

Translocation of nitrogen in the xylem of field-grown cherry and poplar trees during remobilization

PETER MILLARD,^{1,2} RENATE WENDLER,¹ GIACOMO GRASSI,³ GWEN-AELLE GRELET¹
and MASSIMO TAGLIAVINI³

¹ Macaulay Institute, Craigiebuckler, Aberdeen AB15 8QH, U.K.

² Corresponding author (p.millard@macaulay.ac.uk)

³ Dipartimento di Colture Arboree, Università di Bologna, Via Fanin 46, 40127 Bologna, Italy

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Summary Studies of small trees growing in pots have established that individual amino acids or amides are translocated in the xylem sap of a range of tree species following bud burst, as a consequence of nitrogen (N) remobilization from storage. This paper reports the first study of N translocation in the xylem of large, deciduous, field-grown trees during N remobilization in the spring. We applied ¹⁵N fertilizer to the soil around 10-year-old *Prunus avium* L. and *Populus trichocarpa* Torr. & Gray ex Hook var. *Hastata* (Dode) A. Henry × *Populus balsamifera* L. var. *Michauxii* (Dode) Farwell trees before bud burst to label N taken up by the roots. Recovery of unlabeled N in xylem sap and leaves was used to demonstrate that *P. avium* remobilizes N in both glutamine (Gln) and asparagine (Asn). Sap concentrations of both amides rose sharply after bud burst, peaking 14 days after bud burst for Gln, and remaining high some 45 days for Asn. There was no ¹⁵N enrichment of either amide until 21 days after bud burst. In the *Populus* trees, nearly all the N was translocated in the sap as Gln, the concentration of which peaked and then declined before the amide was enriched with ¹⁵N, 40 days after bud burst. Xylem sap of clonal *P. avium* trees was sampled at different positions in the crown to assess if the amino acid and amide composition of the sap varied within the crown. Sap was sampled during remobilization (when the concentration of Gln was maximal), at the end of remobilization and at the end of the experiment (68 days after bud burst). Although the date of sampling had a highly significant effect on sap composition, the effect of position of sampling was marginal. The results are discussed in relation to N translocation in adult trees and the possibility of measuring N remobilization by calculating the flux of N translocation in the xylem.

Keywords: amides, amino acids, internal cycling of N, ¹⁵N labeling, *Populus*, *Prunus*, xylem sap.

Introduction

Nitrogen (N) demand for tree growth can be met either by uptake of external sources such as ammonium, nitrate and or-

ganic N (Gessler et al. 1998, Nasholm et al. 1998), or by remobilization of internal stores (Millard 1996). Several studies have shown that remobilization can provide the majority of N used for growth each year by a range of tree species (Millard and Proe 1991, Neilsen et al. 1997, Weinbaum and van Kessel 1998, Dyckmans and Flessa 2001). However, measurement of the N storage capacity of a tree is difficult and studies have usually involved the use of isotopic tracers coupled with sequential destructive harvesting of small trees growing in sand culture (Millard 1996). Because N content of the perennial organs of the tree increases as the plant grows (Beets and Pollock 1987, Smith et al. 1988, Harrison et al. 2000), it is believed that large, mature trees rely more on the remobilization of stored N for their growth each spring than do small, juvenile trees (e.g., Miller 1986). Unfortunately, N budgets constructed for larger trees growing in soil are often imprecise. For example, there have been several attempts to quantify remobilization by measuring N withdrawal from senescing leaves (e.g., Côté and Camiré 1987, Helmissaari 1992), without accounting for the N uptake in autumn that contributes directly to storage, which has been shown to occur in a range of species (e.g., Millard and Thompson 1989, Millard and Proe 1991). Even when ¹⁵N labeling is used, N budget studies in the field are difficult to interpret because of the difficulty of uniformly labeling all the soil N pools and the incomplete recovery of tree roots (estimated as being less than 65% of root mass, e.g., Le Goff and Ottorini, 2001). Therefore, there is a need to develop a new approach to quantify N storage by trees growing in soil.

An alternative approach to measuring N remobilization by trees is to consider N flux in the xylem during bud burst and leaf growth. In evergreen trees, N is often stored in the leaves (Millard et al. 2001, Warren et al. 2003) and is translocated from old to new leaves during remobilization. This translocation is likely to be restricted to short pathways, the N demand of newly grown leaves being met by translocation from old leaves nearby. In contrast, deciduous trees store N in their roots and bark (Millard and Proe 1991, Cooke and Weih 2005) from where it is translocated in the xylem over the entire height of the tree. A sharp peak in the concentration of N in the

Au: Helmissaari 1992 is not in the References.

xylem sap during bud burst has been observed and attributed to N remobilization (Glavac and Jockheim 1993, Schneider et al. 1994, Drambrine et al. 1995). Use of ^{15}N tracers to label N storage pools in young trees has confirmed that remobilization in *Betula pendula* Roth. (Millard et al. 1998) and *Malus domestica* Borkh. (Malaguti et al. 2001) coincides with these peaks in xylem sap amino acid concentrations. Specific amino acids and amides in xylem sap associated with N remobilization have been identified based on their ^{15}N labeling pattern (Millard et al. 1998, Malaguti et al. 2001, Frak et al. 2002, Grassi et al. 2002). The flux of remobilized N in the xylem has recently been calculated by measuring sap flux and the concentration of amino acids translocated during remobilization. This new approach to quantifying remobilization gave good agreement with quantification based on ^{15}N and destructive harvesting for young *Prunus avium* L. and *Juglans nigra* L. \times *regia* L. grown in sand culture (Frak et al. 2002, Grassi et al. 2002) and young *Malus domestica* grown in soil (Guak et al. 2003). This new method for quantifying remobilization in deciduous trees has potential for application in field experiments, particularly to measure the N storage capacity of trees, given that remobilization is a source-driven process (Millard et al. 2001). However, for the method to be useful, it is first necessary to establish if the N translocation patterns found in small trees during remobilization are also found in larger, more mature trees growing in soil. In addition, it has been shown that the composition of xylem sap can vary, depending on the position in the tree (Glavac et al. 1989, Schill et al. 1996, Smith and Shortle 2001). Therefore, to develop a sap sampling strategy for larger trees it is also necessary to assess the spatial variation in the amino compounds present in xylem sap by sampling the sap at different positions in the tree crown.

The aim of the study was to assess the pattern of N translocation in xylem and to determine if specific amino compounds are translocated as a consequence of N remobilization from storage as opposed to N uptake by the roots. We sampled xylem sap of 10-year-old *Prunus avium* and *Populus trichocarpa* Torr. & Gray ex Hook \times *balsamifera* L. trees growing in the field during their period of N remobilization. Additionally, sap was collected from different positions within the crown of *P. avium* trees during remobilization to determine if the concentration of amino compounds in xylem sap varied with position in the crown.

Materials and methods

^{15}N labeling experiment

Ten-year-old cherry trees (*Prunus avium* L.), growing at the Macaulay Institute in Aberdeen, Scotland (57°08' N, 2°09' W) at 52 m a.s.l. with a mean annual rainfall of 800 mm, in a soil classified as a humus-iron podzol of the Countesswells series were used. The trees were 4.5–5.7 m tall and were growing in a stand of 20 trees with a spacing of 2 m between individuals. In addition, 10-year-old hybrid poplar trees (*Populus trichocarpa* var. *Hastata* (Dode) A. Henry \times *Populus balsamifera* var. *Michauxii* (Dode) Farwell) growing at Hartwood in

North Lanarkshire, Scotland (55°48' N, 3°50' W), at 210 m a.s.l. with a mean annual rainfall of 1100 mm, in soil classified as a poorly drained gley of the Rowanhill series were studied. The trees, which were 5.8–6.2 m tall, were growing in experimental plots described by Proe et al. (1999), with a spacing of 1.5 m between individuals.

When the trees were still dormant, four of each species were chosen from within the respective stands. A square area of ground with a tree at the center was then marked out. The areas were 8.0 and 4.5 m² for cherry and poplar, respectively, corresponding to the main rooting area as assessed by soil excavations elsewhere in the stand. The area was hand-weeded to remove all herbaceous plants and the soil surface was raked. Within each square, three progressively smaller concentric squares were marked out. The dimensions of the squares gave four equal areas. Diagonals of all squares were marked out to provide four triangles around each tree, resulting in 16 equally sized areas (four subdivisions in four main areas). This allowed for a subsequent randomization of the soil sampling (see below). The time of bud burst was determined for each of the chosen trees of each species. On April 3, before bud burst for each cherry tree, four randomly chosen twigs between 20 and 30 cm in length were removed and xylem sap collected (see below). Ten randomly chosen buds/leaves were removed from each tree on the same day. These leaf samples were freeze-dried, weighed and milled before ^{15}N analysis. Immediately after taking the samples, a solution of $^{15}\text{NH}_4^{15}\text{NO}_3$ (150 g N m⁻²) enriched with ^{15}N to 6.453 atom percent excess (APE) was applied to the cleared area. This same procedure was repeated for the poplar trees on April 11, except that the N was applied at 120 g N m⁻² and a ^{15}N enrichment of 6.025 APE. At bud burst (designated as the first day when between 5–10% of all buds were open), which occurred between April 8–11 for cherry and on April 16 in poplar, four twigs for xylem sap and 10 leaves for ^{15}N analysis were removed as described previously. Thereafter, the cherry trees were sampled twice a week until the end of June, then once a week until the end of July (26 harvests in total). The poplar trees were sampled twice a week until June 10, then once a week until the end of June (21 harvests in total). To ensure complete randomization of leaf and twig sampling, the following method was used. All main branches on each tree were counted. Each branch was divided into quarters along its length and each quarter of each branch was given a number so that the total number for each tree was the number of the main branches \times 4. These numbers were used to generate random numbers for identifying positions for leaf and twig sampling at each harvest date. Numbers of leaves/twigs removed during sampling were considered insignificant in relation to the total biomass.

At each xylem sampling date, two soil cores were taken, with a screw auger, from one randomly chosen subdivision within each of the four main areas to a depth of 20 cm. The samples were sieved, bulked and split to give duplicate samples for each tree. One of the samples was oven-dried overnight at 105 °C to determine water content. The other sample was extracted in 1 mol m⁻³ KCl for 2 h, filtered and stored overnight in a plastic bottle at 4 °C. This extract was used to

determine the concentration and ^{15}N enrichment of ammonium and nitrate in the soil as described by Williams et al. (1999).

Spatial variation of sap composition

The spatial variation in xylem sap composition during remobilization was assessed by sampling trees in a 7-year-old clonal cherry plantation, located at the University of Bologna's research station at S. Marino, Bologna, Italy, (44°35' N, 11°27' E, 33 m a.s.l., annual rainfall 552 mm). The soil in the experimental area is an alluvial clay loamy soil (Calcaric Fluvisol), very deep, well-drained and slightly calcareous. Within the plantation, eight trees from a row were selected for uniformity (three whorls, height 4.9–5.4 m, diameter at 1.30 m 47–55 mm) and assigned to one of two groups of four trees each. One group was sampled before the other, to allow the xylem sap to be sampled during remobilization in the same individuals and so reduce any variability in the data caused by tree-to-tree differences in the timing of the peak of N concentration. From March 2002, the selected trees were monitored every 1–2 days. The day when a leaf had first emerged from the apical shoot was designated as the date of bud burst (which occurred between March 16–18). On days 5, 11 and 19 after bud burst, xylem sap was collected from a lateral 2-year-old branch of the intermediate whorl (position "b" in Figure 1) of the four trees in the first group. The same procedure was applied on days 29, 50 and 68 after bud burst on the trees of the second group. In addition, on three dates (days 11, 19 and 68 after bud burst), xylem sap was also collected along a vertical and a radial gradient, i.e., from positions "a" (1-year-old twig), "c" (3-year-old branch) and "d" (2-year-old branch).

Analysis of leaf and soil samples

The quantity of ^{15}N and total N in bud and leaf samples and soil extracts was determined with a Tracer MAT continuous flow mass spectrometer (Finnigan MAT, Hemel Hempstead, UK). The uptake of labeled N was calculated from the ^{15}N enrichment (Millard and Neilsen 1989).

Collection and analysis of xylem sap

During the ^{15}N labeling experiments, sap was collected between 0830 and 1030 h and, in the spatial study, sap was collected between 1000 and 1200 h to avoid confounding of the results due to diurnal variation in sap composition. Xylem sap was extracted from excised twigs with a Scholander pressure chamber (described in Millard et al. 1998) after removing the bark to avoid contamination with phloem sap. The pressure in the vessel was increased until xylem sap was exuded and collected in micro capillary tubes. A pressure of 0.2 MPa was not exceeded, because initial tests (cf. Malaguti et al. 2001) on both tree species showed that up to this pressure there was no contamination of sap by cellular components. During the ^{15}N labeling experiments, all xylem sap collected from an individual tree at each sampling was pooled and weighed. We determined ATP content of a small aliquot with the Sigma Luciferine-Luciferase kit, as a test for contamination with phloem

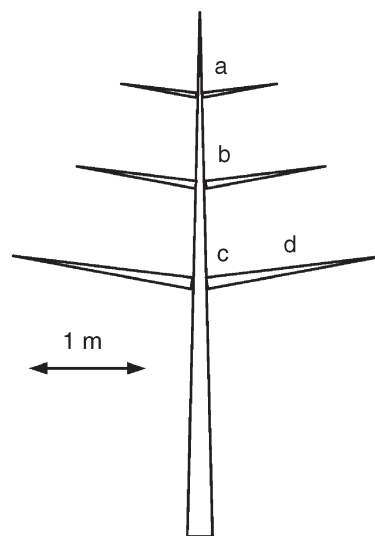


Figure 1. The positions from which xylem sap was sampled from the cherry trees to determine the spatial variation in sap composition. Tree height and whorl insertions are to scale.

sap. Sap samples were stored at $-80\text{ }^{\circ}\text{C}$ and amino acids analyzed by gas chromatography linked to mass spectrometry (GC-MS) for the ^{15}N labeled material or by gas chromatography of unlabeled samples. Particulate material was removed by centrifugation for 5 min at 5800 g (MSE Micro-Centaur centrifuge). Samples (20 mg) were then diluted with 0.5 cm³ of demineralized water and a 100 mm³ aliquot of the dilute sap along with an internal standard of nor-valine (25 mm³ containing 0.18 µg) was added to a 1-CWV, clear glass, crimp-top, tapered vial (Chromacol Ltd, Welwyn Garden City, U.K.) and freeze-dried. The derivatization reagent (100 mm³, consisting of *N*-(*tert*-butyldimethylsilyl)-*N*-methyltrifluoroacetamide containing 1% *tert*-butyldimethylsilyl chloride (Sigma-Aldrich, Gillingham, England) in acetonitrile (1:4 v/v), was added to the dried material and left at room temperature for 10 min. The solution was then heated at 70 °C for 35 min to convert the free amino acids to their *tert*-butyldimethylsilyl derivatives (*t*-BDMS).

The derivatives were analyzed by GC-MS with a Trace 2000 gas chromatograph, fitted with an AS 2000 autosampler and interfaced to a Finnigan Trace Quadrupole mass spectrometer in the single ion recording (SIR) mode. Separation of the *t*-BDMS derivatives was effected with a fused silica Zebron ZB-S capillary column, 30 m × 0.25 mm id × 0.25 µm phase thickness (Phenomenex, Macclesfield, U.K.). The column was operated with a temperature programme of 60 °C for 1 min, increased to 225 °C at 10 °C min⁻¹, held for 1 min, increased to 325 °C at 7.5 °C min⁻¹ and held for 5 min. The sample was introduced to the column by a splitless technique (splitless for 1 min followed by an 80:1 split). The injector temperature was held at 240 °C and the interface line temperature was a constant 250 °C. The mass spectrometer was operated in electron impact ionization mode with an ionization energy of 70 eV and a source temperature of 200 °C. Enrichments of ^{15}N in in-

dividual amino acids were calculated from the ratio of the ion monitored at natural abundance and in enriched amino acids (Campbell 1974). Amino acid concentrations were calculated by use of response factors derived from the analysis of solutions containing known weights of amino acids. Quality control was assured by analyzing a standard solution of amino acids. Under the GC-MS conditions employed, the precision of the isotope ratio analyses were 0.70 APE, so the APE value of an individual compound had to be higher than this value before it was considered enriched. Unlabeled derivatives were analyzed by gas chromatography under the conditions described previously.

Statistical analyses

Data for xylem sap amino N concentrations and ^{15}N enrichment and the recovery of labeled and unlabeled N in buds/new grown shoots or leaves, were related to the stage of leaf development based on number of days from bud burst (D) as a measure of time. For the ^{15}N labeling experiment, amino N concentrations and N contents were expressed as a proportion of the maximum value measured for that type of data for each tree. Because sap and shoot sampling on the one hand and bud burst, on the other hand, were synchronized for all four poplar trees, means and standard errors were calculated based on four replicates for each value of D. For cherry trees, bud burst was spread over 3 days. When the number of observations (either amino N concentration in the xylem or bud/leaf N content) for each value of D was less than 3, means were calculated after grouping observations made over 2 or 4–5 D, before and after 80 D, respectively. Means and standard errors of cherry data were therefore compiled over 3–4 replicates. The timing of events, such as peak amino N concentration or appearance of enriched amino N in the sap, was determined for each replicate tree based on original data and a mean D value and its standard error was then calculated as an average of the four replicate trees.

To compare the patterns of appearance of labeled and unlabeled N in the buds, new leaves or shoots, the relationships between (1) labeled or (2) unlabeled N content and D were analyzed by curve regression analysis using Genstat for Windows Release 6.1 (VSN International, Oxford). The analysis comprised two steps. First, the best fit curve was determined, assuming a similar type would fit both (1) and (2) for the same tree species. Second, the significance of setting different values for the parameter of the selected curve for relationship (1) or (2) was assessed.

For analysis of the spatial variation in sap composition, the effect of harvest date on the concentration of amino compounds in xylem sap was assessed by a one-way ANOVA, after \log_{10} transformation of the data to ensure homogeneity of variance and normal distribution of the residuals. To assess whether sampling position affected amino N concentration in the three main amino N compounds (Asn, Gln and Asp) found in cherry xylem sap and whether that effect differed between the three compounds, a Residual Maximum Likelihood (REML) analysis was carried out with sampling date and position, and

N compound as factors. Sample number and tree number were added in the random model to account for tree to tree variations and sample to sample variation. Statistical significance for each factor and their interaction was tested using χ^2 tests on Wald statistics. Before analysis, \log_{10} transformation of the data was required to comply with the assumption of homogeneity of variance and normal distribution of the residuals.

Results

^{15}N labeling experiment

The concentration and ^{15}N enrichment of nitrate and ammonium recovered from the soil during the experiment are shown in Figure 2. At both sites, concentrations of nitrate were higher than concentrations of ammonium, but both were enriched with ^{15}N for the duration of the experiment. For nitrate, the enrichments were relatively constant with a mean value of 3.98 ± 0.101 APE at the cherry site and 4.29 ± 0.067 APE at the poplar site. The ^{15}N enrichment of ammonium at the first sampling was similar to that of nitrate at both sites, but thereafter declined concomitantly with a decrease in the concentration of ammonium. The reason for this decline in both the concentration and enrichment of ammonium is unclear, but may be associated with a combination of preferential uptake by the trees and the balance between immobilization and mineralization by the soil microbial biomass. As a consequence, mean enrichment of ammonium was lower than that of nitrate, being 2.94 ± 0.228 APE at the cherry site and 2.54 ± 0.241 APE at the poplar site. However, because both the soil ammonium and nitrate pools were enriched with ^{15}N for the duration of both experiments, recovery of labeled N in xylem sap was indicative of the translocation of N from root uptake.

Table 1 shows the amino acids and amides recovered in the xylem sap of the cherry trees. The majority of N was translocated as glutamine (Gln) and asparagine (Asn) and together, these amides accounted for 88% of the N recovered during the initial peak in N concentration in the xylem sap (14 D) and 76% after the N concentration had declined (58 D). Figure 3 shows the change with time in Gln and Asn concentrations and APE in cherry xylem sap. Immediately following bud burst, the concentration of Gln increased, peaked at 14 ± 2 D and then declined rapidly (Figure 3A). The concentration of Gln ranged from 51 to $195 \mu\text{g g}^{-1}$ sap for the four trees at bud burst, whereas the maximal concentration of Gln varied between 245 and $1074 \mu\text{g g}^{-1}$ sap after bud burst. The total duration of the peak was about 25 days. Glutamine was not significantly enriched with ^{15}N until 21 ± 2 D, demonstrating that the peak in Gln concentration in the xylem sap mainly represented N translocation as a consequence of remobilization. Interpretation of the patterns of the concentration and enrichment of Asn (Figure 3B) were less clear than for Gln. At bud burst, the concentration of Asn ranged between 3 and $28 \mu\text{g g}^{-1}$ sap. The concentration then increased to a maximum ranging from 259 to $460 \mu\text{g g}^{-1}$ sap, although the timing of the peak varied between trees (between 26 and 82 D with a mean of 48 D). As a consequence, there was no single peak in the concentration of

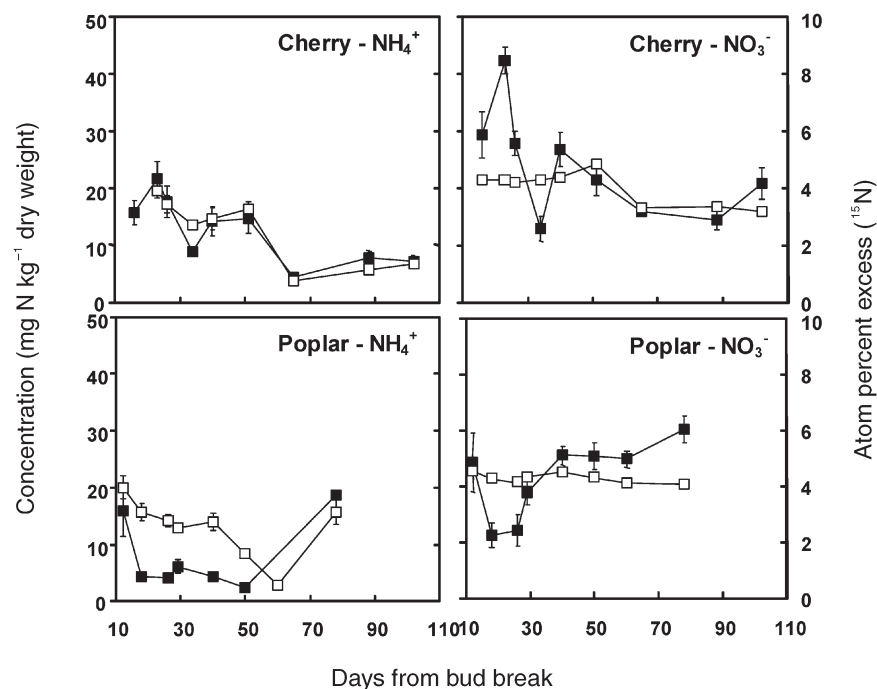


Figure 2. Variation in the concentration (■) and ¹⁵N enrichment (□) of ammonium (NH₄⁺) and nitrate (NO₃⁻) in the soil beneath the cherry and poplar trees. Each value is the mean and standard error of four replicates.

sap Asn, as was found with Gln. No enrichment of Asn was found until 21 ± 2 D (the same date as for Gln), suggesting that the initial translocation of Asn was due to remobilization. This was confirmed by the pattern of recovery of ¹⁵N in buds and leaves (Figure 3C). No ¹⁵N was found until 25 ± 2 D, whereas the unlabeled N started to increase 18 ± 1 D, shortly after the peak in Gln concentration in the xylem sap, but before the sap was enriched with ¹⁵N. Once individual leaves could be distin-

guished and sampled separately, the increases in both labeled and unlabeled leaf N concentration followed a similar pattern, suggesting that both sources of N reached the leaves through the same N flux.

Table 2 shows the main amino acids and amides recovered in the xylem sap of the poplar trees. The majority of N was translocated as Gln, accounting for 93% of the N recovered during the initial peak in concentration of sap N (at 27 D) and

Table 1. Comparison of the recovery of N in amino acids and amides ($\mu\text{g N g}^{-1}$ sap) in the xylem sap of *Prunus avium* and *Populus trichocarpa* × *Populus balsamifera* trees harvested during the period of N remobilization and after remobilization had finished. Data are means ± standard errors of four replicates. Abbreviation: D = days from bud burst.

Compound	<i>Prunus</i>		<i>Populus</i>	
	During (14 D) ¹	After (58 D)	During (27 D)	After (49 D)
Alanine	0.5 ± 0.1	0.9 ± 0.1	1.4 ± 0.5	0.5 ± 0.1
Glycine	0.2 ± 0.1	0.2 ± 0.0	1.0 ± 0.2	0.3 ± 0.1
Valine	4.2 ± 0.7	0.4 ± 0.1	5.2 ± 0.4	2.3 ± 0.3
Leucine	0.5 ± 0.1	0.1 ± 0.0	2.1 ± 0.2	0.9 ± 0.2
Isoleucine	1.6 ± 0.3	0.1 ± 0.0	2.8 ± 0.3	1.2 ± 0.2
Gaba	0.2 ± 0.1	0.3 ± 0.1	0.5 ± 0.2	0.2 ± 0.1
Proline	4.5 ± 1.0	1.6 ± 0.3	0.5 ± 0.2	0.2 ± 0.0
Serine	0.5 ± 0.2	0.4 ± 0.1	1.3 ± 0.5	0.4 ± 0.1
Threonine	6.9 ± 2.4	0.9 ± 0.2	4.2 ± 0.4	2.5 ± 0.4
Phenylalanine	1.5 ± 0.6	0.1 ± 0.0	2.7 ± 0.4	0.9 ± 0.2
Aspartic acid	96.0 ± 21.0	9.1 ± 3.2	2.1 ± 0.8	0.8 ± 0.2
Glutamic acid	6.4 ± 1.3	2.1 ± 0.5	2.6 ± 1.1	1.1 ± 0.3
Ornithine	1.9 ± 1.1	0.9 ± 0.3	0.9 ± 0.1	0.9 ± 0.3
Asparagine	189.6 ± 45.2	45.2 ± 7.9	2.6 ± 1.4	2.9 ± 1.3
Glutamine	693.0 ± 211.4	8.7 ± 2.3	436.0 ± 84.7	54.3 ± 13.5
TOTAL	1007.3 ± 263.8	71.0 ± 14.6	466.6 ± 263.8	69.4 ± 17.0

¹ Number within brackets indicates sampling date.

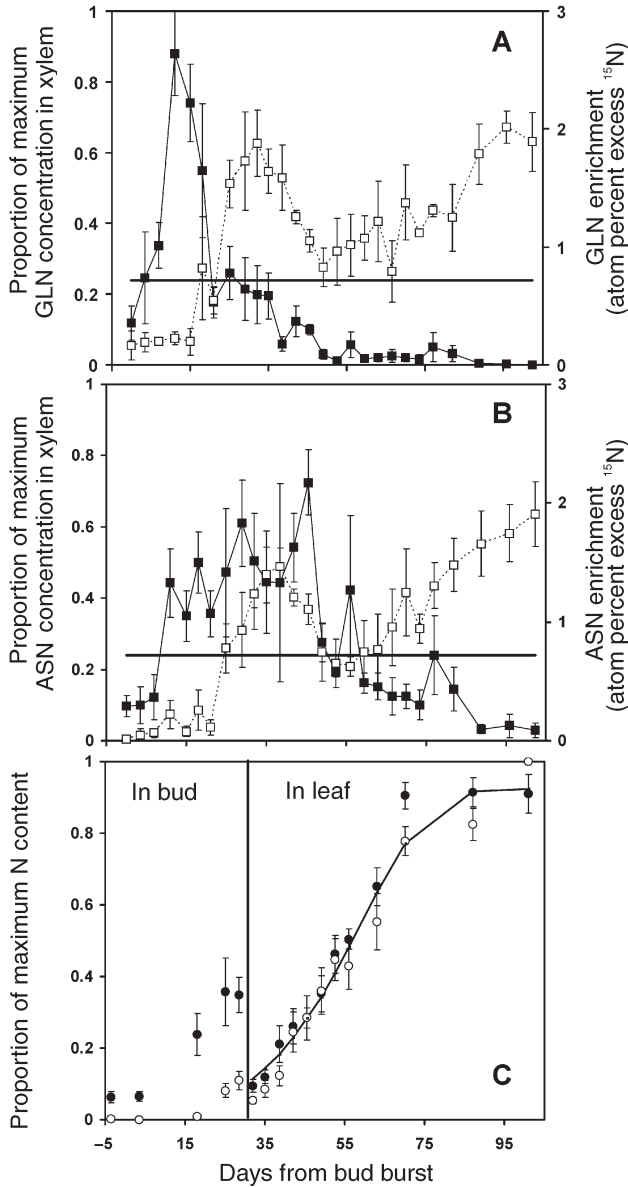


Figure 3. Time courses of concentration (■) and ^{15}N enrichment (□) of glutamine (GLN) (A) and asparagine (ASN) (B) in the xylem sap of cherry trees, and recovery of unlabeled (●) and labeled (○) N per bud or leaf (C). In A and B, each value is the mean and standard error of 3–4 replicates. Concentrations of glutamine and asparagine are expressed as a proportion of the maximum value measured for each tree for each molecule. The horizontal lines show the enrichment threshold (based on the precision of the ^{15}N determinations) below which the ^{15}N atom percent excess is not considered significantly different from zero. In C, each value is the mean and standard error of four replicates. A logistic curve ($y = 0.92 - 0.97 \exp(-e^{0.06(x-60)})$) was the best fitting curve ($r^2 = 85.7$) to describe the increase in leaf N through time, with no significant differences between unlabeled and labeled N in the leaf.

78% after the concentration of sap N had decreased (at 49 D). Figure 4A shows the changes in concentration and APE of Gln in poplar xylem sap at each sampling date. Following bud burst, the concentration of Gln increased rapidly and remained

high until 35 D, after which it fell rapidly. At bud burst, the concentration of Gln ranged from 36 to 44 $\mu\text{g g}^{-1}$ sap and then increased to reach a maximum ranging from 210 to 423 $\mu\text{g g}^{-1}$ sap. Glutamine was not significantly enriched with ^{15}N until 40 ± 2 D, showing that the initial peak in Gln concentration represented N translocated as a consequence of remobilization, as was also been found for the cherry trees. This was confirmed by the pattern of recovery of ^{15}N in the leaves (Figure 4B). There was no recovery of ^{15}N in the leaves until 36 ± 2 D, whereas the unlabeled N started to increase at 18 ± 3 D, after the concentration of Gln increased in the xylem sap, but before the sap was enriched with ^{15}N . The increases in labeled and unlabeled N concentrations in the new tissues followed time courses that differed significantly, indicating that the two N sources reached poplar leaves in separate fluxes of N.

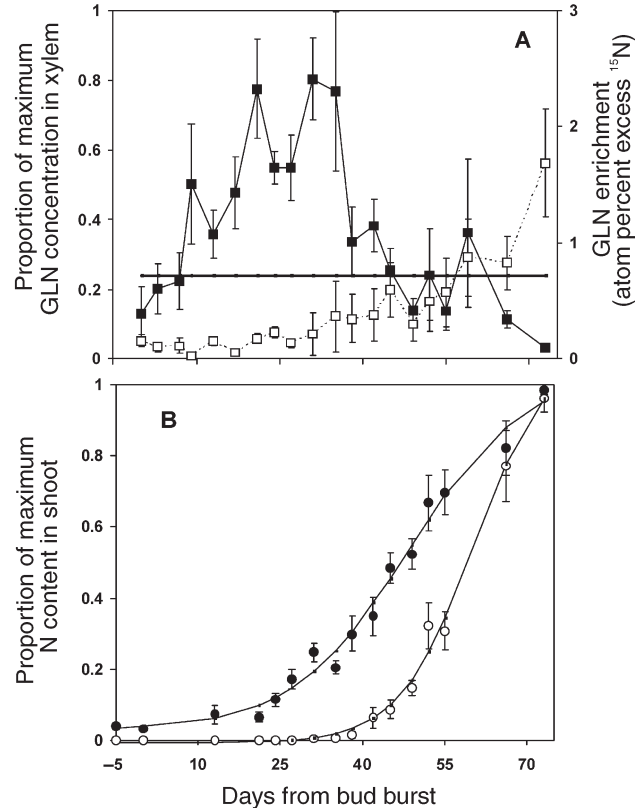


Figure 4. Effects of bud burst on the concentration (■) and ^{15}N enrichment (□) of glutamine (GLN) in the xylem sap of poplar trees (A) and the recovery of unlabeled (●) and labeled (○) N in the shoot (B). Each value is the mean and standard error of four replicates. In A, the concentration of glutamine is expressed as a proportion of the maximum concentration measured for each tree. The horizontal line shows the enrichment threshold (based on the precision of the ^{15}N determinations) below which the ^{15}N atom percent excess is not considered significantly different from zero. In B, Gompertz curves were the best fit ($r^2 = 94.2$) to describe the increase in shoot N through time, which differed significantly between unlabeled and labeled N, ($P < 0.001$; unlabeled N: $y = 0.03 + 1.01/(1 + e^{(-0.09(x-155)})}$) and labeled N: $y = 0 + 1.11/(1 + e^{(-0.15(x-167)})}$).

Spatial variation in cherry sap composition

The cherry trees used for the study of the spatial variation in sap composition exhibited a similar pattern of N translocation in the xylem during their growth following bud burst as described for poplar. There was an increase in the concentration of N found in Asn and Gln following bud burst with the peak lasting a maximum of 19 D (data not shown). The sap collected for analysis of their composition from different positions within the crown were taken when the Gln and Asn concentrations were high within the peak corresponding to remobilization (at 11 D), immediately after the end of remobilization (at 19 D) and at the end of the experiment (68 D). Differences in sap concentrations of the three major amino compounds (AA) Asn, Gln and aspartic acid (Asp), sampled at the three dates (Date) and four positions (Position) were analyzed by three-way REML analysis (Figure 5). The effects of Date and AA were highly significant, as was the interaction between Date and AA, mainly because the magnitude of variation through time differed between Asn and Gln on the one hand, and Asp on the other hand. There was no effect of crown position on Asn, Gln or Asp. However the interaction between Position and Date was significant, because of a difference in concentration between the different positions at 2 D, whereas sap amino N concentration remained unaffected by sampling position at 11 D (peak remobilization) and at 68 D (Figure 5).

Discussion

Temporal variation in sap composition

In both cherry and poplar, the concentration of amino compounds peaked in the xylem sap shortly after bud burst. Similar patterns have been reported for smaller trees (Millard et al. 1998, Malaguti et al. 2001, Frak et al. 2002, Grassi et al. 2002). The peaks were caused by two factors, N remobilization and dilution of sap as a result of increases in transpiration rates as leaves grew. Increases in sap velocity as a consequence of leaf growth caused a dilution of solutes in the sap, resulting in overall lower sap amino acid and amide concentrations after remobilization had finished compared with soon after bud burst. However, the qualitative differences in sap composition through time demonstrated that sap composition was also influenced by the balance between N remobilization and root uptake of N.

In cherry trees, the main amino N compounds recovered in the xylem sap were Gln and Asn. The pattern of N translocation in the xylem sap of the large trees in our study was similar to that observed by Grassi et al. (2002) for saplings in sand culture. In both studies, N from remobilization was recovered in the growing leaves before that from current root uptake (18 days by Grassi et al. (2002) and 7 days in our study). Grassi et al. (2002) suggested that changes in xylem sap composition reflect the shift in N sources from remobilization to root uptake. In our study, Asn was the second most abundant N compound in the xylem sap when the N concentration peaked at 14 D, but it was also the predominant amino compound after N remobilization ended. In our first experiment, the concentra-

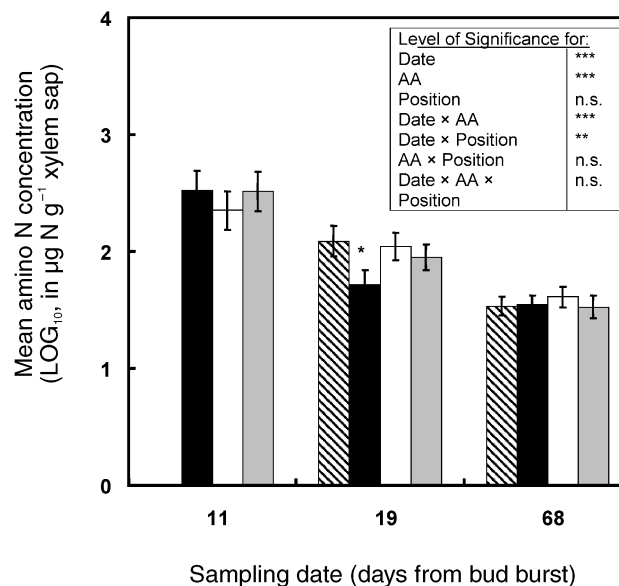


Figure 5. Effect of sampling position on mean amino-N concentration in the xylem sap of cherry trees. Data are the means and standard errors of the concentrations of aspartic acid, asparagine and glutamine of four replicates trees after log₁₀ transformation. Xylem sap was sampled in positions a (hatched bars), b (plain bars), c (clear bars) and d (shaded bars) as described in Figure 1, at 11, 19 or 68 days following bud burst. The asterisk indicates that the mean amino-N concentration for that position differs significantly ($P < 0.05$) from all other positions within the same sampling date. The F probabilities for the effects of sampling date (Date), sampling position (Position) and amino-N compound (AA) are given at $P > 0.05$ (ns), $P < 0.05$ (*), $P < 0.01$ (**) and $P < 0.001$ (***), respectively. Data were analyzed by Residual Maximum Likelihood after sample number and tree number were added in the random model to account for tree-to-tree variations and sample-to-sample variation.

tion of Asn in the xylem sap increased during N remobilization and, although it varied between sampling periods, remained relatively high for about 60 D. The ¹⁵N enrichment (indicating translocation of N from root uptake) of both Asn and Gln in sap occurred concurrently, when Gln concentrations had already fallen, but Asn concentrations were still relatively high. The probability that N was derived from soil sources other than the ¹⁵N-labeled ammonium or nitrate taken up during the course of the experiment is remote. The trees were grown in mineral soils with relatively low organic matter content and were well supplied with fertilizer N. Hence the uptake of organic N, via their mycorrhizal partners or through direct root uptake was unlikely to have been sufficient to alter the enrichment pattern of xylem amino-N. Therefore, our data indicate that Asn translocation was initially due to remobilization, but switched to N from root uptake. In contrast, Gln translocation was due almost entirely to remobilization (cf. Grassi et al. 2002). The amount of N translocated as nitrate by cherry trees is unknown.

In poplar trees, the majority of N translocated in the xylem was as Gln (94 and 77% of total amino-N at 27 D and 49 D, respectively). Similarly, Sauter and van Cleve (1992) found

more than 75% of sap N was in the form of Gln during the whole vegetative season. A peak in the concentration of Gln in the sap of poplar of up to 13 mol m^{-3} has been reported in the period after bud break and during leaf expansion (Schneider et al. 1994) and was explained as Gln resulting from protein body mobilization before being released from the ray cells into the vessels (Sauter and van Cleve 1992). Our ^{15}N studies showed that, in poplar, remobilization started before root uptake of N, but that Gln was translocated in the xylem as a consequence of both processes. Besides this amide, poplar uses nitrate to transport N to the shoot during periods of rapid root N uptake (Siebrecht et al. 2003) and regardless of soil N availability, nitrate reductase activity in poplar leaves is more than 10-fold higher than in stems and roots (Black et al. 2002). Nitrate is also a significant component of sap in other tree species (e.g., Glavac and Jochheim 1993, Prima Putra and Botton 1998, Toselli et al. 1999), although in each of these studies greater concentrations of amino-N were recovered than of nitrate-N. It has been suggested that a high capacity for leaf nitrate reduction (and hence xylem transport of N as nitrate) is associated with fast-growing, pioneer species, whereas late-successional species translocate amino-N (Aidar et al. 2003). In poplar, Siebrecht et al. (2003) reported concentrations of nitrate varying between 1 and 4 mol m^{-3} . Although they did not measure amino compounds in their study, these values are roughly one tenth the maximum total amino-N concentrations we measured in poplar sap during remobilization (33 mol m^{-3}), but roughly equivalent to the values found after remobilization had finished (5 mol m^{-3}). Therefore, it is likely that, although N derived from remobilization is translocated as Gln by poplar, a significant proportion of N from root uptake is translocated as nitrate.

Spatial variation in cherry sap composition

Several studies have reported variations in the composition of xylem sap at different positions within the crown; however, these studies involved compounds other than amino acids (e.g., sugars, see Schill et al. 1996), for which the physiological controls of xylem translocation, loading and offloading may differ from those for amino acids. Others have studied spatial variation of amino-N in the xylem, by either direct measurement of amino-N concentration or indirect quantification such as elemental N concentration or sap density xylem sap (Lousteau et al. 1998, Lu et al. 2000, Smith and Shortle 2001, McDonald et al. 2002, Escher et al. 2004); however, all these studies focused on evergreen broad-leaved or coniferous species. Because of the potentially short distance over which N is translocated in the xylem after remobilization in evergreen trees (i.e., from old to new leaves), amino-N concentration in xylem sap is likely to be spatially heterogeneous. Hence, the composition of xylem sap at any one point would not necessarily reflect the flux of remobilized N reaching the buds and growing leaves.

In deciduous trees, spatial variation in the concentration of N compounds in xylem sap has been studied in 5-month-old poplar (Siebrecht et al. 2003) and 35–42-year-old beech (Gla-

vac and Jochheim 1993). Nitrate concentration in the xylem was found to be spatially variable in the beech trees, although tree-to-tree variation was greater than spatial variations within each tree. Spatial variation in the concentration of amino acids in the xylem was also assessed by Siebrecht et al. (2003), and shown to increase by about 50% toward the growing apex of poplar seedlings. Escher et al. (2004) compared the concentrations of amino acids in xylem sap sampled below or above the point of attachment of mistletoe on poplar branches in a 60-year-old lowland forest and found no significant spatial variation in xylem sap amino-N concentration. We observed little spatial variation (about 15%) in amino acid concentration in the xylem sap of field-grown cherry trees, because the spatial variation occurred on only one sample date during N remobilization. At that time, amino acids were, on average, less concentrated in the xylem of lateral branches growing at mid-height within the crown than in the upper crown.

Developing a method to measure N remobilization in field-grown trees

Our data indicate that the understanding of N remobilization gained through studying young trees growing in pots also applies to larger trees growing in soil. Because the temporal patterns of N translocation in the xylem of cherry during remobilization were similar to those reported by Grassi et al. (2002), it appears likely that the approach to measuring N remobilization based on the flux of N in the xylem would also apply to larger trees. However, the small but significant spatial variation in the composition of cherry sap during remobilization, along with the poplar data reported by Siebrecht et al. (2003) indicate that designing a sap sampling strategy for deciduous trees may also have to take the position within the crown into account. Nevertheless, because xylem sap exudates were obtained by application of a pneumatic pressure to cut twigs, the error in sap composition introduced by the sampling technique might be greater than the spatial variation observed in our study, or might be inconsistent throughout the crown. Moreover, xylem sap composition in cut twigs might differ from that of transpiring trees because of lateral exchange (Schurr 1998).

Siebrecht et al. (2003) suggested that variation in sap flow, not composition, regulated N flux in the xylem of poplar trees, but this has never been demonstrated for any tree during the remobilization period. However, attempts to develop a technique to quantify N remobilization by trees based on sap N flux (Frak et al. 2002, Grassi et al. 2002), have revealed that variations in sap velocity have a greater influence on the estimation of remobilization than the changes in concentration of amino compounds in sap. This is because sap flux following bud burst increases by several orders of magnitude more than the variation in the concentration of amino-N compounds (Frak et al. 2002, Guak et al. 2003). The amount of N allocated to storage in the autumn affects the amount remobilized the following spring (Millard 1996). However, the N status of the tree can also affect leaf phenology directly. This can be seen by a delay in leaf senescence in autumn when trees are replete

with N (Millard and Thomson 1989) and earlier bud burst (Lumme and Smolander 1996, Grassi et al. 2002) and faster leaf growth in the spring (Millard and Proe 1991, Lobit et al. 2001, Grassi et al. 2002). These phenological effects are likely to have a large impact on transpiration and hence sap velocity in the spring. Although sap flux of amino compounds depends on both sap velocity and amino acid concentrations, if velocity varies more than do amino acid concentrations it may be possible to compare N remobilization between different trees based on their cumulative sap flux, thereby eliminating the need to calculate the flux of amino compounds; however, such an approach would require careful validation.

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