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Natural variation in the long-distance transport of nutrients and photoassimilates in response to N availability

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Abstract:

The phloem and the xylem are involved in the allocation of nutrients and photoassimilates between organs. However, the regulation of the long-distance transport of C and N and its interplay with central metabolism is largely unknown. We exploited the natural variation of *Arabidopsis thaliana* accessions to analyze the metabolite profiles of phloem and xylem sap in two conditions of nitrogen (N) supply. Changing N supply from limiting to high availability led to a lower metabolite exudation rate from the phloem, indicating a lower mass flow of carbon (C) towards sink organs. However, the accessions did not all respond in the same way, consistent with reports showing a variability in the ability of natural accessions to cope with N abundance for improved growth. Distinct consequences of N availability were observed in the xylem sap and phloem exudate. This study revealed that the N metabolism response, set up to cope with N availability, is associated with a regulation of the phloem transport and may be an adaptive trait. Our study also highlighted an unexpected variability in the translocation of organic acids in response to N availability, suggesting that both phloem sugar transport and respiratory metabolism participate in the adaptive response to mineral nutrition.

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Introduction

The acquisition of vascular tissues by land plants, which allow long distance transport of water, mineral ions and photoassimilates among source and sink organs, has been a critical adaptive trait in their evolution (Van Bel, 2003). Both root-to-shoot transport of water, minerals and nutrients by the xylem and the phloem translocation of photoassimilates from photosynthetic organs to heterotrophic ones provide carbon (C) skeletons and primary metabolites fueling the growth and development of organs. Carbohydrate allocation is mainly achieved by the phloem, even if small amounts of sugars, mostly glucose, are translocated by the xylem transpiration stream (Alvarez et al., 2008; Krishnan et al., 2011). Organic forms of Nitrogen (N), mainly amino acids, are transported by advection as solutes in both phloem and xylem (Tegeder, 2014). Besides metabolites, both phloem and xylem transport ions, peptides, hormones and macromolecules, acting as signal molecules for long-distance communication pathways (Notaguchi and Okamoto, 2015; Turnbull and Lopez-Cobollo, 2013).

Many reports have shown that the phloem and xylem sap compositions fluctuate depending on the environmental conditions or on genetic background (Fernandez-Garcia et al., 2011; Guelette et al., 2012; Hunt et al., 2009, 2006; Lam et al., 1995; Rellán-Álvarez et al., 2011; Vilaine et al., 2013; Zhang et al., 2010). General models for flow of nutrients between roots and shoots have been proposed in *Ricinus communis* by Peuke, suggesting a central role of the phloem for adjusting nutrition status (Peuke, 2010), however a comprehensive model taking in account the diversity of molecular factors involved in the coordinated regulation of this process is still lacking.

Most efforts to unravel the processes involved in allocation of C and organic forms of N in the plant have focused on the translocation of sugars and amino acids, which is mainly driven by sugar and amino acids transporters located in phloem or xylem cells. Sugar transporters, such as the SUC/SUT and the SWEET transporters for sugars (Braun et al., 2014; Lemoine et al., 2013), MST transporters for the sugar alcohols (Noiraud et al., 2001), and several families of amino acid and ureide transporters (Tegeder, 2014; Tegeder and Masclaux-Daubresse, 2018) have been described. Plasmodesmata at the interface of the vascular transport cells and surrounding cells also participate for specific steps to the intercellular trafficking of metabolites (van Bel, 2021). In addition, evidence has also accumulated that the availability of certain metabolites is critical. N remobilization from source to sink organs is associated with the activity of glutamine and asparagine synthetases that produce the major transportable forms of organic N (Brugière et al., 1999; Gaufichon et al., 2017; Masclaux-Daubresse et al., 2006). On the other hand, sugar loading and unloading drives phloem flow, therefore a strong coupling exists between C and N transport, relying on sugar gradients along the phloem pathway and N assimilation and remobilization. However, we still have a poor understanding of the intricate interplay between sugar transport, N metabolism and transport of organic N.

Exploring natural genetic diversity can help to unravel regulatory processes participating in a plant's adaptation to its environment. Nitrogen deprivation is one important condition that affects plant growth and development. As for many other traits, natural variability has been used in Arabidopsis to analyze the plant response to N availability (Chardon et al., 2012; Chietera et al., 2018; Ikram et al., 2012; Sulpice et al., 2013). For example, nitrogen use efficiency (NUE) has been explored in a broad range of accessions, revealing different strategies for plant growth and seed development (Chardon et al., 2012), and demonstrating variability in the efficiency of N remobilization (Masclaux-Daubresse and Chardon, 2011). It remains unclear whether such a response results from the regulation of N assimilation in

source leaves or is a secondary consequence of the adjustment of phloem flow when the plant adjusts root growth and hastens reproduction for fitness to cope with low nitrogen, remains unclear.

In our study we compared the metabolite contents of phloem and xylem exudates of five Arabidopsis accessions, which had previously been characterized by their contrasting performances in response to N deprivation (Ikram et al., 2012). A metabolite profiling using GC-MS was conducted to analyze the contents of the primary metabolites in the exudates (sugars, organic acids and amino acids), the most abundant metabolites in the saps (Dinant et al., 2010). Analysis of the concentration of the metabolites in the xylem sap, of the phloem exudation rates of these classes of metabolites and the metabolite contents of the phloem exudates revealed an unexpected variability between accessions which was related to growth traits and life history of the plant.

Materials and Methods

Growth conditions

Five Arabidopsis thaliana accessions, Burren-0 (Bur-0), Catania-1 (Ct-1), Cape Verde Islands-0 (Cvi-0), Columbia-0 (Col-0) and Edinburgh (Edi-0), were obtained from the Versailles stock center ("Versailles Stock Center," n.d.). They are representative of the groups described by Ikram et al. (Ikram et al., 2012) and belong to the core collection of 24 accessions selected by McKhann et al. (McKhann et al., 2004) to capture the maximum molecular and morphological diversity. Plants were grown in a controlled-growth chamber under long-day condition (16 h light / 8 h dark cycle, 150 μM $m^{-2}s^{-1}$) and were cultivated in soil under the low-N (1 mM nitrate; LN) or high-N (10 mM nitrate; HN) nutrition rates as described by Lemaître et al. (Lemaître et al., 2008).

Growth parameters

Four plants to 8 plants were sampled for each measurement in two separate experiments. Soluble sugar (glucose, fructose, and sucrose) concentration was determined enzymatically as described previously (Vilaine et al., 2013). The projected leaf area of each rosette was measured at 27 days after sowing and quantified using ImageJ ("ImageJ," n.d.). The number of rosette leaves was determined on plants harvested at stage 6.10 (Boyes et al., 2001). For shoot biomass and seed yield, plants were harvested 70 days after sowing. Shoot biomass dry weight (DW) was determined by pooling the aerial parts (rosettes, floral stem and siliques). The harvest index was calculated as the ratio of seed mass to the shoot biomass. Total N and C contents of dried seeds and the rosette leaves were determined with an elemental analyzer (Thermoflash 2000, Thermo Scientific). For C and N content of the leaves, it was determined on a pool of the 6^{th} and 7^{th} leaves sampled on the same plants as those used for exudation. Leaf N index (leaf NI) was determined on a pool of the 6^{th} and 7^{th} leaves by the product of the leaf N content per their weight. For each trait the Net Nutrition Effect (NNE) was calculated as the difference between the average value determined in HN and the one determined under LN.

Xylem sap sampling

Xylem saps were collected from plant via cut petioles of rosette leaves and cut inflorescence stems on plants harvested four weeks after sowing as described (Sunarpi et al., 2005). Exudates were collected from well-watered plants cut off at mid-day. Xylem exuded spontaneously after petioles and inflorescence stems were cut. Up to 30 µl could be collected *per* plant after two hours of exudation. Exudates were frozen in liquid nitrogen. Eight biological replicates for each accession and each nutrition condition were collected except for Cvi-0 grown in HN, for which a weak exudation was observed and for which we could collect only two replicates. A volume of 10 µl of sap from each sample was analyzed by GC-MS.

Collection of phloem exudates

Phloem exudates were collected by EDTA-facilitated exudation, a method that has been successfully applied in Arabidopsis to analyze phloem sap amino acids profiles and phloem soluble carbohydrates flow (Batailler et al., 2012; Vilaine et al., 2013; Xu et al., 2018). Phloem exudates were collected from the petioles of the sixth leaf of each plant harvested at midday, at the same stage as the one used of xylem sap sampling. Petioles were sectioned, recut in a Petri dish filled with exudation buffer (Beneteau et al., 2010) and immediately immersed for two hours of exudation in 80 µl of the exudation buffer (10 mM HEPES, 10 mM EDTA, pH 7.5), containing 4 mg. L^{-1} ribitol as an internal standard for metabolomic analysis. To prevent contaminations from the surrounding tissues, a delay of several minutes of exudation in the cutting dish was applied prior to transfer of the leaf. Four biological replicates were used for each Arabidopsis accession and each nutrition condition. The fresh weight of each leaf used for exudation was measured and the exudates stored at -80°C. Sixty µl of exudates were used for GC-MS analysis.

Metabolite profiling of xylem and phloem exudates by Gas Chromatography-Mass Spectrometry analysis (GC-MS)

For each accession and growth conditions, 4 replicates for the phloem sap exudate and 8 replicates for the xylem sap exudate were analyzed. Chemical derivatization and GC-MS analysis were performed as described by Fiehn (Fiehn, 2006) with the modifications described in (Vilaine et al., 2013). Samples were injected in split-less mode into an Agilent 7890A GC coupled to an Agilent 5975C mass spectrometer. GC-MS analysis was performed on a Rxi-5SilMS column (Restek). For quantification, standards were injected at the beginning and end of the analysis. Data were analyzed with AMDIS ("AMDIS," n.d.) and QuantLynx software (Waters). For phloem samples, the analysis provides the amounts of metabolites collected after two hours of exudation, which corresponds to an exudation rate for each metabolite (expressed in mM. g^{-1} of leaf fresh weight . hr^{-1} of exudation). For xylem samples, the analysis provides the concentration of metabolites in the sap (expressed in mM).

Normalization of phloem sap exudate data

To identify, within the metabolite profiles of the phloem sap-enriched exudates, specific differences in their proportion, independent of the exudation rate, the profiles were adjusted using a method of normalization that has been developed for the analysis of phloem sap-enriched exudates (De Marco et al., 2021; Yesbergenova-Cuny et al., 2016). This method enabled a comparison of the content of metabolites, by using the method originally developed for normalizing multiple target microarray experiments (Martin-Magniette et al., 2008). Data were log-transformed and each metabolomic profile was corrected with respect to the mean

metabolomic profile using locally weighted scatterplot smoothing (LOWESS). Log_2 transformation and normalization were carried out using R statistical software version 3.1.2 ("R statistical software, version 3.1.2," n.d.). The normalized values are termed "content" and are expressed in relative units (R.U.).

Assessment of carbon (C) and organic nitrogen (N) content from metabolite profiles

The C and organic N contents in the phloem and xylem were obtained from the elemental composition of metabolites (carbohydrates, amino acids, organic acids) that have been quantified in the xylem and the phloem fluids, using the method of A. Peuke (Peuke and Jeschke, 1993). For phloem flow, C or organic N were calculated from carbohydrates, amino acids and organic acids that have been quantified in the exudate ($\mu M.g^{-1}$ fresh weight. hr^{-1}). For xylem flow, C or organic N were calculated from carbohydrates, amino acids nd organic acids that have been quantified in xylem saps (expressed in mM).

Statistical analysis and clustering

Statistical analyses were done using R software. One way and two-way analyses of variance (ANOVA) to determine the variability due to the effect of nutrition (N), genotype (G) and interaction between nutrition and genotype (GxN) were done on 60 metabolites for the xylem sap and 44 metabolites for the phloem, after removal of the metabolites with missing values and using BH correction. Data were visualized using ggplot2 R package (Wickham, 2016). Pairwise comparisons were done with a Student's t-test, and $p < 0.05$ were considered significant. Correlations were calculated using the Pearson correlation coefficient and tested with the adjusted *p*-value determined with the Holm's method. Correlations with $p < 0.01$ were considered significant.

Results

Metabolite profiles of xylem sap and phloem exudate under low and high N supply

The five accessions chosen in this study are Bur-0, Col-0, Ct-1, Cvi-0 and Edi-0. The plants were grown under long-day conditions, and we observed contrasting growth and yield responses to either low (1 mM NO_3, LN) or non-limiting (10 mM NO_3, HN) N supply (Figure S1), consistent with the observations obtained in short-days conditions (Ikram et al., 2012). Not all accessions benefited to the same extent from HN supply compared to LN regarding growth, biomass production and seed yield. Briefly, Bur-0, Ct-1 and Edi-0 were characterized by a high efficiency to use high N to produce more biomass and higher seed yield, while Cvi-0 was less efficient to use N, with Col-0 being intermediate (Figure S1). With a delayed flowering time, Bur-0 produced more biomass and larger seeds under HN compared to the other accessions. For each accession in each N nutrition, we collected the xylem sap and the phloem exudate of four plants.

Composition of xylem saps and phloem exudates under contrasting N nutrition

The compositions of the xylem sap and phloem exudate were analyzed by GC/MS, which allowed the identification and quantification of sugars, amino acids and organic acids. Over 100 metabolites were identified in the xylem saps from which 66 were quantified

(Supplementary Table 1). Carbohydrates and amino acids were abundant, with concentrations up to 2 mM. The concentration of organic acids was lower, up to 0.5 mM. Over 79 metabolites were detected in the phloem exudates, among which 61 compounds were quantified (Supplementary Table 2). The rate of exudation for sucrose, the predominant carbohydrate in the phloem exudate, was up to 25.8 µM. g^{-1} fresh weight (FW). hr^{-1}. The rates of exudation of amino acids and organic acids were smaller, no more than 5.2 µM. g^{-1} FW. hr^{-1} and 2.1 µM. g^{-1} FW. hr^{-1} respectively. Amino acids were more abundant under HN than LN both in xylem sap and phloem exudates (2 fold higher in HN), in contrast to organic acids that were less abundant (about half in HN compared to LN). In the xylem saps the total concentration of carbohydrates was similar in HN and LN, while the exudation rate of carbohydrates in the phloem exudates was smaller in HN than in LN (Supplementary Tables 1 and 2). This lower value was mainly due to a two-fold reduction of the sucrose exudation rate.

As expected, the data confirmed large differences in the proportions of sugars, amino acids and organic acids between xylem and phloem sap (Figure 1A-B), with a large excess of carbohydrates, over 75% of the quantified metabolites, in the phloem samples, characteristic of phloem sap, by contrast to the xylem sap (less that 50% of the quantified metabolites). The proportion of amino acids was higher in the xylem saps, especially under HN (over 45%). The proportion of organic acids remained small, with 5-6% of the total amounts of metabolites in the phloem exudates and 5 to 13% of the amounts of metabolites in the xylem sap.

Metabolite profiles

The metabolite profiles confirmed the differences between xylem sap and phloem exudates for carbohydrates, amino acids and organic acids (Figure 2 A-F). Glucose was the most abundant carbohydrate in the xylem sap while sucrose was the most abundant one in the phloem exudates (Figure 2A, D). The proportion of glutamine was higher in the xylem saps compared to phloem exudates both under LN and HN, and Asp was the dominant amino acid under LN (more than 12-fold increase compared to HN conditions). Interestingly, organic acid composition in the xylem saps and the phloem exudates was similar, with comparable proportions of malate, citrate and fumarate in the xylem saps under LN and HN and in the phloem exudates under LN. The most striking differences were the higher proportion of citrate and lower proportion of fumarate in the phloem exudates in HN compared to LN, and the higher proportion of succinate in the xylem saps under HN compared to LN.

Natural variation in amino acids, organic acids and carbohydrates transporterd by the xylem and the phloem

Variability in the proportions of metabolites in xylem sap and phloem exudate

We compared the metabolite profiles in xylem saps and phloem exudates between accessions. We observed a variability in the proportions of amino acids, carbohydrates, and organic acids in these accessions under HN and LN (Figure 3A-B). The variations were larger under HN. For example, the proportion of amino acids under HN was higher in the xylem sap of Col-0 compared to the other accessions (Figure 3A). The proportion of amino acids under HN was smaller in the phloem exudates of Edi-0 compared to the other accessions (Figure 3B).

Interestingly in the xylem saps, we also observed a variability in the total concentration of metabolites between accessions, revealing an effect of the nutrition, the genotype and an

interaction between genotype and nutrition on the xylem metabolite total content (Figure 3C). The highest values were observed in Cvi-0 in HN, indicating that more important amounts of metabolites are xylem-transported root-to-shoots in this accession under HN. For the phloem exudates, less variability was observed in the total metabolite exudation rates (Figure 3D). However, higher values were observed in the phloem exudates of Edi-0 than in the others, either in LN or HN, indicating that this accession has a higher capacity to transport metabolites by the phloem than the others (Figure 3D). The data showed an effect of the nutrition and the genotype in the metabolite exudation rate of the phloem.

Variability due to genotype, nutrition and interaction genotype per nutrition on the total concentration of carbohydrates, amino acids and organic acids

We observed a high variability due to nutrition and to genotype on the total concentrations of amino acids, carbohydrates and organic acids in the xylem saps and in the phloem exudates, effects that were highly significant (Figure 4A-F). For example, carbohydrate concentration in the xylem sap of Cvi-0 was higher under HN than in the other accessions, and the exudation rates of amino acids were smaller in Cvi-0 and Col-0 under HN than in the other accessions. More variability was observed for amino acids and carbohydrates under HN than under LN, in both xylem sap and phloem exudates. More interestingly we observed a strong interaction between genotype and nutrition on the xylem carbohydrate and organic acids total contents (for GxN $p<0.001$, Figure 4B,C) and on the amino acids exudation rates (for GxN $p<0.001$, Figure 4D).

*Variability of **metabolite contents***

Analysis of the metabolite contents in phloem exudates

We also looked at which metabolites vary the most according to genotype and nutrition in the phloem exudates. Because of the high variations in the exudation rates of the phloem exudates due to genotype and nutrition (Figure 3D), the metabolite profiles of phloem exudates were adjusted using a method of normalization that has been developed for the analysis of phloem sap-enriched exudates (De Marco et al., 2021; Yesbergenova-Cuny et al., 2016) (Supplementary Table 3). In the following text, these normalized data, named "metabolite contents", are expressed in relative units (R.U.).

Comparison of metabolite contents in both phloem exudates and xylem saps

To compare the effects of N nutrition on metabolite contents in the phloem exudate and concentrations in xylem sap, we calculated for each metabolite and each accession the fold changes in HN compared to LN. The heat map (Figure 5) confirmed that the content of many amino acids (Ala, Asn, Gln, GABA, Pro, Thr, Ser for example) was higher under HN than under LN, in both phloem exudates or in xylem saps, although the the content of Glu and of 2-oxoglutarate was stable in the phloem exudates. Interestingly, the content of several carbohydrates was lower under HN than LN, i.e. for raffinose, melibiose, galactinol, in both the phloem exudates and xylem saps. Lower values were also observed for malate and fumarate under HN than LN, in both the phloem exudates and xylem saps. Interestingly, for HN, contents of branched amino acids (Leu, Ile and Val) and aromatic amino acids (Trp, Phe and Tyr) were higher in xylem saps and lower in phloem exudates, whereas Asn, Gln, Ala, GABA and Pro were higher accumulation in both xylem saps and phloem exudates.

316

317 Effects of nutrition and genotype on the metabolite contents

318 A two-way ANOVA was applied to the data to determine whether the variations in the
319 metabolite contents observed in the xylem saps and phloem exudates were due to the
320 genotype (G), the nutrition (N) or their interaction (GxN). The effects due to each factor were
321 reported as an heat-map for each metabolite (Figure 6). This analysis confirmed the main
322 effect of the nutrition on the content of several amino acids, such as Pro, GABA, Tyr, Thre,
323 Asn and Gln, in both xylem saps and phloem exudates. However, it reavealed a strong effect
324 of genotype on the contents of arginine, lysine, serine, cystein, tryptophane, glycerate,
325 fumarate and pipecolate. The ANOVA showed a strong interaction GxN for Asn, Lys, His,
326 pipecolate and succinate. This analysis confirmed the role of nutrition on metabolite contents
327 transported by both phloem and xylem, impacting both amino acids and sugar contents, and
328 showed that the genotype alone, or together with the nutrition treatment, significantly
329 impacted the transport of several organic acids in both xylem and phloem.

330 Correlations between the contents of phloem and xylem metabolites

331 The link between the transport of organic acids and that of sugars and amino acids is
332 poorly understood. Our data showed that malate, citrate, fumarate and succinate are not
333 influenced in the same way by nutrition and genotype (Figure 6). Of these four organic acids,
334 only malate shows a predominant effect of nutrition both in phloem exudate and xylem sap
335 (75% and 57% of the variance explained by N in the phloem and xylem, respectively). An
336 equally strong effect of nutrition was observed for Gln, GABA, Pro, Asn, Ala, which is
337 expected, as well as for sucrose and raffinose, which is unexpected (Figure 6). In contrast,
338 succinate showed a strong effect GxN in phloem exudate (54% of the variance) while
339 fumarate showed a strong effect G in xylem exudate (50% of the variance). We also
340 investigated whether there were correlations between the contents of organic acids with
341 abundant sugars and amino acids (Figure 7A and Figures S2 and S3). Interestingly, in the
342 xylem saps, both fumarate and malate were positively correlated to Asp, in contrast to the
343 phloem exudates where the correlations were negative (Figure 7A). Finally, we found high
344 positive correlations between malate and fumarate contents and raffinose contents, both in
345 the xylem saps and in the phloem exudates.
346
347 Another interesting example was pipecolic acid (Pip) which showed high variability in
348 the saps depending both on G and GxN (Figure 6, with 23 and 26% of the variance explained
349 by G, and 26% and 14% explained by GxN, respectively, in phloem exudates and xylem
350 saps). The high G and GxN effects reflect the dependence of Pip content on the accessions,
351 in HN compared to LN, both in the xylem sap or phloem exudates (Figure 5). Interestingly,
352 its accumulation in the xylem sap was correlated to the accumulation of the Gln, Glu and
353 Asn, a link that was not observed in the phloem exudates (Figure 7B).
354

355 ***Relationship between the translocation of C and organic N and plant growth***

356 Relationship between the phloem metabolite content and seed yield and biomass

357 We then tested whether a correlation exists between the metabolite exudation rates or
358 the metabolite phloem contents and seed yield or biomass production at harvest time
359 measured on plants grown on the same conditions (Figure S1). The rates of exudation of the
360 abundant amino acids in the phloem sap, alanine, asparagine, aspartate and glutamine showed

positive correlations with seed yield and biomass at harvest (Table 1), with asparagine showing the most significant correlation with seed yield.

Consequences of N nutrition on the translocation of C and organic N

With an abundant supply of mineral N, more N is expected to be translocated in both phloem and xylem, and giving a higher N storage in the leaves and in the seeds (Figure S1 G,H). However, the effect of N nutrition on the translocation of C by the phloem and xylem could be altered in different ways. In order to explore this possibility, we used a method that was initially developed to study the flow of C and N between roots and shoots (Peuke and Jeschke, 1993), converting carbohydrates, amino acids and organic acids into equivalent C and organic N units (Figure S4). As expected, N nutrition had a strong effect on the translocation of organic N, with more organic N that was transported in both xylem saps and phloem exudates under HN compared to LN (Figure 8A,B, Supplementary Figure S4) and more N in leaves and seeds (Figure 8C, Supplementary Figure S1 G,H). The nutrition had less effect on C translocation (Figure 8D), with more C that was transported in xylem for most accessions under HN compared to LN (Figure 8E, Supplementary Figure S4), less C that was transported by the phloem under (Figure 8E, Supplementary Figure S4) and less carbon in leaves and seeds under HN (Figure 8F, Supplementary Figure S1 I,J).

This indicated that more C is translocated to sinks, such as roots and seeds under LN, while less C is remobilized from root-to-shoot. We observed a variability for the translocation of C and organic N between accessions, both in the xylem or phloem fluids (Supplementary Figure S4). For example, we observed a higher C flow in the xylem sap for Cvi-0 and Edi-0 and a lower phloem flow for Cvi-0 (Figure 8C), and less carbon in the seeds for Bur-0 and Cvi-0. Interestingly, the main factor explaining the variability of C flows *via* the phloem or xylem was the genotype (25% and 40% of the variance of xylem and phloem flows, respectively), with lower effects of nutrition (about 20% of the variance) and GxN interaction (about 10% of the variance). These findings contrasted with the flows of organic N *via* the phloem or xylem, that were mostly explained by nutrition (85% and 57% of variance in xylem and phloem flows, respectively) (Figure 8A,D).

Discussion

In this study, we used five Arabidopsis accessions that responded differently to N supply, reflecting different strategies to cope with N availability, in order to determine the natural variability of the metabolite composition of the saps under two conditions of nitrogen supply and to assess whether Arabidopsis has developed adaptive transport strategies to cope with nitrogen excess or deficit. We analyzed the metabolite contents in both phloem and xylem saps. We also determined the phloem exudation rate for each metabolite to reflect phloem sugar export and flow of metabolites (De Marco et al., 2021; Xu and Liesche, 2021).

Phloem and xylem sap metabolite composition in contrasting N supply

In response to HN, all accessions showed higher transport of organic nitrogen by the phloem and the xylem compared to LN, with higher values in amino acids proportions both in the phloem and xylem, higher concentration of amino acids in the xylem saps, and higher phloem exudation rate of amino acids, except for Col-0 and Cvi-0 (Figure 4 A,D). In the phloem exudates, variations in N supply were associated with slight variations in the proportions of Glu, Gln and Asp, the most abundant amino acids, and in the xylem sap it was

associated with more Asp in LN and more Gln in HN (Figure 9). Our findings confirm that the transport of organic N in the phloem and the xylem also reflects differences in the N assimilation and remobilization processes, which have been shown to be tightly regulated by the action of enzymes and transporters, a number of which being specifically expressed in the phloem and xylem cells (Tegeder and Masclaux-Daubresse, 2018).

By contrast, the effect of N supply on carbohydrates in phloem and xylem was small (Figure 4 B,E), revealing homeostasis of glucose concentration in the xylem sap and of sucrose content in phloem exudates (Figure 5). Interestingly there was a higher phloem exudation rate of carbohydrates under LN (Figure 4E), associated with more C transport to the roots by the phloem (Figure 8E). Plants generally respond to a shortage in mineral nutrition by allocating more resources to the organs involved in mineral acquisition (Lemoine et al., 2013), and our data are consistent with an increased root/shoot ratio under limiting N supply (Smolders and Merckx, 1992), and with more resources being used for root growth. The findings of less carbon in the xylem sap in LN further supports the hypothesis that more C is used in the roots under LN to promote root growth.

Among other interesting features of sap compositions, we not that in the xylem sap more malate, citrate and fumarate were observed in LN, consistent with reports that malate and other organic acids are used as a counter-ion for the transport of K^+ instead of NO_{3-} when N is limiting (Vitor et al., 2018). In the phloem exudates N deficiency was associated with more raffinose, while N sufficiency was associated with more Pro and GABA, consistent with more accumulation of raffinose and less accumulation of Pro in the leaves of Arabidopsis under chronic N starvation (Luo et al., 2021). As is common in plants of the 1-2a type of apoplasmic loaders, Arabidopsis can synthesize and translocate small amount of raffinose and galactinol by the phloem (Haritatos et al., 2000), but their synthesis in the phloem, and subsequent translocation, is still poorly understood.

Natural variations in phloem and xylem transport

The differential behaviors in the Arabidopsis accessions may reflect adaptive responses in N uptake and N assimilation under contrasting conditions, and could also reflect differences in the strategies of allocation of C and N throughout the plant. A higher efficiency of N remobilization under low N has been described in Arabidopsis accessions (Chietera et al., 2018; Masclaux-Daubresse and Chardon, 2011), implicating both variations in N metabolism in mature leaves and N transport via the phloem. Such a response increases resource allocations to roots to promote soil colonization (Ikram et al., 2012) or to reproductive organs to produce more seeds (Masclaux-Daubresse and Chardon, 2011). Our findings confirm that N nutrition has a strong effect on the amounts of amino acids that are transported *via* the phloem and provide additional evidence on the effect of N nutrition on the translocation of amino acids *via* the xylem. Hence, the translocation of Gln, Pro, Asn, Ala and GABA mostly depended on the N supply (Figure 6). Interestingly, our findings provide further evidence of a genotypic variability for the translocation of Asp, Lys, Try, Phe and Arg, *via* phloem and xylem, with a high GxN effect for the phloem Asp content (Figure 6). This demonstrates intraspecific variability for several abundant molecules that transport assimilated N, in both phloem and xylem. These observations, as well as other reports demonstrating that other amino acids predominate in phloem saps, for example Ala in *Zea mays* or Pro in *Citrus sinensis* (Hijaz and Killiny, 2014; Yesbergenova-Cuny et al., 2016), revealing an interspecific variability, show the plasticity of the mechanisms involved in the long distance transport of assimilated N. Further work is necessary to determine whether they also contribute to adaptive processes.

We also provided evidence that more C is allocated from shoot-to-root in N limitation than in N excess, but this property is highly dependent on genotypes (I.e. intraspecific variability). Most researchers consider that the associated higher phloem sugar concentration as a stress response (Lemoine et al., 2013; Peuke, 2010). The data indicate that when N is abundant, the accessions have different ability to regulate C allocation, which suggests an adaptive mechanism for promoting shoot biomass production instead of root growth, a strategy to enhance seed yield and improve plant fitness. This hypothesis is further supported by the observation of a significant high effect of the genotype on the variability of the C flows both *via* the phloem and the xylem (Figure 8D).

Interestingly, not all accessions showed the same responses to chronic high N supply. In Cvi-0, the remobilization of organic N by the phloem did not respond to N availability, while C allocation was lower in HN compared to LN (Figure 8B,E). We also observed in this accession compared to the others a different regulation of the transport of amino acids, carbohydrates and organic acids to N availability (Figure 4). These findings confirmed a variability among accessions to take advantage of high N supply for growth, which is related to the regulation of the phloem and xylem transport. The accessions with high seed yield and biomass in HN, i.e. Bur-0, Ct-1 and Edi-0, had the highest exudation rate of Gln, Asn and Ala, which suggests a more efficient remobilization of N. But these three accessions also showed the highest sucrose exudation rates, indicating more efficient phloem mass flow. We can conclude that the more efficient growth of these accessions under HN could be due to a combination of more active N remobilization *via* the phloem and maintaining phloem flow, driven by higher sugar loading or unloading.

These findings suggest variations in the regulation of phloem transport combined with variations in N metabolism. Sugar loading and unloading depends on the activity of sugar transporters, belonging to the SUC2/SUT1 family (Milne et al., 2018; Zhang and Turgeon, 2018), with the SUC2 sucrose transporter being a key regulator of sugar export in Arabidopsis. The transcription of these transporters has been shown to be regulated under stress conditions (Xu et al., 2018). Furthermore, recent reports showed that the activity of SUC transporters is also regulated at post-translational levels (Xu and Liesche, 2021). Our observation in Arabidopsis of a genetic variability in the export of sugars by the phloem in non-limiting N supply should provide new approaches to investigate the processes involved in the regulation of sugar export.

Association between sugar transport, respiratory metabolism and the export of organic acids

Quite surprisingly, the transport of organic acids in the phloem and xylem has so far been little studied, while a considerable effort has been made on the transport of sugars and amino acids (van Bel, 2021), and information about the molecular mechanisms of long-distance transport of organic acids is still limited. We also know little about the interplay between sugar transport respiratory metabolism, including the TCA cycle and glycolysis, and the export of organic acids. Recently we have shown in antisense lines of tomato deregulated for the expression of the sucrose transporters SUT1 and SUT2 a modification of organic acid contents in the phloem exudates, and in particular of the dicarboxylic acids glycolate and glyoxylate (De Marco et al., 2021). The same was observed in tomato plants infected by the Stolbur phytoplasma (De Marco et al., 2021), which indicates that an impairment of phloem sugar transport triggers metabolic changes that in turn leads to downstream changes in the export of organic acids.

Here, we investigated both the organic acid contents of the xylem saps and phloem exudates in two N nutritional conditions and also the natural variation of these compounds. Interestingly, in the xylem sap the malate and fumarate concentrations were positively correlated to sucrose, raffinose and Asp concentrations, in contrast to succinate that was not correlated to them (Figure 7). In the phloem exudates, there was a negative correlation between malate and fumarate and Gln and Asn. There was no correlation between these amino acids and succinate (Figure 7). Interestingly, there were differential effects of genotype, nutrition or the GxN interaction for citrate, succinate, malate and fumarate contents in the xylem saps and phloem exudates (Figure 6).

These findings revealed that the different accessions cope with N availability by exporting in the phloem different combinations of organic acids, even if there is little variation in the overall exudation rate of organic acids (Figure 4F). We observed in Cvi-0 the highest concentrations of malate, citrate and fumarate in the xylem sap (twice as much as in the other accessions) in HN, with more root-to-shoot transport of organic acids (Figure 4C), which could contribute to an unbalance in C allocation and changes in the root primary metabolism. Because nutrition explains most of the variability of malate content both in the xylem and phloem exudates, the export of this compound is likely coupled to N metabolism. By contrast, the high effect of the genotype on the variability of succinate and fumarate both in the xylem and phloem exudates suggests that the capacity to export these organic acids could be adaptive. Our study highlighted the unexpected variability in the translocation of organic acids in response to N availability, suggesting that both phloem sugar transport and respiratory metabolism participate to the adaptive response to mineral nutrition. Future studies concerning the coupling of sugar export and TCA organic acid transport and metabolic regulation of sugar export *via* vascular cells, will likely result in new insights into the control of organic acids export in both phloem and xylem, and carbon allocation in general.

Revealing the natural variability of mobile signal molecules

The non-protein amino acid pipecolic acid (Pip), a lysine catabolite, is an essential component of systemic acquired resistance (SAR), priming resistance in *Arabidopsis thaliana* against (hemi)biotrophic pathogens (Vlot et al., 2021). Pip is a mobile long-distance signal moving either in the xylem (Abeysekara et al., 2016) or in the phloem (Návarová et al., 2013). Interestingly we detected Pip in both xylem saps and phloem exudates. Our analysis demonstrated a high natural variability in the accumulation of pipecolate in both exudates, with a strong effect of nutrition, genotype and a combined effect GxN. Meanwhile, Pip accumulation in the xylem sap was highly correlated to the accumulation of the most abundant amino acids (Gln, Glu, Asn and Asp), but did not occur in the phloem exudates. This suggests that there is a major difference between the regulation of Pip synthesis in shoot and roots. Pip is a strong inducer of plant immunity by priming SAR. Variability in the steady state level of Pip depending on the nutrition or the genotype may well affect the susceptibility to plant pathogen (Fagard et al., 2014; Farjad et al., 2021, 2018; Verly et al., 2020) as well as causing natural variation in susceptibility of Arabidopsis to plant pathogens (Rigault et al., 2017).

Conclusion

Our findings show a significant intraspecific variability in carbon allocation, both in the transport of sugars and amino acids in the phloem and xylem. This variability is much greater

than that observed in N allocation. Unfortunately, how the export of sugars, especially sucrose, is coordinated with that of amino acids and organic acids remains an open question.

Supplementary Materials:

Supplementary Table 1. Mean (+/- SE) of metabolite concentrations of 66 metabolites identified in xylem sap in LN and HN.

Supplementary Table 2. Phloem metabolite exudation rate. Mean values (+/- SE) of the exudation rate (of 62 metabolites identified in phloem sap exudates in LN or HN (n=20 with 4 biological replicates per accession and 5 accessions, for each condition).

Supplementary Table 3. Phloem exudate metabolite content. Mean values (+/- SE) of the content of 61 metabolites identified in phloem exudates in LN or HN

Figure S1. Net nutrition effects on the growth and yield of Arabidopsis Accessions.

Figure S2. Correlogram of Xylem metabolite concentrations

Figure S3. Correlogram of Phloem metabolite contents

Figure S4. C and organic N translocation in xylem and phloem exudates

Author Contributions: S.D. conceived and supervised the experiments. A.M. performed C and N quantification. S.D. and F.C. analyzed metabolic profiles. F.V., R.L.H and S.D. performed phenotyping of the plants. Writing and editing of the manuscript was done by S.D.., R.L.H., F.C., F.V. and C.B. All authors contributed to the corrections.

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Abbreviations

LN	Low nitrogen supply
HN	High nitrogen supply

WT	wild type
TCA	tricarboxylic acid cycle
SUC2	sucrose transporter 2

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Figure Legends

Figure 1. Xylem sap and phloem exudate metabolite profiles under contrasting N nutrition

Pie chart with mean proportions of amino acids, organic acids and carbohydrates under low nitrogen (LN) and high nitrogen (HN) conditions in the xylem sap and the phloem exudate. (**A**) Xylem sap metabolite composition under LN or HN (*n*=52) and (**B**) Phloem exudate metabolite composition under LN and HN (*n*=20). Carboh: carbohydrates. AA: amino acids. OA: organic acids. Oth.: other metabolites.

Figure 2. Proportions of carbohydrates, amino acids and organic acids in the xylem sap and phloem exudates

The pie charts show the proportions of carbohydrates (**A and D**), amino acids (**B and E**) and organic acids (**C and F**) in the xylem sap (**A-C**) and in the phloem exudates (**D-F**). They are drawn from the values of the 5 accessions in LN or HN (for xylem saps: *n*=52 for LN and *n*=46 for HN; for phloem exudates: *n*=20 for both LN and HN). Only metabolites accounting for at least 2% of the content are reported on the charts. LN: low nitrogen nutrition (1 mM), HN: high nitrogen nutrition (10 mM).

Figure 3. Variability of the composition of the xylem sap and phloem exudate

A and B: Stacked bar graph showing for each accession the proportions of amino acids, organic acids and carbohydrates under low nitrogen (LN) and high nitrogen (HN) in the xylem sap (**A**) and the phloem exudate (**B**). C and D: Graph showing for each accession in LN and HN the mean cumulative concentration (+/- SD) of amino acids, organic acids and carbohydrates in the xylem saps (**C**) and the mean cumulative rate of exudation (+/- SD) of amino acids, organic acids and carbohydrates the phloem exudates (**D).** For xylem saps, *n*= 8-12 and for phloem exudates, *n*=4. **C and D**: Above each bar: result of a Tukey HSD post-hoc test (blue letters). Above each graph: results of two-way ANOVA for the contribution of genotype (G), the N nutrition (N) and their interaction (GxN) on the variance, with ns: not significant, * for $p < 0.05$, ** for $p < 0.01$ and *** for $p < 0.001$.

Figure 4. Variability of amino acids, carbohydrates and organic acids in xylem sap and phloem exudation rates

Boxes and whisker plots of amino acids, carbohydrates and organic acids concentrations in the xylem saps (**A-C**) and phloem exudation rates (**D-F**) under low nitrogen (LN) or high nitrogen (HN). In **A-C,** concentrations are expressed in mM and in D-F, exudation rates are expressed in $\mu M.g^{-1}$ FW. hr^{-1}. The black lines inside represent the medians; the top and bottom ends of the boxes represent the first and third quartiles, respectively; and the whisker extremities represent the maximum and minimum data points. For xylem saps, *n*= 8-12 and for phloem exudates, *n*=4. Above each panel: results of the two-way ANOVA for the contribution of genotype (G), the nutrition (N) and their interaction (GxN) on the variance, with ns: not significant, * for $p < 0.05$, ** for $p < 0.01$ and *** for $p < 0.001$. Above each box is indicated the result of a Tukey HSD post-hoc Test. FW: fresh weight.

Figure 5. Heat map of the fold-changes in the metabolite contents in phloem and xylem exudates

Inserted heat maps represent the fold changes in HN compared to LN for each metabolite. The five squares from left to right represent fold changes in Bur-0, Col-0, Ct-1, Cvi-0 and Edi-0 groups, respectively, with phloem exudate values in the first line and xylem exudate values in the second. The lower and upper limits of the color range of heat maps was -3 (in yellow) and +3 (in blue), with yellow colors indicating a lower content under HN, blue

colors indicating a higher content and white color indicating no significant variations (based on a *T*-test). Normalized log_2 values were used for phloem exudates and log_2 concentrations were used for xylem saps.

Figure 6. Heat map of the effects of the genotype (G), nutrition (N) and interaction (GxN) on metabolite contents

Sap contents are represented on a blue or orange scale, respectively for phloem and xylem exudates, with effects due to G, N and GxN, respectively, shown from left to right. Effects on phloem content are on upper bar and effects on xylem concentration are on lower bar. Heat map colors represent the sum of the squares calculated by a 2-way ANOVA for each effect, shown as the percentage of the total variance. Crossed out boxes : metabolite for which the ANOVA was not determined. The stronger the color, the stronger the effect for each factor (see legend panel). The lower and upper limits of the color range of heat maps were 0 % (in white) and 100% (in blue for phloem and orange for xylem exudates), with 0% corresponding to non-significant effects.

Figure 7. Correlations between metabolites in the xylem saps and phloem exudates

(A) Pearson correlation between malate, citrate, fumarate and succinate concentrations in the xylem sap with that of other abundant metabolites in the xylem sap and between malate, citrate, fumarate et succinate contents and that of selected metabolites in the phloem exudates. (**B**): Pearson correlation between pipecolate concentration in the xylem sap and that of other abundant metabolites in the xylem sap and between pipecolate content and that of selected abundant metabolites in the phloem exudates. *: adjusted *p*-values (Holm's method). In grey background: negative correlations.

Figure 8. Effect of the nutrition on C and N flows

(**A,D**): Two-way ANOVA of estimated organic N (**G**) and C translocation (**H**) in xylem and phloem exudates. The analysis was done on the raw metabolite data for xylem concentrations and phloem exudation rate. The percentage of variance (based on the sum of squares) due to genotype (G), nutrition (N), genotype by nutrition interaction (GxN) and residual (res) are represented in white, light grey, dark grey and black, respectively. Significant percentages are indicated in bold letters., with *** for $p < 0.001$. **(B, C, E, F and H)**: The histograms show for each trait the Net Nutrition Effect (NNE). For each trait, NNE is the difference between the mean values in HN and LN, with positive values indicating a gain due to HN and negative values indicating a loss. **B**: Translocated organic N by xylem or phloem, **C**: Stored organic N in leaves and seeds, **E**: Translocated C by xylem or phloem, **F**: Stored C in leaves and seeds, **G**: Shoot Biomass at harvest time and, Seed yield. Next to each bar, stars in blue indicate the result of a *t-test* with * indicating significant differences when comparing HN and LN ($p < 0.05$).

Figure 9. Schematic representation of metabolite transport in phloem and xylem under contrasting N nutrition

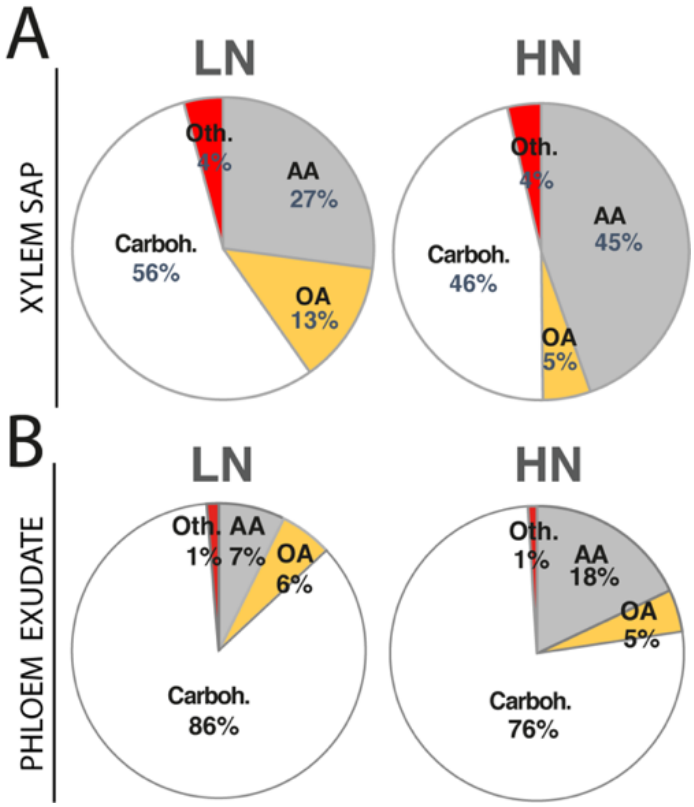
In **A** (LN) and **B** (HN), the phloem is represented with blue-green cell walls and xylem with yellow cell walls. The directions of the sap flows are arbitrary. Sugars are reported in black, amino acids in red and organic acids in blue. Letters size indicate the relative abundance of the main components under each condition. Mal: malate. Fum: fumarate. Cit: citrate.

Tables and Figures

Table 1. Correlations between phloem metabolite contents or exudation rates with seed yield and biomass

(n=10, using for each trait the mean value calculated for each genotype and each condition). Adjusted p-values (Holm's method): * p <0.05, ** p <0.01 and *** p < 0.001.

Pearson correlation (r)	Seed yield		Biomass	
Phloem content				
Alanine	0.838	*	0.915	**
Asparagine	0.878	*	0.916	**
Aspartate	0.591	ns	0.680	ns
GABA	0.971	ns	0.809	ns
Glutamine	0.937	**	0.946	**
Phloem exudation rate				
Alanine	0.906	**	0.903	**
Asparagine	0.950	***	0.891	*
Aspartate	0.890	*	0.889	*
GABA	0.941	**	0.950	**
Glutamine	0.948	**	0.918	**



894

895 **Figure 1. Xylem sap and phloem exudate metabolite profiles under contrasting N**
896 **nutrition**

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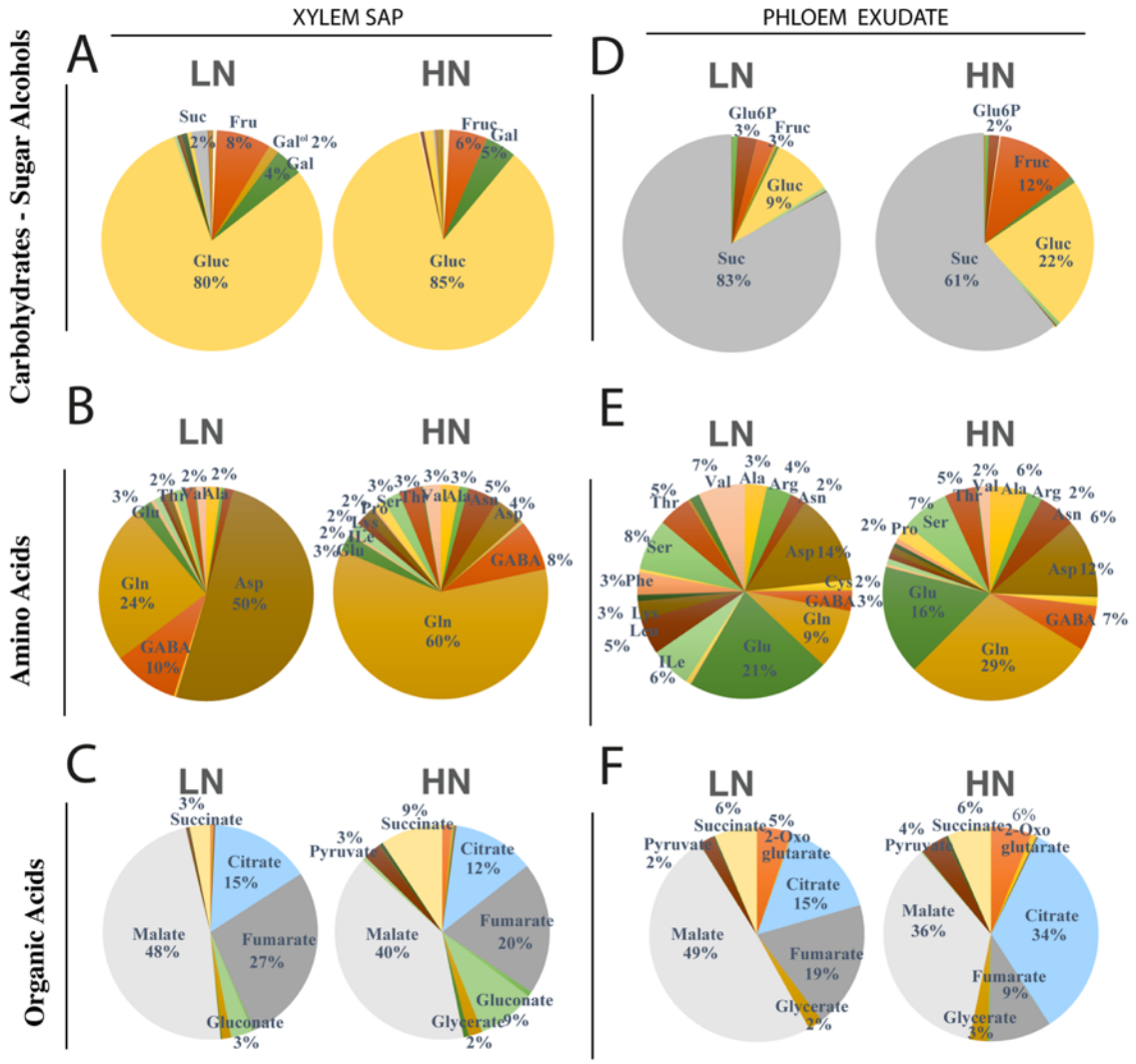

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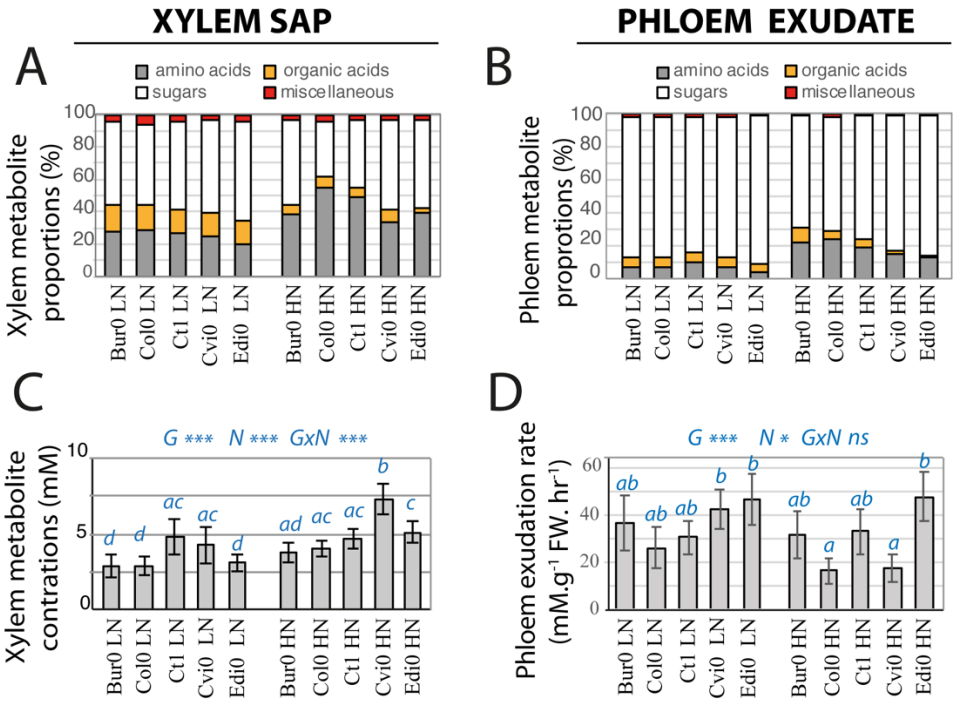

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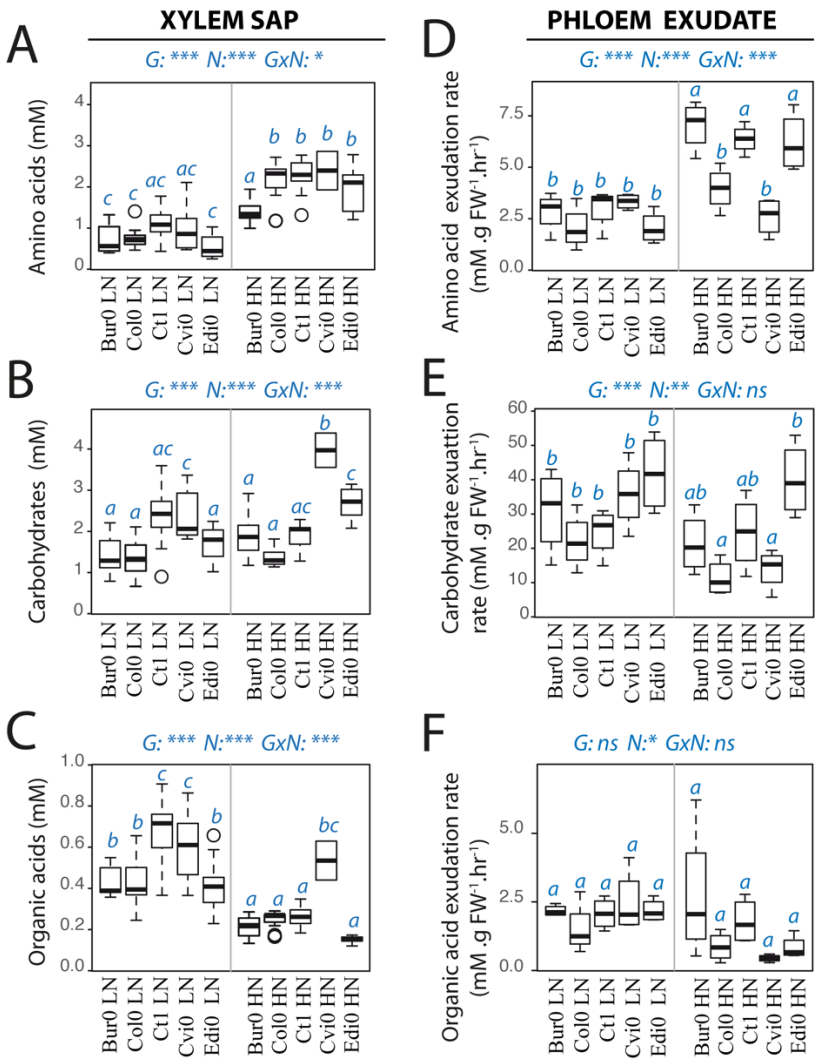

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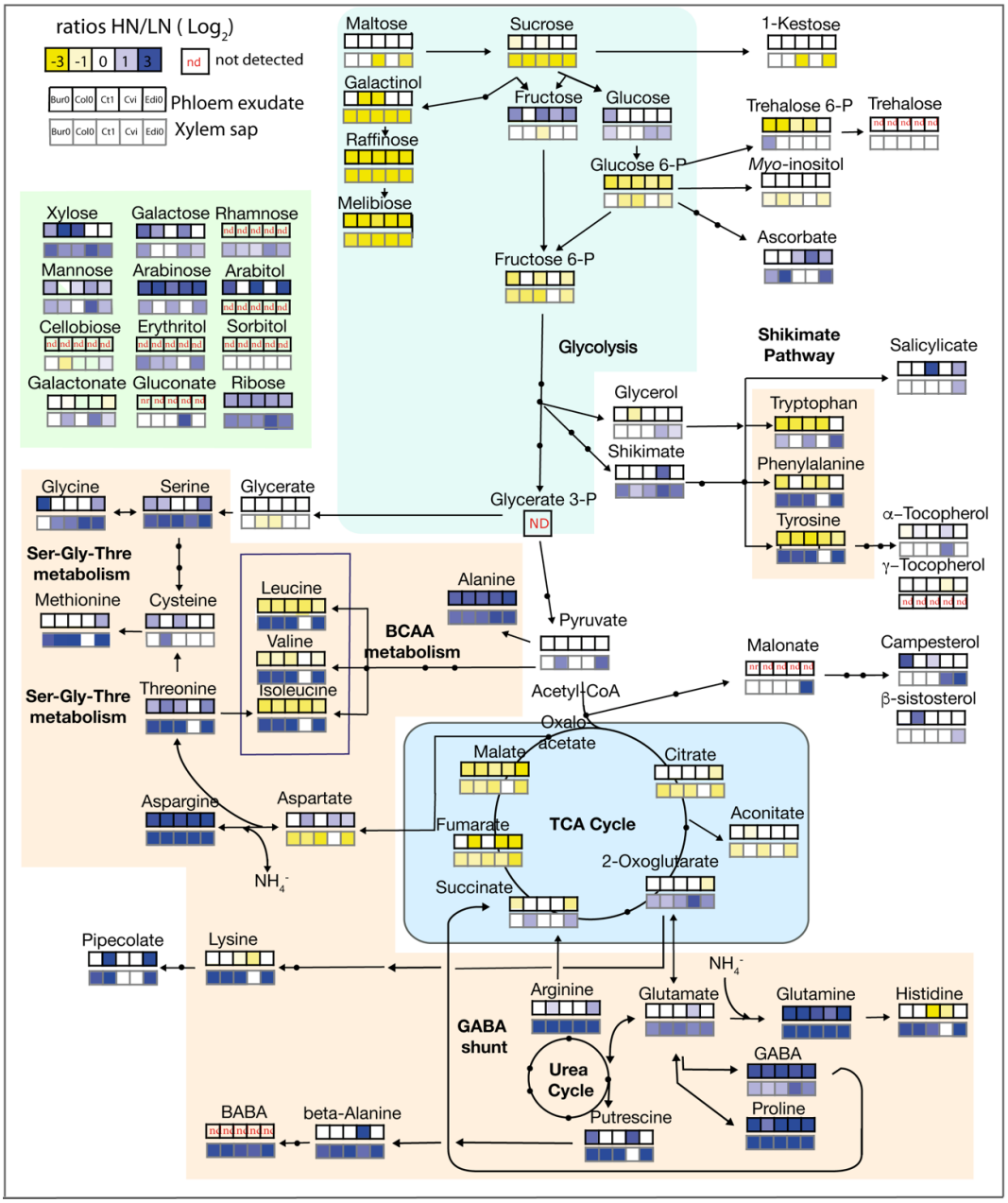

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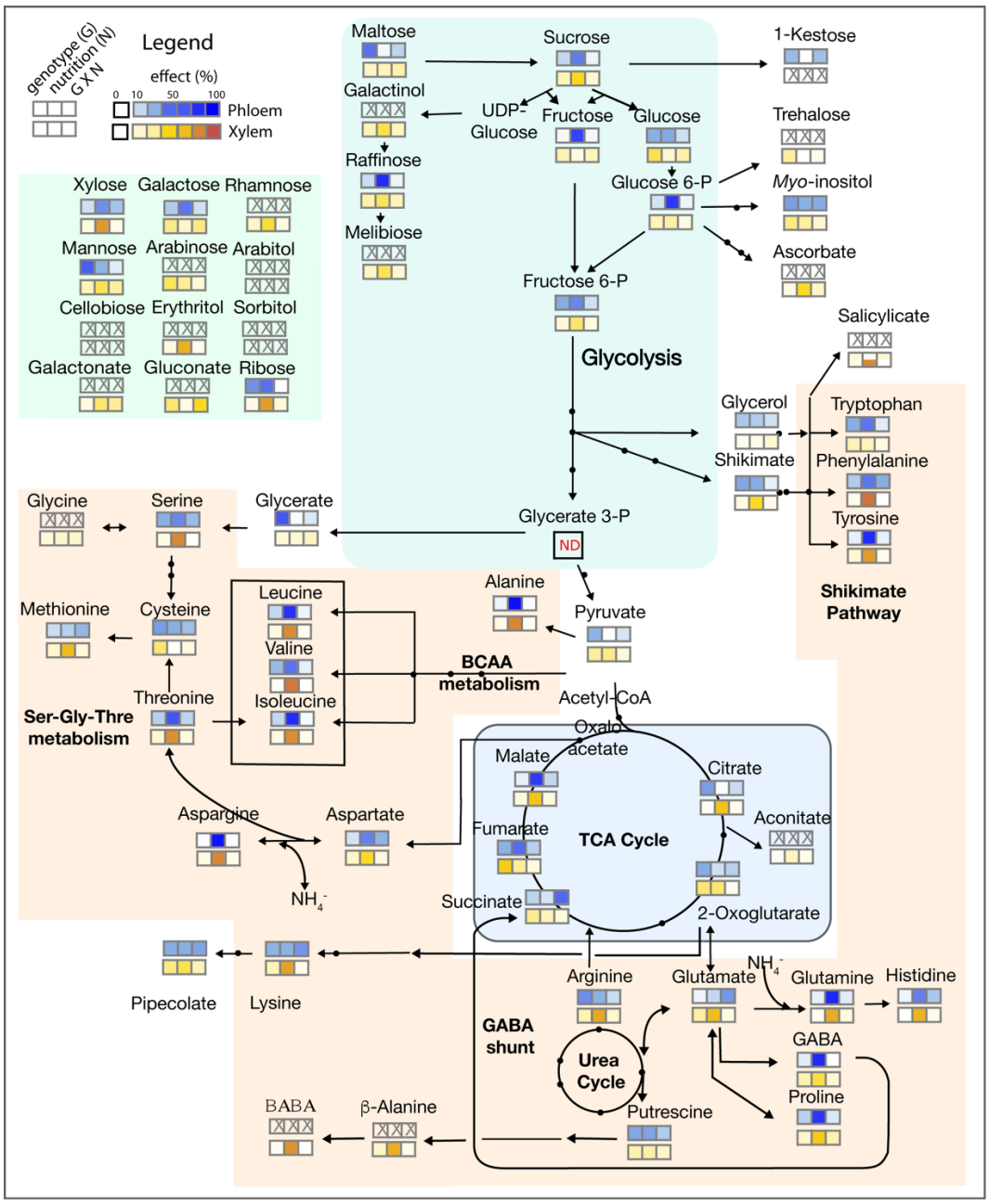
Inserted heat maps represent the fold changes in HN compared to LN for each metabolite. The five squares from left to right represent fold changes in Bur-0, Col-0, Ct-1, Cvi-0 and Edi-0 groups, respectively, with phloem exudate values in the first line and xylem exudate values in the second. The lower and upper limits of the color range of heat maps was -3 (in yellow) and +3 (in blue), with yellow colors indicating a lower content under HN, blue colors indicating a higher content and white color indicating no significant variations (based on a *T*-test). Normalized $\log_2$ values were used for phloem exudates and $\log_2$ concentrations were used for xylem saps.



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974

A

Pearson Correlation (*r*)		Xylem (*n*=98)	Phloem (*n*=40)
Abundand amino acids			
Asparagine	Glutamine	0.9363 (***)	0.9519 (***)
Aspartate	Asparagine	-0.5673 (***)	0.671 (***)
Aspartate	Glutamate	-0.4791 (***)	0.671 (***)
TCA Organic acids and abundant Amino Acids			
Fumarate	Asparagine	-0.389 (**)	-0.7496 (***)
Fumarate	Aspartate	0.6104 (***)	-0.8027 (***)
Fumarate	Glutamine	-0.327 (*)	-0.6382 (***)
Fumarate	Glutamate	-0.3407 (*)	-0.6655 (***)
Malate	Asparagine	-0.669 (***)	-0.9152 (***)
Malate	Aspartate	0.7553 (***)	-0.7265 (***)
Malate	Glutamine	-0.6397 (***)	-0.912 (***)
Malate	Glutamate	-0.5144 (***)	-0.4225 (ns)
Succinate	Asparagine	0.5062 (***)	-0.2563 (ns)
Succinate	Aspartate	-0.1 (ns)	0.1504 (ns)
Succinate	Glutamine	0.4585 (***)	-0.3654 (ns)
Succinate	Glutamate	0.6072 (***)	0.0828 (ns)
TCA Organic acids and sucrose and raffinose			
Fumarate	Sucrose	0.4568 (***)	0.1772 (ns)
Fumarate	Raffinose	0.6213 (***)	0.7995 (***)
Malate	Sucrose	0.6382 (***)	0.5039 (ns)
Malate	Raffinose	0.7236 (***)	0.825 (***)
Succinate	Sucrose	-0.1349 (ns)	0.1852 (ns)
Succinate	Raffinose	-0.1418 (ns)	0.1031 (ns)

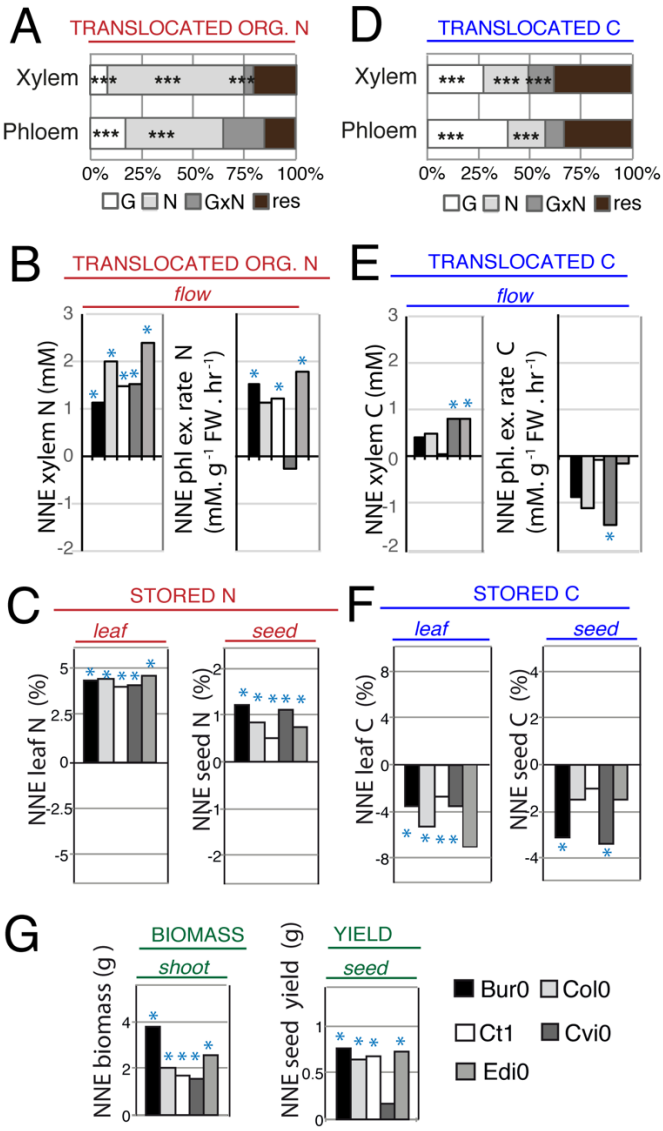
B

Pearson Correlation (*r*)		Xylem (*n*=98)	Phloem (*n*=40)
Pipecolate and abundant amino acids			
Pipecolate	Asparagine	0.4524 (***)	0.4716 (ns)
Pipecolate	Glutamine	0.4107 (**)	0.4513 (ns)
Pipecolate	Aspartate	-0.3512 (*)	0.6082 (**)
Pipecolate	Glutamate	0.5745 (***)	0.3209 (ns)
Pipecolate and TCA Organic acids			
Pipecolate	Citrate	-0.4078 (**)	-0.2116 (ns)
Pipecolate	Fumarate	-0.2956 (ns)	-0.6868 (***)
Pipecolate	Malate	-0.382 (**)	-0.5635 (*)
Pipecolate	Succinate	0.3512 (*)	-0.0752 (ns)
Pipecolate and Sugars			
Pipecolate	Sucrose	-0.4846 (**)	-0.1146 (ns)
Pipecolate	Raffinose	-0.4158 (**)	-0.6395 (***)

975

976 **Figure 7. Correlations between metabolites in the xylem saps and phloem**
977 **exudates**

978 (**A**) Pearson correlation between malate, citrate, fumarate and succinate
979 concentrations in the xylem sap with that of other abundant metabolites in the
980 xylem sap and between malate, citrate, fumarate et succinate contents and that of
981 selected metabolites in the phloem exudates. (**B**): Pearson correlation between
982 pipecolate concentration in the xylem sap and that of other abundant metabolites
983 in the xylem sap and between pipecolate content and that of selected abundant
984 metabolites in the phloem exudates. *: adjusted *p*-values (Holm's method). In grey
985 background: negative correlations.



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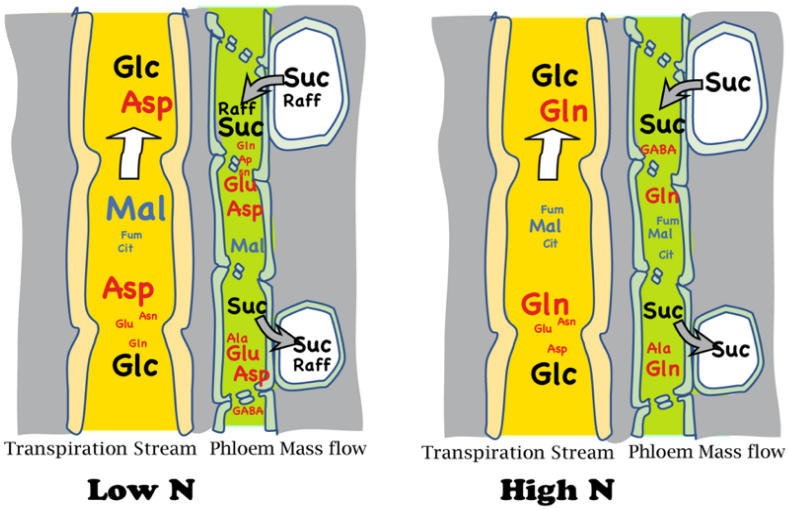
(**A,D**): Two-way ANOVA of estimated organic N (**G**) and C translocation (**H**) in xylem and phloem exudates. The analysis was done on the raw metabolite data for xylem concentrations and phloem exudation rate. The percentage of variance (based on the sum of squares) due to genotype (G), nutrition (N), genotype by nutrition interaction (GxN) and residual (res) are represented in white, light grey, dark grey and black, respectively. Significant percentages are indicated in bold letters., with *** for p <0.001. **(B, C, E, F and H)**: The histograms show for each trait the Net Nutrition Effect (NNE). For each trait, NNE is the difference between the mean values in HN and LN, with positive values indicating a gain due to HN and negative values indicating a loss. **B**: Translocated organic N by xylem or phloem, **C**: Stored organic N in leaves and seeds, **E**: Translocated C by xylem or phloem, **F**: Stored C in leaves and seeds, **G**: Shoot Biomass at harvest time and, Seed yield. Next to each bar, stars in blue indicate the result of a *t-test* with * indicating significant differences when comparing HN and LN (p <0.05).



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1014 **Supplementary Information**

1015

1016 **Supplementary Table 1.** Mean (+/- SE) of metabolite concentrations of 66 metabolites
1017 identified in xylem sap in LN and HN. (n=46 for HN and 52 for LN, with 8-12 biological
1018 replicates per accession and 5 accessions, for each condition). *T*-test: comparison of the data
1019 obtained in HN compared to LN. *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$). Carbohydrates°:
1020 sugars; sugar alcohols and phosphorylated sugars. Ratios >1 are in red, Ratios <1 in blue.

Concentration (mM) In xylem sap	LN			HN			Ratio HN/LN	*T*-test
SUM	3.5390	+/-	0.1639	4.5423	+/-	0.2323		***
AMINO ACIDS (SUM)	0.8101	+/-	0.0548	1.9788	+/-	0.1199		***
Alanine	0.0178	+/-	0.0011	0.0566	+/-	0.0016	3.18	***
Arginine	0.0043	+/-	0.0006	0.0260	+/-	0.0016	6.09	***
Asparagine	0.0108	+/-	0.0014	0.0927	+/-	0.0042	8.59	***
Aspartate	0.4079	+/-	0.0342	0.0888	+/-	0.0052	0.22	***
b-aminoisobutyrate	0.0002	+/-	0.0000	0.0006	+/-	0.0000	3.81	***
beta-Alanine	0.0015	+/-	0.0002	0.0053	+/-	0.0002	3.64	***
Cysteine	0.0025	+/-	0.0004	0.0027	+/-	0.0003	1.08	ns
GABA	0.0797	+/-	0.0045	0.1509	+/-	0.0077	1.89	***
Glutamine	0.2262	+/-	0.0402	1.1819	+/-	0.0661	5.22	***
Glutamate	0.0203	+/-	0.0012	0.0523	+/-	0.0026	2.58	***
Glycine	0.0038	+/-	0.0006	0.0066	+/-	0.0004	1.75	***
Histidine	0.0005	+/-	0.0001	0.0014	+/-	0.0001	2.80	***
Isoleucine	0.0082	+/-	0.0016	0.0338	+/-	0.0014	4.11	***
Leucine	0.0052	+/-	0.0010	0.0245	+/-	0.0010	4.72	***
Lysine	0.0078	+/-	0.0014	0.0331	+/-	0.0018	4.27	***
Methionine	0.0003	+/-	0.0000	0.0018	+/-	0.0001	5.18	***
Phenylalanine	0.0019	+/-	0.0002	0.0064	+/-	0.0002	3.40	***
Proline	0.0030	+/-	0.0003	0.0337	+/-	0.0031	11.30	***
Serine	0.0120	+/-	0.0010	0.0508	+/-	0.0016	4.23	***
Threonine	0.0124	+/-	0.0015	0.0668	+/-	0.0028	5.39	***
Tryptophan	0.0010	+/-	0.0003	0.0012	+/-	0.0001	1.14	ns
Tyrosine	0.0014	+/-	0.0002	0.0047	+/-	0.0002	3.40	***
Valine	0.0123	+/-	0.0018	0.0528	+/-	0.0015	4.28	***
CARBOHYDRATES ° (SUM)	1.8211	+/-	0.0939	2.0634	+/-	0.1588		ns
Fructose-6-P	0.0007	+/-	0.0001	0.0002	+/-	0.0000	0.34	***
Glucose-6-P	0.0020	+/-	0.0002	0.0010	+/-	0.0001	0.47	***
Ribose-5-P	0.0000	+/-	0.0000	0.0000	+/-	0.0000	1.28	ns
Trehalose-6-P	0.0000	+/-	0.0000	0.0000	+/-	0.0000	1.24	ns
Arabinose	0.0101	+/-	0.0008	0.0147	+/-	0.0007	1.46	***
Cellobiose	0.0001	+/-	0.0000	0.0001	+/-	0.0000	0.75	ns
Fructose	0.1454	+/-	0.0088	0.1136	+/-	0.0054	0.78	***
Galactinol	0.0278	+/-	0.0033	0.0011	+/-	0.0002	0.04	***
Galactose	0.0766	+/-	0.0045	0.0950	+/-	0.0034	1.24	***
Glucose	1.4563	+/-	0.0791	1.7617	+/-	0.0966	1.21	*
Kestose-1	0.0001	+/-	0.0000	0.0000	+/-		0.03	
Maltose	0.0086	+/-	0.0007	0.0043	+/-	0.0008	0.49	***
Mannose	0.0062	+/-	0.0003	0.0098	+/-	0.0004	1.58	***
Melibiose	0.0056	+/-	0.0006	0.0005	+/-	0.0000	0.10	***
Raffinose	0.0153	+/-	0.0020	0.0006	+/-	0.0001	0.04	***
Rhamnose	0.0005	+/-	0.0000	0.0008	+/-	0.0000	1.59	***
Ribose	0.0113	+/-	0.0005	0.0278	+/-	0.0011	2.46	***
Sucrose	0.0430	+/-	0.0037	0.0064	+/-	0.0007	0.15	***

Trehalose	0.0033	+/-	0.0005	0.0029	+/-	0.0002	0.88	ns
Xylose	0.0089	+/-	0.0005	0.0218	+/-	0.0007	2.44	***
Sorbitol	0.0007	+/-	0.0001	0.0040	+/-	0.0014	6.00	*
ORGANIC ACIDS (SUM)	0.5037	+/-	0.0235	0.2317	+/-	0.0200		***
2-Oxoglutarate	0.0018	+/-	0.0002	0.0035	+/-	0.0002	1.92	***
Aconitate	0.0007	+/-	0.0001	0.0004	+/-	0.0000	0.53	***
Ascorbate	0.0002	+/-	0.0000	0.0006	+/-	0.0000	2.76	***
Citramalate	0.0012	+/-	0.0001	0.0005	+/-	0.0000	0.44	***
Citrate	0.0765	+/-	0.0039	0.0286	+/-	0.0014	0.37	***
Fumarate	0.1383	+/-	0.0123	0.0463	+/-	0.0051	0.33	***
Galactonate	0.0012	+/-	0.0000	0.0017	+/-	0.0001	1.43	***
Gluconate	0.0166	+/-	0.0013	0.0203	+/-	0.0030	1.23	ns
Glycerate	0.0071	+/-	0.0007	0.0048	+/-	0.0002	0.68	***
Glycolate	0.0013	+/-	0.0002	0.0022	+/-	0.0005	1.64	ns
Malate	0.2408	+/-	0.0110	0.0925	+/-	0.0053	0.38	***
Malonate	0.0001	+/-	0.0001	0.0006	+/-	0.0003	4.91	ns
Pipecolate	0.0004	+/-	0.0001	0.0014	+/-	0.0001	3.31	***
Pyruvate	0.0021	+/-	0.0002	0.0063	+/-	0.0007	3.00	***
Salicylate	0.0003	+/-	0.0000	0.0005	+/-	0.0001	1.40	ns
Shikimate	0.0006	+/-	0.0001	0.0013	+/-	0.0000	2.01	***
Succinate	0.0156	+/-	0.0010	0.0217	+/-	0.0014	1.39	***
MISCELLANEOUS (SUM)	0.1449	+/-	0.0069	0.1642	+/-	0.0086		*
Erythritol	0.0002	+/-	0.0000	0.0004	+/-	0.0000	2.05	***
Ethanolamine	0.0170	+/-	0.0008	0.0355	+/-	0.0011	2.09	***
Glycerol	0.0637	+/-	0.0027	0.0718	+/-	0.0027	1.13	*
myo-Inositol	0.0579	+/-	0.0040	0.0356	+/-	0.0020	0.62	***
Putrescine	0.0063	+/-	0.0018	0.0188	+/-	0.0027	3.00	***

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Supplementary Table 2. Phloem metabolite exudation rate. Mean values (+/- SE) of the exudation rate of 62 metabolites identified in phloem sap exudates in LN or HN (n=20 with 4 biological replicates per accession and 5 accessions, for each condition). A T-test was realized to determine the effect of the nutrition. (*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$). Carbohydrates°: sugars; sugar alcohols and phosphorylated sugars. Traces: values close to the limit of detection, detected occasionally. Ratios >1, in red background, correspond to metabolites that exudate more under HN than LN, Ratios <1, in blue background, correspond to metabolites that exudate less under HN than LN.

Exudation rate (µM.g^{-1} FW. hr^{-1})	LN			HN			Ratio HN/LN	*T*-test
SUM	36.3854	+/-	2.6832	29.1366	+/-	3.1904	0.80	ns
AMINO ACIDS (SUM)	2.6573	+/-	0.2049	5.2350	+/-	0.4300	1.97	***
Alanine	0.0886	+/-	0.0072	0.2966	+/-	0.0289	3.35	***
Arginine	0.1002	+/-	0.0099	0.1222	+/-	0.0142	1.22	ns
Asparagine	0.0622	+/-	0.0058	0.2936	+/-	0.0230	4.72	***
Aspartate	0.3671	+/-	0.0327	0.6196	+/-	0.0440	1.69	***
β-Alanine	traces			0.0047	+/-	0.0005		***
Cysteine	0.0481	+/-	0.0037	0.0727	+/-	0.0127	1.51	ns
GABA	0.0807	+/-	0.0083	0.3567	+/-	0.0556	4.42	***
Glutamine	0.2411	+/-	0.0371	1.5044	+/-	0.1359	6.24	***
Glutamate	0.5677	+/-	0.0450	0.8637	+/-	0.0622	1.52	***
Glycine	0.0094	+/-	0.0039	0.0137	+/-	0.0022	1.46	**
Histidine	0.0192	+/-	0.0023	0.0078	+/-	0.0009	0.40	***
Isoleucine	0.1640	+/-	0.0120	0.0462	+/-	0.0045	0.28	***
Leucine	0.1431	+/-	0.0116	0.0458	+/-	0.0051	0.32	***
Lysine	0.0736	+/-	0.0067	0.0538	+/-	0.0065	0.73	ns
Methionine	0.0255	+/-	0.0023	0.0326	+/-	0.0034	1.28	ns
Phenylalanine	0.0909	+/-	0.0098	0.0402	+/-	0.0047	0.44	***
Proline	0.0130	+/-	0.0015	0.1270	+/-	0.0229	9.79	***
Serine	0.2051	+/-	0.0189	0.3745	+/-	0.0452	1.83	**
Threonine	0.1312	+/-	0.0107	0.2455	+/-	0.0237	1.87	***
Tryptophan	0.0117	+/-	0.0013	0.0037	+/-	0.0007	0.31	***
Tyrosine	0.0390	+/-	0.0038	0.0112	+/-	0.0014	0.29	***
Valine	0.1865	+/-	0.0155	0.0989	+/-	0.0093	0.53	***
CARBOHYDRATES ° (SUM)	31.1270	+/-	2.5061	22.2402	+/-	2.8041	0.71	*
Arabitol	0.0017	+/-	0.0004	0.0138	+/-	0.0024	7.94	***
Fructose-6-P	0.2763	+/-	0.0304	0.1171	+/-	0.0112	0.42	***
Glucose-6-P	0.8935	+/-	0.0966	0.3525	+/-	0.0308	0.39	***
Glycerol-3-P	0.0009	+/-	0.0001	0.0013	+/-	0.0001	1.43	*
myo-Inositol-1-P	0.0014	+/-	0.0002	0.0007	+/-	0.0001	0.50	ns
Sucrose-6-P	0.0026	+/-	0.0003	0.0009	+/-	0.0001	0.36	*
Trehalose-6-P	0.0028	+/-	0.0005	0.0004	+/-	0.0001	0.14	***
Arabinose	0.0094	+/-	0.0008	0.0438	+/-	0.0068	4.65	***
Fructose	0.8034	+/-	0.0936	2.6591	+/-	0.3385	3.31	***
Galactinol	0.1501	+/-	0.0285	traces				***
Galactose	0.1198	+/-	0.0123	0.2489	+/-	0.0418	2.08	**
Glucose	2.7691	+/-	0.2765	4.9926	+/-	0.5982	1.80	**
Kestose-1	0.0007	+/-	0.0001	0.0006	+/-	0.0001	0.79	ns
Maltose	0.1760	+/-	0.0284	0.1399	+/-	0.0294	0.80	ns
Mannose	0.0185	+/-	0.0024	0.0267	+/-	0.0029	1.44	*
Melibiose	0.0076	+/-	0.0010	0.0014	+/-	0.0006	0.18	***
Raffinose	0.0889	+/-	0.0128	0.0068	+/-	0.0034	0.08	***
Ribose	0.0243	+/-	0.0031	0.0458	+/-	0.0049	1.88	***

Sucrose	25.7894	+/-	2.3708	13.5407	+/-	2.2242	0.53	***
Xylose	0.0138	+/-	0.0036	0.0431	+/-	0.0078	3.13	**
Xylulose	0.0006	+/-	0.0001	0.0006	+/-	0.0000	1.09	ns
ORGANIC ACIDS (SUM)	2.1095	+/-	0.1595	1.3592	+/-	0.2961	0.64	*
2-Oxoglutarate	0.1095	+/-	0.0111	0.0831	+/-	0.0134	0.76	ns
Aconitate	0.0044	+/-	0.0008	0.0115	+/-	0.0032	2.61	*
Ascorbate	0.0013	+/-	0.0001	0.0022	+/-	0.0002	1.73	***
Citramalate	traces			0.0035	+/-	0.0007		***
Citrate	0.3205	+/-	0.0413	0.4564	+/-	0.1313	1.42	ns
Erythronate	0.0001	+/-	0.0000	0.0001	+/-	0.0000	0.86	ns
Fumarate	0.4031	+/-	0.0548	0.1261	+/-	0.0360	0.31	***
Galactonate	traces			0.0064	+/-	0.0005		***
Glycerate	0.0461	+/-	0.0047	0.0366	+/-	0.0029	0.79	ns
Malate	1.0346	+/-	0.0868	0.4809	+/-	0.1076	0.46	***
Pipecolate	0.0019	+/-	0.0005	0.0030	+/-	0.0005	1.55	ns
Pyruvate	0.0466	+/-	0.0102	0.0559	+/-	0.0119	1.20	ns
Salicylate	0.0034	+/-	0.0011	0.0019	+/-	0.0003	0.56	ns
Shikimate	0.0045	+/-	0.0007	0.0061	+/-	0.0006	1.36	*
Succinate	0.1342	+/-	0.0125	0.0875	+/-	0.0069	0.65	**
MISCELLANEOUS (SUM)	0.4915	+/-	0.0639	0.3022	+/-	0.0289	0.61	*
α-Tocopherol	0.0004	+/-	0.0003	0.0001	+/-	0.0000	0.19	ns
Ethanolamine	0.0026	+/-	0.0007	0.0226	+/-	0.0045	8.72	***
Glycerol	0.2461	+/-	0.0399	0.1181	+/-	0.0129	0.48	**
myo-Inositol	0.2262	+/-	0.0312	0.1364	+/-	0.0134	0.60	*
Porphine	0.0066	+/-	0.0018	0.0070	+/-	0.0013	1.07	ns
Putrescine	0.0019	+/-	0.0005	0.0023	+/-	0.0003	1.22	ns
Urate	0.0001	+/-	0.0000	0.0001	+/-	0.0000	1.02	ns
Threonate	0.0011	+/-	0.0002	0.0012	+/-	0.0001	1.17	ns
β-Sitosterol	traces			0.0045	+/-	0.0013		
Campesterol	0.0003	+/-	0.0001	0.0004	+/-	0.0002	1.59	ns
Sinapinate-cis	0.0002	+/-	0.0000	0.0004	+/-	0.0001	2.38	*
Sinapinate-trans	0.0018	+/-	0.0001	0.0048	+/-	0.0011	2.68	**
Galactosylglycerol	0.0003	+/-	0.0001	0.0005	+/-	0.0002	1.60	ns
Digalactosylglycerol	0.0097	+/-	0.0010	0.0104	+/-	0.0036	1.07	ns

Supplementary Table 3. Phloem exudate metabolite content. Mean values (+/- SE) of the content of 61 metabolites identified in phloem exudates in LN or HN (n=20 with 4 biological replicates per accession and 5 accessions, for each condition). The data used for this analysis are expressed as relative units (R.U.) determined after Log_2 transformation and normalization. A *T*-test was realized to determine the effect of the nutrition (*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$). Carbohydrates°: sugars, polyols and phosphorylated sugars. Positive fold changes are in red, negative fold changes in blue.

Metabolite Content (R.U.) in phloem exudates	LN			HN			FOLD CHANGE	*T*-test HN/LN
AMINO ACIDS								
Alanine	-2.126	+/-	0.050	-0.817	+/-	0.072	1.31	***
Arginine	-2.430	+/-	0.093	-2.205	+/-	0.102	0.23	**
Asparagine	-2.611	+/-	0.098	-0.773	+/-	0.090	1.84	***
Aspartate	-0.301	+/-	0.050	0.092	+/-	0.082	0.39	***
β–Alanine	traces			-6.759	+/-	0.104		
Cysteine	-3.515	+/-	0.101	-3.158	+/-	0.124	0.36	**
GABA	-2.247	+/-	0.127	-0.735	+/-	0.106	1.51	***
Glutamine	-0.470	+/-	0.155	1.290	+/-	0.108	1.76	***
Glutamate	0.356	+/-	0.054	0.499	+/-	0.067	0.14	*
Glycine	-6.106	+/-	0.695	-5.698	+/-	0.229	0.41	ns
Histidine	-5.210	+/-	0.176	-6.229	+/-	0.180	-1.02	***
Isoleucine	-2.321	+/-	0.071	-3.553	+/-	0.114	-1.23	***
Leucine	-2.448	+/-	0.084	-3.614	+/-	0.105	-1.17	***
Lysine	-3.051	+/-	0.065	-3.454	+/-	0.118	-0.40	**
Methionine	-4.318	+/-	0.117	-4.132	+/-	0.091	0.19	*
Phenylalanine	-2.999	+/-	0.143	-3.819	+/-	0.116	-0.82	***
Proline	-4.709	+/-	0.113	-2.594	+/-	0.250	2.12	***
Serine	-1.098	+/-	0.077	-0.718	+/-	0.123	0.38	***
Threonine	-1.837	+/-	0.058	-1.185	+/-	0.108	0.65	***
Tryptophan	-6.127	+/-	0.239	-7.665	+/-	0.235	-1.54	***
Tyrosine	-4.270	+/-	0.128	-5.836	+/-	0.107	-1.57	***
Valine	-1.836	+/-	0.089	-2.410	+/-	0.091	-0.57	***
CARBOHYDRATES °								
Arabitol	-6.468	+/-	0.446	-5.303	+/-	0.180	1.16	***
Fructose-6P	-1.461	+/-	0.120	-2.181	+/-	0.170	-0.72	***
Glucose-6P	0.395	+/-	0.089	-0.803	+/-	0.129	-1.20	***
Sucrose-6P	-8.529	+/-	0.201	-8.807	+/-	0.317	-0.28	ns
Trehalose-6P	-8.579	+/-	0.263	-10.512	+/-	0.354	-1.93	***
Arabinose	-5.100	+/-	0.097	-3.867	+/-	0.097	1.23	***
Fructose	1.149	+/-	0.083	1.957	+/-	0.092	0.81	***
Galactinol	-2.380	+/-	0.220	traces				
Galactose	-2.035	+/-	0.134	-1.249	+/-	0.125	0.79	***
Glucose	2.587	+/-	0.106	2.986	+/-	0.112	0.40	**
Kestose	-9.863	+/-	0.206	-10.045	+/-	0.199	-0.18	ns
Maltose	-2.213	+/-	0.187	-2.276	+/-	0.238	-0.06	ns
Mannose	-4.884	+/-	0.143	-4.357	+/-	0.149	0.53	**
Melibiose	-6.970	+/-	0.215	-9.690	+/-	0.437	-2.72	***
Raffinose	-4.459	+/-	0.192	-8.034	+/-	0.375	-3.57	***

Ribose	-4.369	+/-	0.139	-3.533	+/-	0.112	0.84	***
Sucrose	4.984	+/-	0.040	4.691	+/-	0.059	-0.29	***
Xylose	-5.626	+/-	0.464	-4.095	+/-	0.182	1.53	***
ORGANIC ACIDS								
2.Oxoglutarate	-2.674	+/-	0.107	-2.801	+/-	0.115	-0.13	ns
Aconitate	-6.888	+/-	0.245	-6.472	+/-	0.272	0.42	*
Ascorbate	-8.311	+/-	0.064	-7.754	+/-	0.127	0.56	**
Citramalate	traces			-7.781	+/-	0.126		
Citrate	-0.737	+/-	0.177	-0.998	+/-	0.216	-0.26	ns
Fumarate	-1.488	+/-	0.191	-2.989	+/-	0.307	-1.50	***
Galactonate	traces			-6.902	+/-	0.102		
Glycerate	-3.796	+/-	0.111	-3.769	+/-	0.161	0.03	ns
Malate	0.590	+/-	0.121	-0.667	+/-	0.133	-1.26	***
Pipecolate	-8.430	+/-	0.332	-7.654	+/-	0.305	0.78	**
Pyruvate	-3.899	+/-	0.241	-3.840	+/-	0.179	0.06	ns
Salicylate	-8.349	+/-	0.485	-8.242	+/-	0.274	0.11	ns
Shikimate	-7.194	+/-	0.241	-6.311	+/-	0.232	0.88	**
Succinate	-2.420	+/-	0.116	-2.487	+/-	0.132	-0.07	ns
MISCELLANEOUS								
α–Tocopherol	traces			-14.188	+/-	0.624		
β–Sitosterol	nd			-7.569	+/-	0.362		
Campesterol	-11.489	+/-	0.289	-10.947	+/-	0.391	0.54	ns
Ethanolamine	-6.402	+/-	0.311	-5.133	+/-	0.149	1.27	***
Glycerol	-1.664	+/-	0.251	-2.140	+/-	0.170	-0.48	*
Myo-Inositol	-1.716	+/-	0.121	-1.935	+/-	0.082	-0.22	**
Putrescine	-8.635	+/-	0.358	-7.942	+/-	0.161	0.69	**

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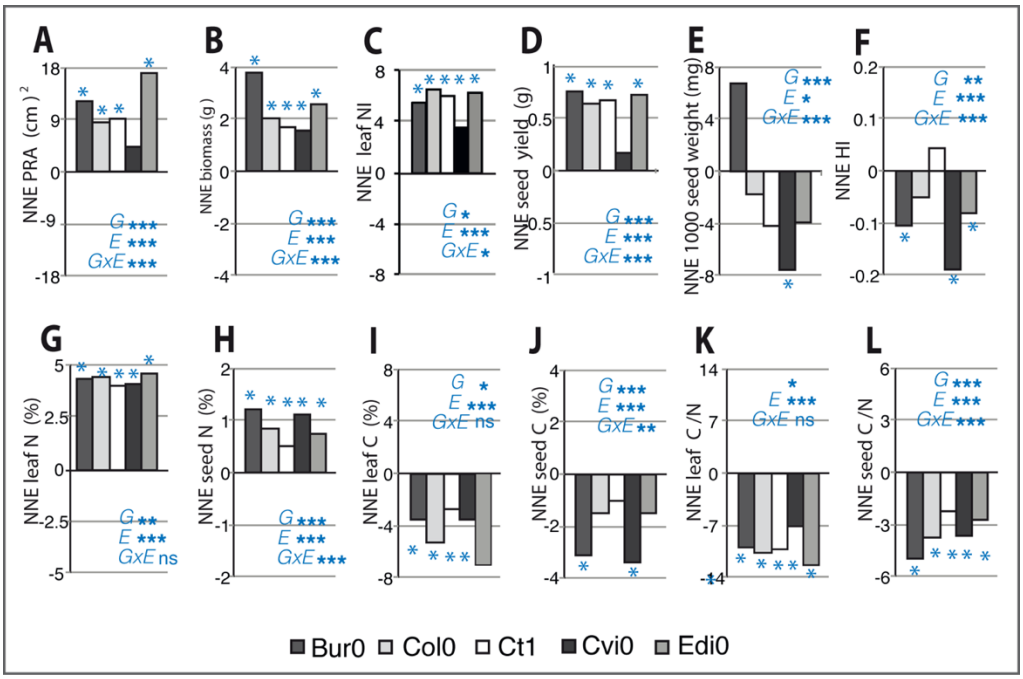

Figure S1. Growth and yield traits of *Arabidopsis* accessions

Data are shown as histograms showing the response of each accession under LN or HN of different growth and yield traits. The Net Nutrition Effect (NNE) represents the gain for a trait provided by non-limiting supply of N (HN) o the plants compared to limiting N nutrition (LN). For each trait it was calculated as the difference between the mean value in HN and the mean value in LN. Positive values for NNE indicate a gain due to HN and negative values indicate a loss. (**A**): NNE on Projected Rosette Area (PRA) at 27 DAS, (**B**): Shoot biomass after seed harvest at 45-50 DAS, (**C**): Leaf nitrogen index (Leaf NI), (**D**): Seed yield, (**E**): 1000-seed weight, (**F**): Harvest index (HI), (**G**): Leaf N percentage; (**H**): Seed N percentage, (**I**): Leaf C percentage, (**J**): Seed C percentage, (K): Leaf C/N ratio, (**L**): Seed C/N. Above each box is indicated the result of a Tukey HSD post-hoc Test indicating significant differences when comparing HN and LN. Next to each panel: results of the two-way ANOVA for the contribution of genotype (G), the nutrition (N) and their interaction (GxN) on the variance, with ns: not significant, * for p <0.05, ** for p <0.01 and *** for p <0.001.

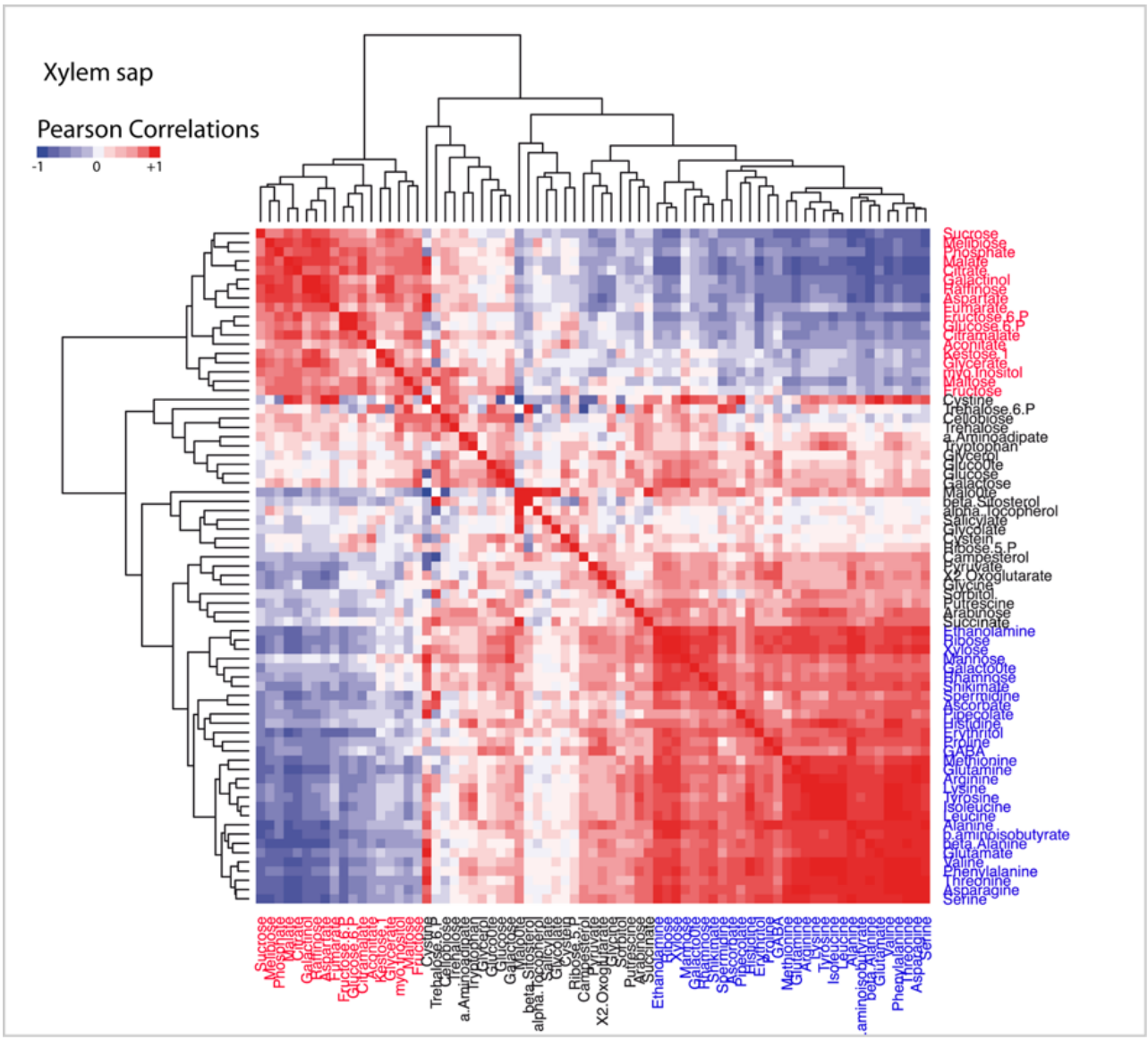

Figure S2. Correlogram of metabolite concentrations in xylem saps

Heatmap of pairwise correlation values of 66 metabolites in xylem sap. The Pearson correlation coefficients were calculated for all values from the five accessions in HN and LN (*n*=98).

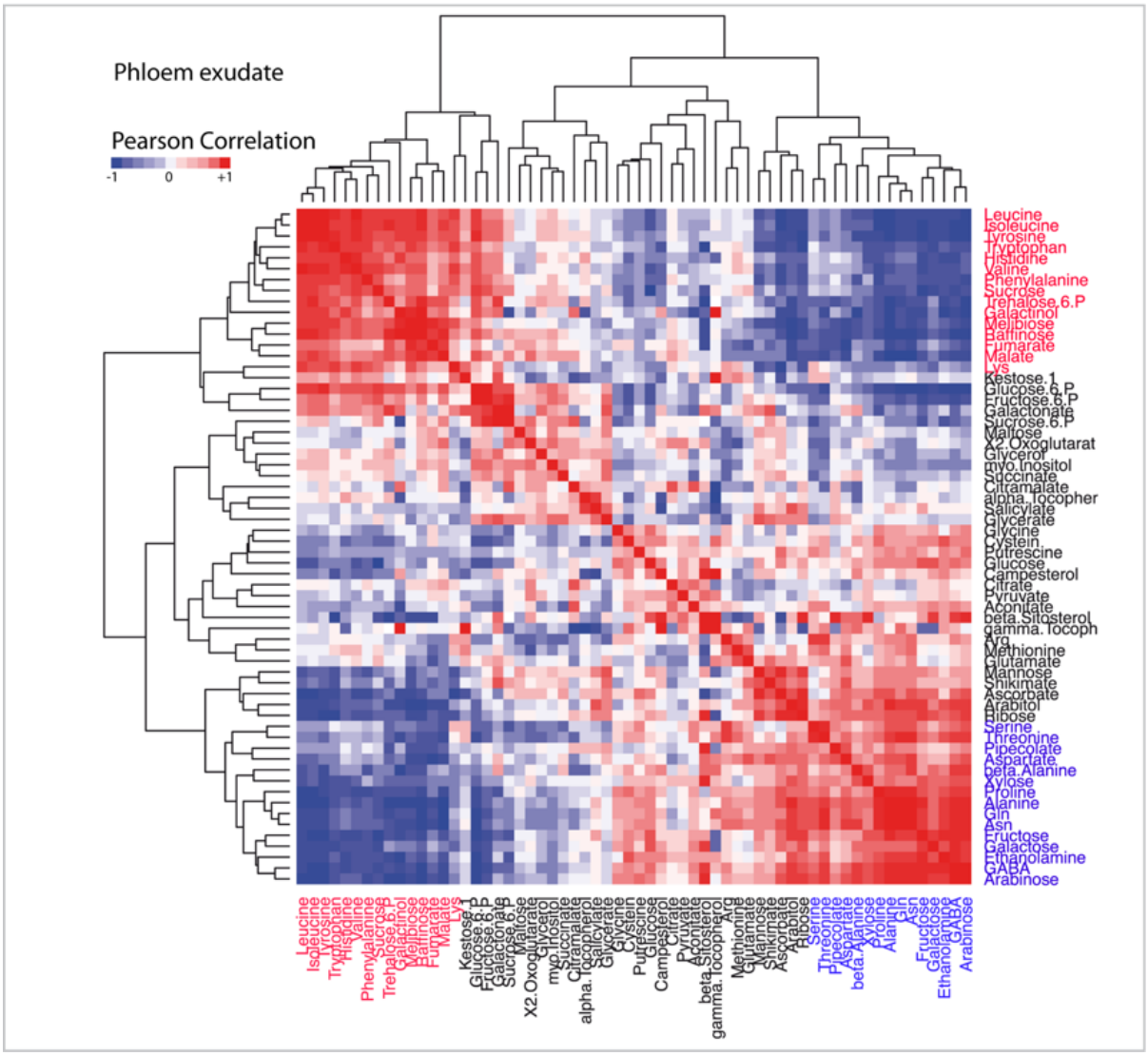

Figure S3. Correlogram of metabolite contents in phloem exudates

Heatmap of pairwise correlation values of 61 metabolites in phloem exudates using normalized dataset. The Pearson correlation coefficients were calculated for all values for five accessions in HN and LN (n=40).

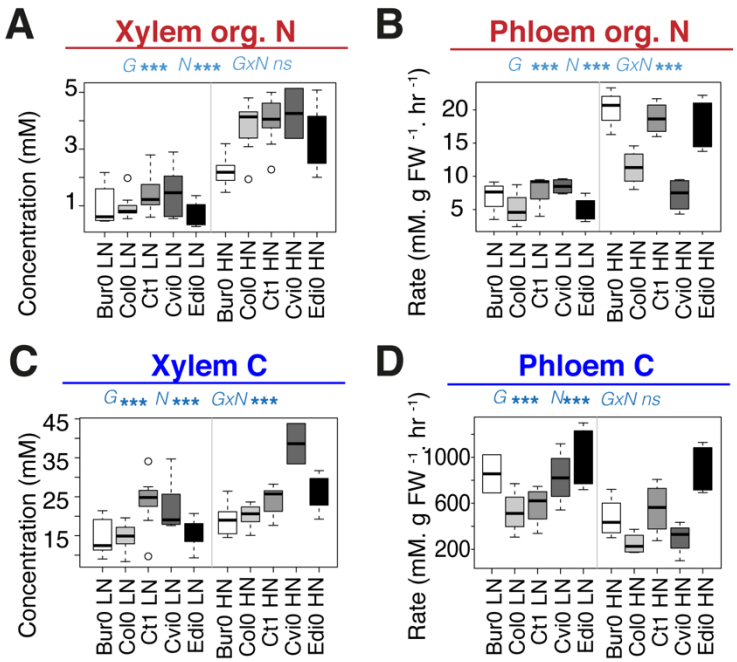

Figure S4. C and organic N translocation in xylem and phloem exudates

(**A-D**) Variations of estimated C and organic N are shown as boxes and whisker plots showing the distribution of the biological replicates, under low nitrogen (LN) or high nitrogen (HN). **(A-B):** Organic nitrogen translocated in xylem and phloem exudates with organic N estimated from xylem metabolite concentrations (**A**) and phloem metabolite exudation rates (**B**). **(C-D):** Carbon translocated in the xylem and phloem exudates with C estimated from xylem metabolite concentrations (**C**) and phloem metabolite exudation rates (**D**). The black lines inside represent the medians; the top and bottom ends of the boxes represent the first and third quartiles, respectively; and the whisker extremities represent the maximum and minimum data points. Above each panel in blue: results of two-way ANOVA for the contribution of genotype (G), the N nutrition (N) and their interaction (GxN) on the variance, with ns: not significant, * for p <0.05, ** for p <0.01 and ***. org: organic.

Natural variation in the long-distance transport of nutrients and photoassimilates in response to N availability

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Abstract:

The phloem and the xylem are involved in the allocation of nutrients and photoassimilates between organs. However, the regulation of the long-distance transport of C and N and its interplay with central metabolism is largely unknown. We exploited the natural variation of *Arabidopsis thaliana* accessions to analyze the metabolite profiles of phloem and xylem sap in two conditions of nitrogen (N) supply. Changing N supply from limiting to high availability led to a lower metabolite exudation rate from the phloem, indicating a lower mass flow of carbon (C) towards sink organs. However, the accessions did not all respond in the same way, consistent with reports showing a variability in the ability of natural accessions to cope with N abundance for improved growth. Distinct consequences of N availability were observed in the xylem sap and phloem exudate. This study revealed that the N metabolism response, set up to cope with N availability, is associated with a regulation of the phloem transport and may be an adaptive trait. Our study also highlighted an unexpected variability in the translocation of organic acids in response to N availability, suggesting that both phloem sugar transport and respiratory metabolism participate in the adaptive response to mineral nutrition.

Keywords: Allocation; Transport; Pipecolate; Succinate, Sucrose, Raffinose

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: