c_i^M) \approx \frac{\delta(\Delta p^M)}{RT} + \delta(c_s^M). \qquad \text{(5h)}$$

Having established the governing equations (5a-d) and error estimates (5e-h), we are now able to estimate the sugar and ion concentrations required to maintain phloem pressure and flow. We first consider the case in which no ions are present (i.e., $c_i^{WT} = 0$) in the wild type plant phloem sap, which makes $x = 1$ from Eq. (4d). In this case, we find $c_s^{WT}$ from equation (5a):

$c_s^{\mathrm{WT}} = 571 \pm 180$ mol/m$^3$

$c_i^{\mathrm{WT}} = 0$ mol/m$^3$

$c_s^{\mathrm{M}} = 37 \pm 14$ mol/m$^3$

$c_i^{\mathrm{M}} = 584 \pm 170$ mol/m$^3$

where the ion concentration thus increases strongly, from 0 to 584 mol/m$^3$, between the wild type and mutant plants.

A more conservative estimate is that, for instance, half, the osmotic potential in the wild type is generated by ions. For $x = 0.5$ we find

$c_s^{\mathrm{WT}} = 285 \pm 90$ mol/m$^3$

$c_i^{\mathrm{WT}} = 285 \pm 90$ mol/m$^3$

$c_s^{\mathrm{M}} = 18 \pm 7$ mol/m$^3$

$c_i^{\mathrm{M}} = 602 \pm 163$ mol/m$^3$

This corresponds to an approximate doubling of the phloem ion concentration between the wild type and mutant plants. The $K^+$ concentrations predicted are quite close to those measured in the wild type (297 ± 74 mol m$^{-3}$) and *sut1* (564 ± 78 mol m$^{-3}$) phloem by XRF (see Fig. 4 in the main text). Substituting the phloem $K^+$ concentrations that we actually measured in plants used for $^{11}$C

studies of sap flow speed plus the modeled sucrose concentration ($c_s^{\mathrm{M}}$), formula 4b finds that $\Delta p^M \simeq 1.42\ MPa$. Then, the flow speed $v$ (m/s) can be predicted from Darcy's law

$$v = \frac{k}{\eta}\frac{\Delta p}{L} \qquad (2)$$

where $k$ ($m^2$) is the conductivity, $\eta$ (Pa s) is the sap viscosity, $\Delta p$ (Pa) is the pressure difference and $L$ (m) is the transport pathway length. Since the presence of the relatively small $K^+$ ions has a negligible effect on viscosity when compared to sucrose, we treat the viscosity $\eta$ as a function of $c_s$ only. Using viscosity for $c_s^{\mathrm{M}}$ determined from published sucrose-viscosity relationship data [87], and $L$ and $k$ based on measured values, and propagating the SEM for $K^+$ concentration we predicted transport speed:

$$v \cong 0.95\ \pm 0.13\ m\ hr^{-1}$$

This calculated $v$ is quite close to the transport speed measured in *sut1* mutants in three independent experiments. On the contrary, if we assume zero $K^+$ and sucrose alone ($c_s^{\mathrm{M}}$) maintains sap flow in the *sut1* mutant, then $v \cong 0.03\ m\ hr^{-1}$, or if $K^+$ remains at WT levels, then $v \cong 0.51\ m\ hr^{-1}$, which is much lower than the observed sap flow velocity of the *sut1* mutant and outside of the 95% confidence intervals.