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# Supplying nitrate before bud break induces pronounced changes in N nutrition and growth of young poplars

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**Abstract.** Tree nutrient research concentrated on endogenous C and N remobilisation in spring has neglected to acknowledge the possibilities of significant effects of N uptake before bud break, especially on the quality of regrowth and N reserve remobilisation. To investigate this subject, experimental studies were performed on young poplars (*Populus tremula* × *Populus alba*, clone INRA 717–1B4) grown with a controlled nutrient supply: (i) without N, ‘control’; (ii) N supplied throughout the course of the experiment, ‘N-supply’; and (iii) N supplied only before bud break, ‘N-pulse’. Results confirm the hypothesis that poplar scions can significantly take up nitrate before bud break, amounting to ~34% of the total N stored the previous year. After bud break, emerging leaves restart the sap flow, which increased nitrate uptake to support the regrowth. N-pulse and N-supply treatments were found to have significant effects shortly after a growth period, i.e. by increasing N content of all tissues (e.g. 37 and 81% in new shoots respectively), leaf area (18 and 29%) and specific leaf area (20 and 35%). Therefore, results confirm the hypothesis that early N supply plays a significant role in the N status and N remobilisation involved in the spring regrowth of young trees.

**Additional keywords:** absorption, nitrogen, Poplar, regrowth, remobilisation, spring.

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## Introduction

Storage processes for nitrogen (N) and carbon (C) compounds are key factors to the growth, development and longevity of trees (Dickson 1989; Dyckmans and Flessa 2001; Cheng and Fuchigami 2002; Millard and Grelet 2010; El Zein *et al.* 2011*b*). For caducifoliate species, the remobilisation of nutrients from roots and the aerial parts of the plants provides necessary nutrients for the growth of leaves through bud break as well as the reactivation of the cambial growth of twigs and trunks (Sauter and Vanclève 1994; Bausenwein *et al.* 2001; Landhäusser and Lieffers 2003; Wong *et al.* 2003). During the bud-break period, plants start to induce several mechanisms to support the full growth season of whole-plant structures, such as the return of water flow through transpiration, the translocation of organic and mineral elements and the regulation of the uptake of ions and water from fine roots (Imsande and Touraine 1994; Ameglio *et al.* 2002; Guak *et al.* 2003; Guérin *et al.* 2007). These mechanisms have been widely described in the literature and vary in expression according to the tree species, age, the climate

of previous years and the growth conditions of the tree (Vizoso *et al.* 2008).

Physiological processes supporting N remobilisation and N uptake by tree roots have been widely studied (Tagliavini *et al.* 1997; Malaguti *et al.* 2001; Grassi *et al.* 2003; Guak *et al.* 2003; Millard *et al.* 2006). Some of these studies used the tree species *Juglans nigra* L. × *Juglans regia* L. (Frak *et al.* 2002) and *Malus domestica* Borkh (Malaguti *et al.* 2001) and illustrated their capacity to concomitantly initiate N uptake and N remobilisation processes in the spring. The relative contribution of N remobilisation and N uptake processes during early bud break depends on the physiological N status and the N requirements of the tree. These conditions change in relation to the development stage of the tree. In addition, the developmental stage of trees is also linked to the quality of young shoots via the mass flow effect of transpiration (Dyckmans and Flessa 2001; Malaguti *et al.* 2001; Grassi *et al.* 2002, 2003; Dong *et al.* 2004; Millard *et al.* 2006; El Zein *et al.* 2011*a*).

Some tree species, such as *Acer pseudoplatanus* L. (Delaire 2005), *M. domestica* Borkh. (Dong et al. 2001) and *J. regia* (Ewers et al. 2001) can take up significant amounts of N before bud break and without leaves transpiration. During the growth season, N uptake impacts the compatibility of active transportation and metabolic activity in roots in response to suitable environmental conditions for plant growth, such as warm temperatures and the availability of N and water in the soil (Dong et al. 2001). However, there are few references in the literature concerning root N uptake before bud break (Delaire 2005). The particular role of N uptake is often neglected in this instance, whereas it could take place in the physiological processes during the transition period from bud dormancy to spring regrowth and the restarting of mass flow.

The experimental design required to study the effect of N uptake before bud break is difficult to achieve in a natural environment because of a limited access at the whole-plant level (roots) and the nitrogen flow inside the soil. Although a natural site provides a range of trees for experiments, these trees are unable to achieve with credibility on some experiment processes, such as balancing the full N content with N uptake and N remobilisation, but using artificial environmental growth conditions can constraint plant and root growth (e.g. container cultivation) and secure chemical and physical parameters to calculate a nitrogen balance. In the present study, we set up experiments in a greenhouse controlled environment using one-year poplar scion (*Populus tremula* L. × *Populus alba* L., clone INRA 717–1B4). Trees were grown in a recirculating nutrient solution (Beaujard and Hunault 1996) to conduct a systemic approach. The experimental design consisted in three treatments, the ‘control’ treatment without N, the ‘N-pulse’ treatment with trees that received N only before bud break and the ‘N-supply’ treatment included trees that received nitrate throughout the experimental period.

The objective of this study was to separate the effect of N uptake before bud break from the N uptake during the start of regrowth-induced transpiration by: (i) correlating the results from the architectural development and the variation of the N concentration of young trees during second-year regrowth and transpiration; as well as (ii) estimating the variation in the total N of each tissue component by modelling N uptake and remobilisation.

## Materials and methods

### Plant material

One-year-old poplar trees (*Populus tremula* L. × *Populus alba* L., clone INRA 717–1B4) originating from *ex vitro* micro-cuttings that were rooted in the spring of 2008 (Galopin et al. 1996) were used as the starting material. Cuttings were cultivated for 1 year in cylindrical containers (diameter × height = 0.2 × 0.3 m) filled with perlite (neutral substrate) and covered with waterproof sand (layer 1 cm, Mursain (GEB SAS, Tremblay en France, France) silex layer, 3-mm grain) to limit evaporation and algae development. Plants were grown in a greenhouse for one growing season in a recirculating nutrient system (six plants per system) and stored immediately after leaf fall in a frost-free greenhouse over the winter. During the

second growing season, a nutrient supply was provided through an automatic drip-irrigation system connected to a tank solution (80 L for six plants) that was recirculated for 1 h six times per day (at 2, 6, 10, 14, 18, 22h) to ensure a non-limiting supply of water and to equilibrate ionic concentrations around the roots. The composition of the nutrient solution was as follows (mmol L<sup>-1</sup>): 1.82 NO<sub>3</sub><sup>-</sup>, 0.19 H<sub>2</sub>PO<sub>4</sub><sup>-</sup>, 0.24 SO<sub>4</sub><sup>2-</sup>, 1.00 K<sup>+</sup>, 0.39 Ca<sup>2+</sup> and 0.355 Mg<sup>2+</sup>, added with micro-nutrients (μmol L<sup>-1</sup>) 20.6 B, 0.65 Cu, 10.7 Fe, 11.6 Mn, 0.28 Mo, 3.20 Zn and EDTA for chelation. Solutions of tanks were renewed every week until 3 September 2008. During the autumn and winter, the environment of roots was kept free of N (see ‘neutral substrate’ above) and were supplied with the following nutrient solution that was less of nitrate: (mmol L<sup>-1</sup>) 0.19 H<sub>2</sub>PO<sub>4</sub><sup>-</sup>, 0.665 SO<sub>4</sub><sup>2-</sup>, 0.97 Cl<sup>-</sup>, 1.00 K<sup>+</sup>, 0.39 Ca<sup>2+</sup>, 0.355 Mg<sup>2+</sup> added with micro-nutrients before-mentioned. New solutions and renewals of the tank content were made every week until the start of the experiment.

The experiment was started on 3 March 2009 (62nd day of year (DOY)) in a temperature-controlled greenhouse (18–20°C). The experimental design consisted in three treatments, the ‘control’ treatment included trees that received nutrient solution without nitrate; the ‘N-pulse’ treatment included trees that were fed with a nutrient solution containing nitrate for 7 days before bud break and then fed with a nutrient solution without nitrate for the rest of the experimental period and finally the N-supply treatment included trees that received a nutrient solution containing nitrate throughout the experimental period. Each treatment contained five trees that were planted within a recirculating nutrient system. The compositions of the nutrient solutions are those given in ‘Plant material’ above.

### N uptake

N uptake was computed from the temporal variation in the quantity of N within the recirculating nutrient system (Beaujard and Hunault 1996). Sample volumes of the nutrient solutions were 0.06 L and the volume of tank was measured twice a day at 1000 and 1800 hours. The samples were analysed on an HPLC Metrohm Bioscan system (Metrohm France-91942, Courtaboeuf, France) with a Metrosep C2 250 × 4.0 mm column. The quantity of N taken up by trees was estimated by the model by Beaujard and Hunault (1996), as follows:

$$Q_{\text{abs}(n)} = \frac{1}{N} \left( - \sum_{i=1}^{i=n} [C_{i-1} \cdot (\Delta V - V_e) + \Delta C \cdot (V_i + V_p)] \right), \quad (1)$$

where  $Q_{\text{abs}}$  (mM tree<sup>-1</sup>) is the cumulative N uptake from the start of the experiment to time  $n$ ;  $C_{i-1}$  (mM L<sup>-1</sup>) is the concentration of N in the tank at sampling time from  $i-1$ ;  $\Delta C$  and  $\Delta V$  is the variations in concentration and volume, respectively, between two sampling times  $T_{i-1}$  to  $T_i$ ;  $V_e$  (L) is the sample volume used for laboratory analysis;  $V_i$  (L) is the solution volume in the tank at the sampling time  $i$ ;  $V_p$  (L) is the volume of the residual solution retained in the substrate of all containers.  $N$  is the number of plants in the recirculating nutrient system.

To ensure data continuity when the nutrient supply was renewed, Eqn 2 was used to compute the fictitious concentration present at the time of renewal:

$$C'_i = \frac{(C_r V_r + V_p C_p)}{V_r + V_p}, \quad (2)$$

where  $C'_i$  ( $\text{mmol L}^{-1}$ ) is the N concentration in the tank like a balanced average of N content in the system,  $C_r$  ( $\text{mmol L}^{-1}$ ) is the concentration of the new solution,  $C_p$  ( $\text{mmol L}^{-1}$ ) is the concentration of the last sample before nutrient solution renewal as so the nutrient solution concentration staying in substrate at the time of nutrient renewal and  $V_r$  (L) is the tank volume of the new solution. Errors on results can be calculated for each step from the uncertainties of measurements of the tank volume ( $\pm 0.5\%$ ) and nitrate concentration ( $\pm 2.5\%$ ) (Beaujard and Hunault 1996).

#### Bud break monitoring

Readings of bud morphology were collected every day during the experiment. Date of bud break was determined as the point at which the first leaf started to emerge from the bud scales. Number of leaves and their area (using a LI-30004 portable area meter, Li-Cor Inc., Lincoln, NE, USA) were measured 15 days after bud break.

#### Sap flow

Leaves growth during early bud break restarts the transpiration and allows the xylem sap flow and the translocation of organic and mineral elements from storages and from the roots to new shoots. Therefore, the sap flow was measured from the start of the experiment until the end to investigate the pattern of water transpiration using a heat balance method, which is based on the energy balance of a stem segment to heat energy supplied by an external annular heater (Sakuratani 1984; Baker and Van Bavel 1987; Valancogne and Nasr 1989).

#### Plant harvest

Three plants were sampled at the start (2 March 2009, 61 DOY) and 15 plants (five per treatment) were sampled 15 days after bud break (8 April 2009, 98 DOY). The length of their main stems were measured *in situ* and diameters of each of them were measured at 20 cm from the base, at the middle of the stem and at 20 cm from the top to allow further calculations of the whole-scion content. Each stem was also sampled at three levels to measure in laboratory weight humidity and chemical contents. For each sample, length and diameter of fresh material and DW were measured both before and after splitting off the stem bark (SB) and stem xylem (SX) to later determine density of dry matter ( $\text{g cm}^{-3}$ ), density of N ( $\text{g cm}^{-3}$ ) and N content ( $\text{g g}^{-1}$ ) of tissues. Roots were separated into two groups: fine roots (FR, diameter  $< 2$  mm) and big roots (BR, diameter  $> 2$  mm). Each group was sampled for N analysis and remains were dried to determine dry matter and N content of each group of roots. At the end of the experiment, samples of new axillary shoots (NS) were harvested from each scion at the 5th, 15th and 30th internodes from the apex to determine their features as described above (dry matter and N content). The remaining samples of each of these groups were also dried and singly weighted to give total biomass and N content. All samples were placed in liquid nitrogen to stop the metabolism, freeze-dried, weighted, ground to a fine powder and finally stored at

$-75^\circ\text{C}$  before further analyses. The remaining tissues were dried in a hot air oven at  $75^\circ\text{C}$  and weighted. DW of the full stem bark and the full stem xylem were computerised as the sum of products of volumes of truncated cones (generated with morphological measurements of scion) by their respective densities (given by analysis of the trunk sampling as the dry weight of a sample divided by its volume).

#### N analysis and N mobilisation

Elementary N, given as the sum of all forms of nitrogen (including reserves), was analysed from all defined samples (FR, fine roots; BR, big roots; SX, xylem of the main axis; SB, bark of the axis; NS, new shoots). Aliquots of 2–3 mg milled material were weighed into tin cartouches (Hekatech, Wegberg, Germany) and assayed with an elemental analyser EA1108 (Carlo Erba Strumentazione, Rodano, Milan, Italy). Atropine (4.816% N; Carlo Erba Strumentazione) was used as a standard.

N mobilisation to new growth in the spring depends on the current N supply and on the amount of storage N, which is remobilised for new growth. When N is not provided during the regrowth period, all N that supports new growth derives from N remobilisation (control treatment). However, if plants take up N before or during bud break, then the N that supports new growth derives from both N remobilisation and N uptake (N-pulse and N-supply treatments).

The relative N net flow of each tissue (as a compartment for modelling) used to support new auxiliary shoot growth was estimated by the difference in the N level of each compartment before and after bud break. To consider variations of the initial size between plants at the start, relative N net flow of each compartment was determined in two steps. First, N content in each compartment of tested plants (control, N-pulse and N-supply) at the start of the experiment was calculated under the assumption that N proportions in tissue compartments would be the same as those determined for the harvested plants at the start of the experiment, assuming that young trees were grown in the same environmental conditions (Eqn 3):

$$A = \frac{C_{\text{Ref}(n)} \cdot \text{DW}_{\text{Ref}(n)}}{\sum_1^p (C_{\text{Ref}(n)} \cdot \text{DW}_{\text{Ref}(n)})} \quad (3)$$

$$N_{\text{Start}(n)} = A \cdot \left( \sum_1^p (C_{\text{End}(n)} \cdot \text{DW}_{\text{End}(n)}) - N_{\text{Up}} \right),$$

Where  $N_{\text{Start}(n)}$  is the estimated N (g) of each compartment ( $n$ ) before bud break at the beginning of the experiment,  $C_{\text{Ref}(n)}$  and  $\text{DW}_{\text{Ref}(n)}$  are the start reference of N content ( $\text{g g}^{-1}$  DW) and DW (g), respectively, of each tissue,  $C_{\text{End}(n)}$  and  $\text{DW}_{\text{End}(n)}$  are the end values of N content ( $\text{g g}^{-1}$  DW) and DW (g), respectively,  $N_{\text{Up}}$  is the N uptake during the experimental period (g).

In the second step, the relative N net flow ( $N_{\text{net\_flow}(n)}$ ) of each compartment before bud break used to support new shoot growth after bud break was calculated by computerising the N content of each compartment at the start (reconstruction) and at the end of the experiment (Eqn 4). In this calculation, negative values indicate an export of nitrogen from tissue and an import for positive values:

$$\%N_{\text{net,low}(n)} = \left(1 - \frac{N_{\text{End}(n)}}{N_{\text{Start}(n)}}\right) \times 100, \quad (4)$$

Where  $N_{\text{Start}(n)}$  and  $N_{\text{End}(n)}$  are the N level of each tissue component at the start and the end of the experiment respectively.

### Statistics

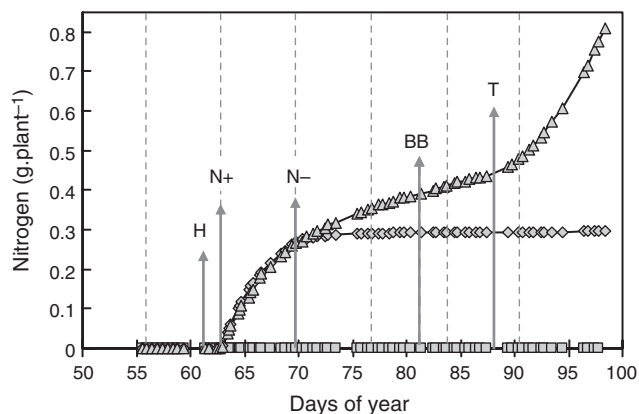
The cumulative N uptake per plant at the end of the experiment was an input of the relative net flow calculation. It was given by the use of the previous working out of modelling of the recirculating nutrient system that used five plants growing on the same system in this case. All data were subjected to analysis of variance. Differences between treatments were assessed by Duncan's multiple-comparison test at the 0.05 probability level. All statistical analyses in this study were performed using the Statistical Analysis System program (SAS, Cary, NC, USA).

## Results

### N uptake

Measurements of cumulative nitrate uptake showed that young poplars could significantly take up nitrate before bud break (Fig. 1). In the N-pulse treatment, nitrate uptake rapidly occurred after nitrate was supplied as a pulse and amounted to 0.30 g of N per plant after 7 days. Nitrate uptake ceased after that period. Trees from the N-supply treatment initially took up nitrate at rates similar to trees from the N-pulse treatment, to reach a rate of 0.39 g of N per plant. However, uptake by trees from the N-supply treatment was still significant after this period, although the rate was lower.

After bud break, nitrate uptake (Fig. 1) by trees from the N-supply treatment dramatically increased soon after the transpiration rate has started to increase (Fig. 2). The last



**Fig. 1.** Cumulative nitrate uptake ( $\text{g plant}^{-1}$ ) of poplar trees from the control ( $\square$ ), N-pulse ( $\diamond$ ) and N-supply ( $\triangle$ ) treatments during the time course of the experiment. The range of uncertainty for each point calculated with errors on measures of volume ( $\pm 0.5\%$ ) and nitrate concentration ( $\pm 2.5\%$ ) was  $\pm 4\%$ . Arrows indicate the point at which temperature was increased in the greenhouse (H); the start of the nitrate-feeding period for the N-pulse and N-supply treatments (N+); the end of the nitrate-feeding period for the N-pulse treatment (N-); the bud break period (BB); and the start of transpiration (T). Dashed lines mark the renewal of nutrient solution in tanks.

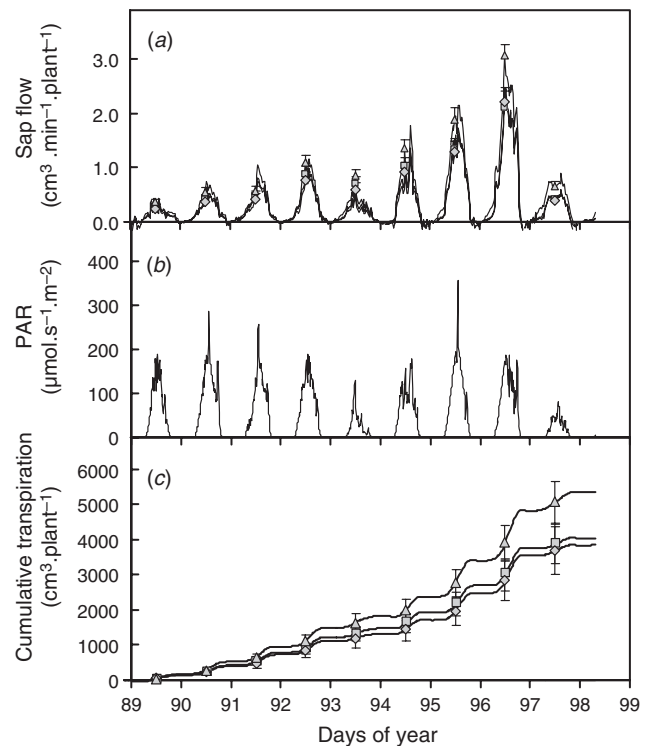
harvest occurred when all leaves existing in buds (8–10) at the start of experiment have been emitted. The amount of nitrate taken up at the end of the experiment by trees from the N-supply treatment amounted to 0.81 g of N per plant.

### Plant architecture

One-year-old poplar scions were 2 m of length and comprised an average of 60 buds. A statistical analysis showed that the amount of new shoots as well as the number of leaves per plant did not differ between the three treatments (Table 1). Analysis of the bud break profiles showed that 50% bud break occurred 19 days (81 DOY) after the beginning of the experiment (62 DOY), whatever the treatment (Fig. 3). In contrast, nitrate treatment increased the leaf area of new shoots (NS) with a statistically significant effect for N-supply.

The leaf area of trees from the N-pulse and N-supply treatments increased by 18 and 29%, respectively, compared with trees from the control treatment. Similarly, the specific leaf area (SLA) of trees from the N-pulse and N-supply treatments was higher than the control trees by 20 and 35% respectively. These two parameters were statistically different between treatments (Table 1).

The changes in dry biomass of the whole plant including new shoots during the regrowth period were not significant between treatments (Fig. 4a). However, the biomass of each compartment at the start of experiments compared with them

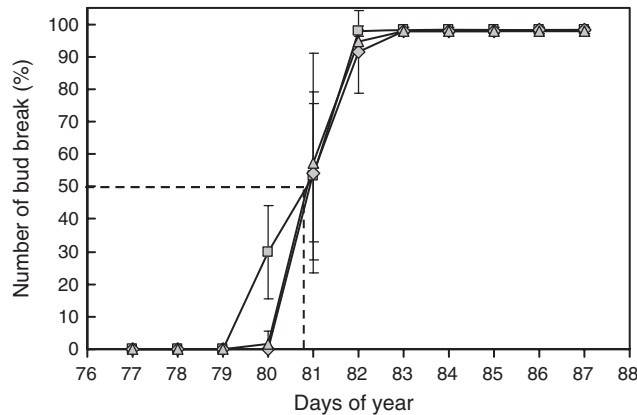


**Fig. 2.** Sap flow (a), light intensity (b) and cumulative water transpiration (c) during the time course of the experiment. Sap flow and water transpiration were measured on poplar trees from control ( $\square$ ), N-pulse ( $\diamond$ ) and N-supply ( $\triangle$ ) treatments. The values represent the mean  $\pm$  s.d. of three plants.



**Table 1. Plant growth parameters for poplar trees for the control, N-pulse and N-supply treatments measured 15 days after bud break**  
Data are means  $\pm$  s.d. of five plants per treatment. *P*-values: ns, not significant; \*\*, *P* < 0.01

	Number of new shoots	Number of leaves	DW of leaf lamina (g)	Leaf area (cm <sup>2</sup> leaf <sup>-1</sup> )	Specific leaf area (cm <sup>2</sup> g <sup>-1</sup> )
Control	44 $\pm$ 3	352 $\pm$ 42	13.93 $\pm$ 1.94	13.2 $\pm$ 0.5	335 $\pm$ 14
N-pulse	44 $\pm$ 3	371 $\pm$ 17	14.86 $\pm$ 1.12	15.8 $\pm$ 0.8	394 $\pm$ 18
N-supply	46 $\pm$ 4	378 $\pm$ 29	15.48 $\pm$ 1.16	17.8 $\pm$ 1.1	433 $\pm$ 7
<i>P</i> -value	ns	ns	ns	**	**



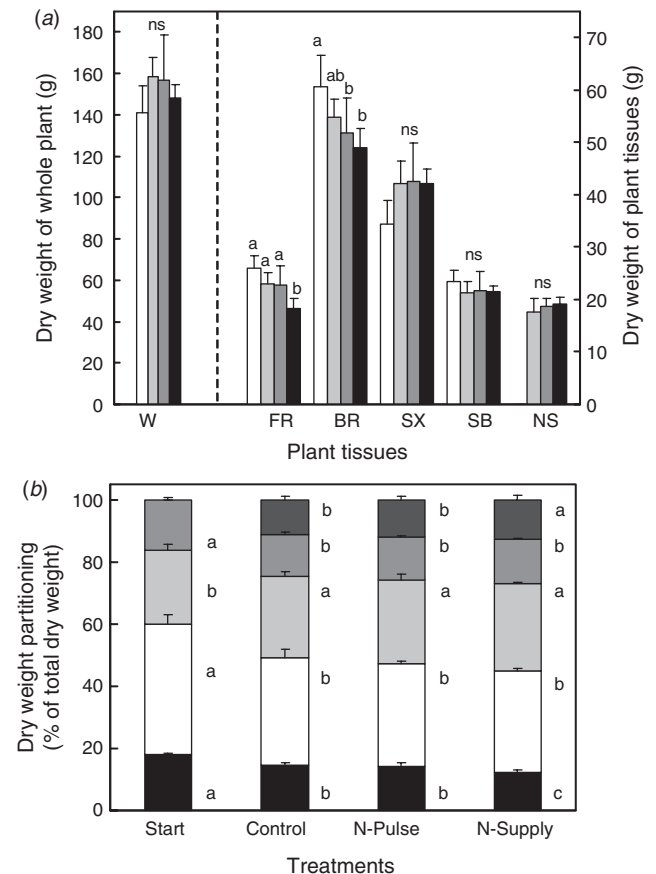
**Fig. 3.** Time course of bud break (%) for poplar trees from the control (□), N-pulse (◇) and N-supply (△) treatments. Bud break was defined as the point at which the first leaf started to emerge from the bud scales. The values represent the mean  $\pm$  s.d. of five plants.

after bud break significantly changed. Before bud break, the aerial biomass of start plants was lower than their root biomass, comprising 40 and 60% of the total DW respectively. In contrast, after bud break, the aerial biomass was always higher than the root biomass. Aerial and root biomasses amounted to 51 and 49%, 53 and 47%, 55 and 45%, for trees from control, N-pulse and N-supply treatments respectively (Fig. 4b). The change in the aerial biomass at the end of the experiment was mostly influenced by the increase in the biomass of new shoots (NS) but also by the increase of biomass of the stem xylem (SX). Some matter was likely to have been transferred from root compartments to aerial parts by the sap flow as shown by the conservation of biomass throughout the experiment.

Most importantly, N-fed trees also exhibit changes in DW partitioning compared with control trees. Fine root biomass (FR) of trees from the N-supply treatment was significantly lower than control trees (*P* < 0.01) by 16% whereas N-pulse biomass of fine roots was intermediate. The same tendency was found with the big root compartment (BR, Fig. 4b).

#### Sap flow

During early bud break, water transpiration could not be measured by the heat balance method, as the leaf area of new shoots was too small and gave a very low transpiration rate. Later, 7 or 8 days after bud break and at the start of the real expansion of lamina, the sap flow could be measured. Results suggest that xylem sap flow of the 1-year-old poplar scion was positively correlated with the leaf area and also depends on the



**Fig. 4.** Plant tissue dry weight (a) of the whole plant (W), or of individual tissues: fine roots (FR; diameter  $\leq$  2 mm), big roots (BR; diameter > 2 mm), stem xylem (SX), stem bark (SB) and new shoots (NS) from poplar trees at the start of the experiment (Start, □), or from the control (□), N-pulse (■) and N-supply (■) treatments. DW partitioning (b) in fine roots (FR), big roots (BR), stem xylem (SX), stem bark (SB) and new shoots (NS) from poplar trees at the start of the experiment (Start), or from the control, N-pulse or N-supply treatments. The values represent the mean  $\pm$  s.d. of three plants of Start and five plants of control, N-pulse and N-supply. Data for control, N-pulse and N-supply trees were collected 15 days after bud break. Different lower case letters indicate differences between treatments (*P* < 0.05).

changes in PAR as light density (Fig. 2a, b). Sap flow increased with the growth of leaves and 15 days after bud break, the cumulative water transpiration of trees from the N-supply treatment was higher than that of trees from the N-pulse and control treatments by 39 and 32% respectively (Fig. 2c).

*N content of plant tissue compartments*

The N content of whole plant (W) before bud break (Start) was significantly lower ( $P < 0.01$ ) than that of trees from the control, N-pulse and N-supply treatments (Fig. 5). After bud break, the N content of the whole plant increased when N was supplied to trees either as a pulse, or continuously, resulting in a 20 and 70% increase for N-pulse and N-supply trees, respectively, compared with the control trees.

When comparing the different compartments, the N content of new shoots of N-pulse trees was 32% (significance  $P < 0.01$ ) higher than that of the control trees (Fig. 5). The N content in other plant tissues (i.e. fine root, big root, stem xylem and stem bark) of N-pulse trees was not significantly different from the control trees. Additionally, the N content of N-supply trees significantly increased in fine roots ( $P < 0.05$ ), big roots ( $P < 0.01$ ), stem bark ( $P < 0.01$ ) and new shoots ( $P < 0.01$ ) by 16, 76, 41 and 37%, respectively, compared with N-pulse trees. The N content in those plant tissues was also higher than that in the control trees by 45, 32, 86 and 81% respectively. In contrast, the N contents in stem xylem after bud break of trees from the control, N-pulse and N-supply treatments were not significantly different (Fig. 5).

*N net flow of plant tissue compartments*

Nitrogen needs during spring regrowth should be fulfilled by an exogenous contribution and by the mobilisation of endogenous N. In our experimental design, trees from the control treatment relied only on internal remobilisation of N stored in plant tissues. Trees from the N-pulse and N-supply treatments relied on both sources.

Using Eqn 4 described above, we were able to estimate the N net flows (NNF) in each compartment, expressed as N percent of N accumulated during the previous year. As a general rule, a

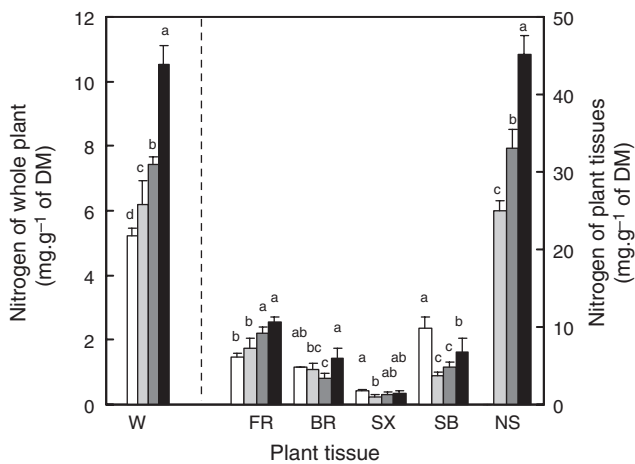
negative net flow indicates N export from a compartment to new shoots whereas a positive value indicates the N load of a compartment from other compartment and N uptake. The total N net flows in trees from the N-supply treatment were statistically different from NNF from the control or N-pulse treatments (SUM, Fig. 6). Total N mobilisation to new growth in control trees amounted to 45% of the total N stored during the previous year. In contrast, the total N net flow that was mobilised to growth of new shoots represented 38 and only 7% of the total N storage in N-pulse and N-supply trees respectively. The differences for NNF between treatments is exemplified in individual tissues, where most tissues exhibited lower NNF for N-pulse and N-supply trees compared with control trees, except for NNF in big roots and stem xylem from the N-pulse treatment (Fig. 6).

**Discussion**

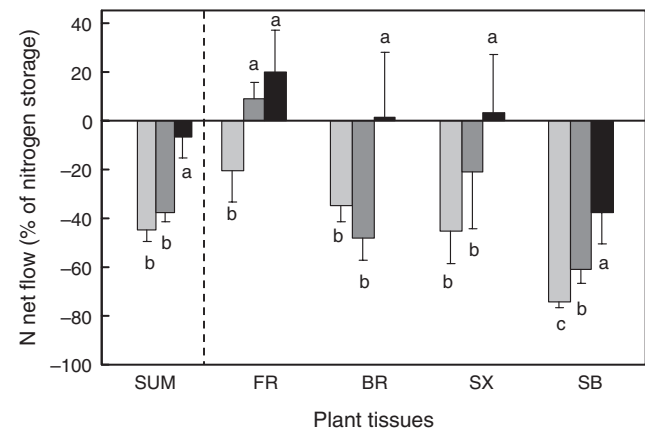
*N uptake before bud break*

The data presented here show that poplars can efficiently take up N before bud break and that this N uptake may account for 34% of the total N stored the previous year. This result is consistent with findings of previous studies obtained with young maple tree (Delaire 2005) and 1-year-old apples tree (Dong *et al.* 2001). Plants that received nutrient solution with nitrate throughout the time course of the experiment (N-supply) could significantly take up N before bud break only within a 10 day period after the start of the nitrate feeding time. Thereafter, N uptake declined until the emergence of new leaves. Leaf emergence, concomitant to the start of transpiration, induced a striking increase in N uptake. Based on this data, we may conclude that N uptake in spring may be controlled and affected by internal factors linked to growth and development.

For instance, our findings indicate that N taken up before bud break cannot be transported by the xylem to other tissues until



**Fig. 5.** Nitrogen content of the whole plant (W), or of individual tissues: fine roots (FR), big roots (BR), stem xylem (SX), stem bark (SB) and new shoots (NS) from poplar trees at the start of the experiment (Start, □), or from the control (▨), N-pulse (▩) and N-supply (▪) treatments. The values represent the mean ± s.d. of three plants from the Start and five plants from the control, N-pulse and N-supply treatments. Data for control, N-pulse and N-supply trees were collected 15 days after bud break.



**Fig. 6.** Nitrogen net flow (percent of nitrogen storage of tissue) for the sum of component tissues (SUM), or of individual tissues: fine roots (FR), big roots (BR), stem xylem (SX) and stem bark (SB) from poplar trees for the control (□), N-pulse (▨) and N-supply (▩) treatments. Data for control, N-pulse and N-supply trees were collected 15 days after bud break. The values represent the mean ± s.d. of five plants. Different lower case letters indicate differences between treatments for a given tissue ( $P < 0.05$ ).

plants have developed an efficient sap flow that occurs with leaf transpiration (Dong *et al.* 2001; Frak *et al.* 2002; Salaün *et al.* 2005; Millard *et al.* 2006; Guérin *et al.* 2007). In addition, N compounds (e.g.  $\text{NO}_3^-$ ,  $\text{NH}_4^+$ , amino acids) accumulated in roots before bud break could have a strong regulatory function, such as feedback inhibition of nitrate uptake and nitrate reduction (Gessler *et al.* 2004; Dłuzniewska *et al.* 2006; Miller *et al.* 2008). Since we observed the same effect on N uptake with ammonium as a N source (data not shown), the effects are most probably linked to the products of nitrate and ammonium assimilation (i.e. amino acids), rather than to the N source itself.

Other external factors, such as the temperature in the root zone (Ter Steege *et al.* 1999; Dong *et al.* 2001; van den Driessche *et al.* 2008; Yin *et al.* 2009) may as well be key determinant in N uptake by plants.

#### *Impact of N supply on the quality of the young shoot*

During regrowth during early spring, the auxiliary buds of 1-year-old poplar scion were broken – usually forming new shoots earlier than the apical bud. This process of regrowth could prepare the main axis to conduct the development of the tree as apical growth and cambial activities. Generally, auxiliary buds are considered as sink sites that require nutrients derived from the mobilisation of storage compounds from other tissues. Some studies have found that C and N storage remobilisation plays a major role in supporting the bud break processes and further growth of new shoots (Dickson 1989; Dyckmans and Flessa 2001; Cheng and Fuchigami 2002; Millard and Grelet 2010; El Zein *et al.* 2011b). However, the results showed that N uptake by roots before and during bud break (under the N-pulse and N-supply treatments) can initially modify the quality of the young shoots with a change in some parameters such as N content and the leaf area involved to photosynthesis response and water efficiency.

Indeed, our data showed that N supply before or during bud break may influence some growth characteristics of new shoots, i.e. by increasing leaf area and SLA within 15 days after bud break. These changes represent an alternation of existing growth patterns by changing the rate of cell division and cell expansion and these mechanisms are affected by the N availability of leaves (Cooke *et al.* 2005). The increase in SLA and in leaf N content is consistent with the findings by Reich *et al.* (1998), Cooke *et al.* (2005) and Ripullone *et al.* (2003), that positively correlated the increase in SLA and N content to the chlorophyll concentration and photosynthetic activity.

In contrast, N supply did not modify the time course of bud breaking, which appears concomitantly in all treatments. It is possible that there is a limitation on the transportation of N accumulated in roots that comes from N uptake before bud break. Therefore, the accumulated N was not completely exported to buds until bud break, as the transpiration of plants increased. In addition, the accumulated N could neither perform functionally nor provide an important chemical that has an effect on bud break. In relation to root pressure, N supply before bud break can stimulate a positive sap pressure as observed with *Acer pseudoplatanus* (Delaire 2005) and may contribute to the rehydration of aerial parts (Ewers *et al.* 2001; Ameglio *et al.*

2002). Additionally, at the start of the experiment, 1-year-old poplar scions were relatively well hydrated. The sap quantity that is mobilised to refill the aerial tissues was, therefore, low and could not ensure a significant transport of nutrients from roots to activate pathways linking buds and root activities. Accordingly, the rehydration of the aerial parts of the tree by positive root pressure before bud break has no influence on the modification of the date and profile of bud break.

The N supply did not affect the number of leaves that emerged during the time course of the experiment. The number of leaves per shoot was equivalent to the quantity of the leaf primordia in the buds that were developed during the previous year and during the winter. Therefore, N uptake did not accelerate foliar biomass production from the primary meristem of axillary shoots. The physiological process of bud break is closely tied to the local N reserve within bud itself and the N reserve of the neighbouring tissues of the stems.

#### *Impact of N supply on N reserve*

Growth of new shoots in spring is the strongest sink for N remobilisation. Xylem sap has been proposed to be the main track for transporting N from exporting tissue (stem and root) to sinks, such as buds and new shoots during regrowth (Malaguti *et al.* 2001; Grassi *et al.* 2002; Grassi *et al.* 2003; Dong *et al.* 2004; Millard *et al.* 2006; El Zein *et al.* 2011a). In comparison with on 1-year-old poplar scion from the control treatment, the quantity of N used for the growth of new shoots was 45% of the total N present at the start of experiments and stored during the previous year. Nitrogen from stem bark and big roots, the main sources of N storage, was mobilised to support new shoot development, providing 52 and 30%, respectively, of the total N transferred to the new shoots. These results support previous findings that deciduous trees increase N concentrations in stems and roots during autumn, whereas the concentrations decrease during spring due to the growth of new shoots (Marmann *et al.* 1997; Dyckmans and Flessa 2001; Frak *et al.* 2002).

N uptake by young poplar trees before bud break has an effect on N net flow, N content, N reserves and remobilisation only 15 days after bud break, which is also an heterotrophic phase for C use. Our study showed that N net flow from whole trees from the N-pulse (38%) and N-supply (7%) treatments apparently contributes to the growth of new shoots with lower rates than the control (45%). However, N content in root tissues increased with N uptake. In addition, nitrate taken up before bud break by trees from the both N treatments induced an increase of the N content of new shoots. Our results do not clarify whether the increase in N content in new shoots shortly after bud break is due to N uptake or stimulation of N remobilisation to new shoots. Unfortunately, N uptake and N remobilisation in tissues could not be resolved in this experiment; further experiments using  $^{15}\text{N}$  are needed to answer this key question. Previous studies performed with *M. domestica* (Malaguti *et al.* 2001; Guak *et al.* 2003), *Prunus avium* (Grassi *et al.* 2003; Millard *et al.* 2006) or *Pyrus communis* (Tagliavini *et al.* 1997) demonstrated that N storage mobilisation for new shoot growth in the spring occurred before the plants may receive N that has been taken up by the roots from an exogenously supplied source. In addition, Millard *et al.* (2006) examined



10-year-old hybrid poplars in the field and supplied them with  $^{15}\text{N}$  at bud break but found that the  $^{15}\text{N}$  was not recovered in leaves until 36 days after bud break, which also corresponded to the autotrophic phase of C acquisition. Similar results were obtained with mature oaks (El Zein *et al.* 2011a). However, these studies did not clarify whether N taken up by roots may have impacted N remobilisation at the start of the spring regrowth.

In contrast, our study showed that sap mobilisation was functional within a 15 day period after bud break. The cumulative water transpiration by 15 days after bud break accounts for a 23- to 33-fold increase of the water capacity of the small trunk. With this water consumption, it is reasonable to hypothesise that sap mobilisation is sufficient to influence N transfer to new shoots that was taken up by roots or originated from remobilisation. In our experiments using nitrogen in the N-pulse and N-supply treatments, the contribution of the stem tissues appears slighter than in trees from the control treatment. At the start of regrowth, shoots use reserves immediately available and initiate xylem sap flow with the start of the transpiration. Gradually, N taken up and N remobilised from different tissues is transported in the sap flow throughout the plant. The transported N forms – mostly amino acids such as asparagine and glutamine in poplar (Couturier *et al.* 2010) – could be exchanged, captured from the xylem sap to supply protein synthesis in living cells or released from tissues to the xylem sap. These exchanges could be driven by the affinity of the tissues for N, which may induce autocatalytic processes for the release of N reserves and perhaps C reserves, along the xylem sap stream. According to our hypothesis, the appearance of a low N contribution of the scion (N net flow) to the regrowth of plants supplied with nitrate could be explain by a differed reload of these compartments in relation to the restarting of water flow through the plant and the nitrogen form of the xylem sap.

The contradictory findings reported by Millard *et al.* (2006) with mature poplar and El Zein *et al.* (2011a) with mature oak may be due to several reasons, such as tree characteristics and history. Our study used 1-year-old poplar scions that were grown in perlite culture in pots and under a controlled environment. Under these conditions, water and nutrients were unlimited and may have had an impact on the plant capability to take up N. Additionally, N taken up by the roots after bud break has a shorter distance to travel to the new shoot because young poplars have a smaller and shorter trunk than older trees. In addition, true history of plants during previous seasons could modify the N affinities of tissues along the path from roots to shoots given at the end a different partitioning of sinks. However, our study may well apply to tree nurseries where growth of young trees may be stimulated by the addition of fertilisers just before bud break and during spring regrowth, rather than applying fertilisers in autumn.

In conclusion, 1-year-old poplar scion can take up N before bud break and the N uptake has a significant effect on the variation increase of the total N (including reserves) in tissue components. Furthermore, the uptake in N before bud break increased the quality of new shoots during early bud break, particularly in the leaf area and the SLA and N contents of leaves, underlining the importance of N uptake before bud break. Future studies could extend to investigate the relative

contribution of N uptake before bud break on particular nutrients, such as carbon storage in plant organs that are remobilised to support early spring growth.

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