The early spring N uptake of young peach trees (*Prunus persica*) is affected by past and current fertilizations and levels of C and N stores

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In deciduous trees, shoot development in early spring is assumed to be achieved mainly at the expense of nitrogen (N) stores. Indeed, the possible compensation for poor autumn N storage by early spring N uptake has been little studied. We therefore determined the dynamics of spring N uptake in relation to spring N supply, carbon and N storage and shoot development. Young peach trees (*Prunus persica* L. Batsch, cv. ‘GF305’) were raised outdoors in a hydroponic set-up during the spring and summer, with an excessive N supply. During the autumn, half of the trees were then N limited. The following spring, the N supply remained either high or low, or changed from high to low or low to high. Between 6 March and 13 May, N uptake was measured automatically on an hourly basis, while shoot growth was monitored once a week. These in situ measurements were completed by three destructive harvests to assess organ composition in N and total non-structural carbohydrates (TNC). Until the end of April, N uptake was dependent on the autumn N treatment, being higher in trees that had been N limited in the autumn. Total non-structural carbohydrate mobilization was also higher in those trees that had lost at least 17 g TNC by 24 April, while TNC levels in non-limited trees remained stable or even rose. Shoot development, estimated by the number of elongated axes and leaves per axis, was also slightly delayed by an N limitation in autumn. After 24 April, N uptake rates increased notably under all treatments and was determined by the spring N supply. In trees receiving a high N supply in the spring, the uptake rates also displayed marked short-term variations. That reduced the differences between treatments and by 13 May no differences could be evidenced between the trees in terms of organ biomass and TNC and N contents, whatever the treatment. We concluded that in the early spring, N uptake may compensate for a deficit of N storage insofar as large quantities of TNC can be mobilized for that purpose.

**Keywords:** bud burst, nitrogen allocation, nitrogen fertilization, non-structural carbohydrate, shoot development.

**Introduction**

Deciduous trees display both common and specific characteristics of the uptake and management of carbon (C) and nitrogen (N) in relation to perennial growth. In particular, specific autumn storage behaviours have been evidenced for both resources. On the one hand, C storage has been observed in perennial organs (stems and roots) in the form of large accumulations of total non-structural carbohydrates (TNC), mainly starch (*Stassen et al. 1981, Kozlowski 1992, Jordan and Habib 1996*). On the other hand, N storage can be inferred from observations that some N is remobilized to perennial organs before leaf fall (*Cheng et al. 2002, Jordan et al. 2011*) and that the tree N content increases during late autumn after the end of shoot growth (*Millard and Proe 1993, Tagliavini et al. 1999, Cheng et al. 2004*). Indeed, young apple and peach trees can absorb up to 45% of their total N content...
within the 2 months preceding leaf fall (Cheng et al. 2002, Jordan et al. 2012).

At the same time, C and N acquisition also differ markedly. Indeed, photosynthetic C acquisition implies that, at leaf fall, trees will lose their only C captor. Therefore, all subsequent C requirements (maintenance respiration during the winter, spring growth flush at bud burst) rely exclusively on the remobilization of previously stored C. This theoretical viewpoint is corroborated by numerous studies that relate spring growth to the importance of autumn C stores (Stassen et al. 1981, Oliveira 1988). In contrast, N acquisition is reliant on the root system, and although the risk of young and fine-root mortality increases during the winter (Wells et al. 2002), most N captors of deciduous trees remain intact so that winter N uptake is possible if soil temperatures are adequate (Weinbaum et al. 1978, Dong et al. 2001). This therefore raises the question of whether winter and early spring N uptake can compensate for limited autumn N storage and contribute to early spring growth.

Some authors have considered that spring N uptake is only dependent on current soil N availability (Millard and Proe 1993, Dyckmans and Flessa 2001), while others think that it is influenced by N cycling (Niederholzer et al. 2001, Stephens et al. 2001). Opinions therefore differ markedly concerning the date at which spring N uptake becomes significant: at the start of N remobilization (El Zein et al. 2011), a few weeks after bud burst (Millard and Neilson 1989, Munoz et al. 1993, Grassi et al. 2003), at rapid shoot growth (Weinbaum et al. 1978) or when leaves exceed 50% of their full expansion (Deng et al. 1989). Such discrepancies arise mainly from mechanistic hypotheses underlying the control of N uptake: the exhaustion of stored N (Millard and Neilson 1989, Munoz et al. 1993, Grassi et al. 2003) or the onset of a significant flow of photosynthates into the roots (Deng et al. 1989). Moreover, although N uptake is a continuous process, it is mostly calculated as mean values over time periods that encompass a few crucial stages when destructive harvests occur, which therefore hampers our vision of N uptake dynamics.

An associated question concerns the effect of autumn N stores on spring regrowth. Indeed, the amount of cycling N is correlated to shoot growth during early spring likely by adjusting the number of leafy axes (Grelet et al. 2003, Jordan et al. 2009). This effect is known to decrease over time and may even disappear at the end of the first growth flush (Niederholzer et al. 2001, Grelet et al. 2003, Jordan et al. 2012). Since N uptake, N cycling and plant growth are intimately related, the contribution of spring N intake to the total N incorporated into current-year shoots and fruits may range from 10 to 74% by the end of April (Weinbaum et al. 1978, Millard and Proe 1993, Tagliavini et al. 1997).

To determine whether autumn stores affect the early spring processes involved in N uptake and growth, we associated the continuous monitoring of N uptake (Adamowicz et al. 2012) over successive seasons with in situ measurements of shoot development and biochemical characterizations of tissue nutrient status. To achieve this, young peach trees (Prunus persica L. Batsch, cv. ‘GF305’) were grown hydroponically at high N (HN) or low N (LN) concentrations during the autumn and spring. Reducing N supply in autumn strongly limited N uptake. As a consequence, the organ N concentration remained almost stable throughout the autumn treatment period in the LN trees but doubled in the HN ones. Starch also accumulated to a greater extent in the LN trees (detailed in Jordan et al. 2011). Spring N uptake dynamics will therefore be evaluated in relation to (i) tree nutrient status at bud burst, (ii) spring N supplies in the rooting medium, (iii) mobilization of C and N stores and (iv) shoot development.

**Materials and methods**

**Tree preparation**

This study was carried out at the INRA research centre in Avignon (South-East France, Lat: 43.95°, Long: 4.817°). On 2 May 2006, eighty 1-year-old peach rootstocks (cv ‘GF 305’) were planted outdoors in a hydroponic system (see below). Each tree had a single axis of 0.8–1 cm in diameter. Until 2 October 2006, they were grown according to the protocol described in Jordan et al. (2011). This mainly comprised severe pruning designed to homogenize both plant architecture and size (Figure 1). The first pruning was performed on 13 June, leaving only two newly grown secondary axes on the main axis. On 12 July, four tertiary axes (two per secondary axis) were selected and their apices removed, once again causing new buds to develop into axes of order 4. On 29 August, two axes of order 4 were selected on each tertiary axis and cut to a length of 30 cm. On each of them, only five buds (i.e., 40 per tree) were allowed to develop freely into axes of order 5.

From 2 October, 48 homogeneous trees were selected and maintained on the hydroponic system where they received a level of N supply that was either low (LNa) or high (HNa) throughout the autumn. Over that period, 12 plants were harvested to assess the effects of the autumn N treatment on tree growth, N and TNC contents (Jordan et al. 2011). On 2 February 2007, the 36 remaining trees were winter pruned, thereby removing 118 g (SE 6) dry weight (DW) on the LNa trees and 159 g (SE 12) DW on the HNa trees. Tree size and shape were rehomogenized, leaving only 16 axes of order 5 per tree (two per parent of order 4); these were trimmed down to 30 cm and their ramifications removed. Buds were allowed only to develop freely on the axes of order 5; on the others, they were removed after burst.

**Hydroponic set-up**

The hydroponic set-up involved the use of the nutrient film technique (NFT) to feed a nutrient solution to four independent
groups of 10 polyvinyl chloride (PVC) plastic troughs. The set-up was outdoors and the troughs were insulated with expanded polystyrene foam and wrapped in a white plastic sheet to prevent thermal shocks and N pollution from rain and dust. Each group received a full nutrient solution containing (mM): 1 NO$_3$, 1 H$_2$PO$_4$, 5.5 SO$_4$, 3 K, 3.5 Ca and 1.5 Mg. Micronutrients were added (0.1 ml l$^{-1}$ Kanieltra 6-Fe, Hydroazote, France) and completed by 16.5 mg l$^{-1}$ EDTA-Fe. This solution was pumped from four tanks (500 l) located in an underground laboratory. Each solution was injected at a constant rate of 2 l min$^{-1}$ into each trough, from which it drained back by gravity to the tank through PVC tubing. Each recycled solution was thoroughly mixed and aerated.

The temperatures, volumes, pH and nitrate concentrations of the solutions were controlled automatically and restored to preset values. Usually, analyses and corrections were performed on an hourly basis using the automatic set-up Totomatix (Adamowicz et al. 2012). The pH was maintained at 5.0. Corrections also ensured major cation repletion. The temperature was controlled at 23 ± 0.5 °C until 29 November and then modified to mimic the pattern measured in the soil (at a depth of 50 cm) at the local INRA weather station: gradual decrease to 11 °C until 20 December, 11 °C until 29 March 2007, gradual increase to 19 °C until 26 April and 19 °C thereafter.

The NO$_3$ uptake rate ($U$ in g day$^{-1}$ tree$^{-1}$) was calculated daily as

$$U = \frac{V_d \times C_d - V_{d+1} \times C_{d+1} + \sum_{t=d+1}^{t=n} I_t}{n} \times 14 \times 10^{-6} \quad (1)$$

with $C_d$, $V_d$, $n$ and $I_t$ being the respective solution [NO$_3$] (µmol l$^{-1}$), volume (l per tank), numbers of trees per tank on day $d$ at midnight and the volume of NO$_3$ (µmol l$^{-1}$) injected to maintain the set concentration at time $t$. The volumes $V_d$ and $V_{d+1}$ were calculated as the difference between their set value (500 l per tank) and the water added to restore the tank level (see details in Adamowicz et al. 2012).

**Nutrition and treatments**

During the preconditioning period (2 May to 2 October 2006), the four tanks contained the same full nutrient solution. On 2 October, we kept this solution in two of the tanks (HNa treatment), while the other two tanks were N limited (LNa treatment), adjusting periodically [NO$_3$] in the LNa treatment in...
order to maintain \(U_{\text{HNs}}/U_{\text{LNs}}\) close to 0.25 (see Jordan et al. 2011 for details). These autumn N regimes lasted until 5 December when this regulation was halted, so that \([\text{NO}_3^-]\) fell during the winter to 0.30 and 0.03 mM in the HLa and LNa tanks, respectively, as a consequence of N uptake.

On 12 March 2007, the solutions were renewed to initiate the spring treatments. Thus each tank received a renewed full nutrient solution containing either 1 mM \(\text{NO}_3^-\) (HNs treatment) or 0.05 mM \(\text{NO}_3^-\) (LNs treatment). In this way, throughout autumn and spring, our protocol established four conditions of contrasting N availabilities: either high, low or mixes from high to low or low to high, coded as follows: HNa/HNs, LNa/LNs, HNa/LNs and LNa/HNs. The regulation of \([\text{NO}_3^-]\) was performed automatically by the Totomatix set-up until 24 May 2007.

**In situ measurements**

Two axes of order 5, referred to parent axes and growing on the same axis of order 4, were selected on each tree. The development of their axillary buds was followed once a week between 2 April and 13 May 2007. Six trees per treatment were followed until the sampling on 24 April (see below); then this number was reduced to three.

In Rosaceae species, each vegetative axillary bud can either stay dormant or develop into a rosette (i.e., a short daughter axis) or a long daughter axis, which will in turn give rise to sylleptic ramifications. The numbers of rosettes, long daughter axes and ramified long daughter axes were noted. Leaves per developing bud were also counted, making a distinction between leaves inserted on the long daughter axis and those on its ramifications. Each developing bud was ranked according to its position (Figure 1) along the parent axis in the 0–10, 10–20 and the 20–30 cm (or 20-apex) segments, respectively.

**Tree samplings**

Four individuals (i.e., two per tank) from the HNa and LNa treatments were sampled on 6 March before the spring treatment application. Two further samplings of three trees per treatment (i.e., 12 trees per date) were taken on 24 April and 13 May, respectively, to assess tree N and TNC status. Before sampling, length and diameter were measured on each older axis (orders ≤4) and its volume was calculated.

The parent axes subjected to in situ measurements were collected with their developing buds and partitioned from base to top into three 10 cm segments (Figure 1). Leaves from the rosettes, the long daughter axes and the ramifications were separated from the current-year stems. An aliquot of roots (i.e., one main root and all its branches) was collected and washed with deionized water. The older axes were sub-sampled to make up a volume of \(-10\) cm\(^3\), set in proportion to the respective contribution of each order (1 to 4) to the total volume of the woody structure. All samples were frozen in liquid N\(_2\), and then kept at \(-20\) °C before freeze drying.

Leaf area was measured with an area meter (LI 3100, Li-Cor, Lincoln, NE, USA) on an aliquot of \(\sim1200\) cm\(^2\) (20 g DW). This sample was then oven-dried (80 °C) and weighed to determine the specific leaf weight (SLW, g m\(^{-2}\)). The rest of the tree was separated into: (i) leaves; (ii) stems of long daughter axes and ramifications; (iii) parent axes; (iv) older axes; and (v) roots. All samples were oven-dried (at 80 °C) and weighed (DW, g). The total leaf area of a tree was calculated from DW and SLW.

**Biochemical analyses**

All freeze-dried samples were finely ground in ball mills (MM301 and PM400/2, Retsch, Haan, Germany) cooled with liquid N\(_2\). The total N concentration was measured using an elemental analyser (Flash EA 1112, Thermo Finnigan, Milan, Italy). Soluble sugars and starch were determined as described by Gomez et al. (2007) and then added together to compute the total TNC pool. Tree N and TNC contents (g organ\(^{-1}\)) were calculated from organ DW (g), N and TNC concentrations (% DW). Tree N and TNC concentrations could then be calculated from tree DW, N and C contents. The parent and older axes were grouped in a single axis pool (orders 1–5).

**Data processing**

Randomization (or permutation) tests (Manly 1991) were used to evaluate the effects of N treatments on DW, total N, and TNC concentrations and contents at each harvest date. Empirical distributions of these variables under the null hypothesis of no treatment effect were derived from 2500 random assignments of the 12 trees to the four treatments (software R 2.11.0 software, www.r-project.org/). This random assignment was justified because the four groups of trees were not significantly different for these variables before treatment since the trees (i) had been raised under the same conditions, (ii) were equivalent in terms of size and (iii) were randomly allocated to groups. The test statistics were the six pairwise differences between the means of the variables per group. Two observed means were considered to be significantly different if their difference was in the distribution tails of the empirical distributions of these differences under the null hypothesis, i.e., in the 2.5% smaller or larger quantiles (62 smaller or larger values obtained by permutations). Permutation tests were performed independently for each harvest date, first at tree level and second for each tree compartment: roots, axes, stems and leaves.

Treatment effects on shoot development (i.e., leaf emission, numbers of rosettes, long daughter axes and ramifications) were evaluated by comparing the means of the four populations at each date (randomization tests). The organ position (rosettes, long daughter axes and ramifications) was compared on 13 May (\(\chi^2\) tests).

**References**

Jordan et al. 2011. Tree Physiol. 34, 61–75. The specific leaf weight (SLW, g m\(^{-2}\)) was performed automatically by the Totomatix set-up until 24 May 2007.

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Results

Nitrogen uptake

Two phases were identified in the spring N uptake. From 16 March to 20 April, N uptake remained low and responsive to the autumn treatment (Figure 2). Indeed, LNa trees which were N limited in the autumn absorbed N earlier and at higher rates than HNa trees.

During the second phase, after 20 April, N uptake rates ($U$, mmol day$^{-1}$ tree$^{-1}$) increased over time and responded to spring N availability since $U_{HNs}$ became higher than $U_{LNs}$. During this second stage, $U$ also displayed marked short-term variations, especially in HNs trees. Indeed, between 3 May and 9 May, $U_{HNs}$ decreased sharply. As a result, no significant differences were observed on 13 May in tree N uptake during the spring, whatever the N treatments (Figure 2).

The N uptake could be calculated by differences between the N content of the trees sampled at the different dates (6 March, 24 April and 13 May). These estimated N uptakes followed the same pattern, but on 13 May they were lower by ~11% than the N uptake measured in situ during the whole treatment period.

Shoot development

Bud burst was completed before 1 April. A mean number of 10.3 ± 0.37 (SD) vegetative buds developed on each parent axis, whatever the N treatment. Transformation of the initial rosettes into long daughter axes was then delayed for both LNa treatments, regardless of the N supply in spring. Thus, on 1 April, LNa trees developed less than two long daughter axes per parent axis, but HNa trees produced more than five (Figure 3a). The difference between treatments decreased over time, and disappeared once the final number of long daughter axes had set, i.e., after 30 April. The number of rosettes decreased concomitantly, until a mean value of 2.6 ± 0.64 per parent axis was reached.

The dynamics of expanded leaves (Figure 3b) is a good marker of daughter axis elongation, being delayed at first and then favoured by N limitation in autumn. Indeed, until the end of April, the number of expanded leaves was smaller in LNa than in HNa trees, but thereafter it gradually increased. At the last count on 17 May, both groups of LNa treatments bore significantly more expanded leaves per long daughter axis than HNa/HNs trees.

Ramification, i.e., the development of axillary meristems along previously emerged long daughter axes, started at around 7 May. The number of ramifications per long daughter axis and the number of expanded leaves on those ramifications were not affected by the treatments, but both numbers varied markedly in the population of long daughter axes (Table 1).

The treatments also affected axis topology, i.e., their positioning on their parents. However, these effects only concerned

Figure 2. Cumulative N uptake calculated from the analysis of nutrient solutions during the spring treatment period (6 March–13 May). Each solution was fed to seven trees until 24 April; this number was then reduced to four. Autumn LN treatments are plotted using broken lines, i.e., dot-dashed for the LNa/HNs treatment and long-dashed for the LNa/LNs treatment. Thin lines indicate that further N limitation was applied in the spring. Solid thick and thin lines represent the HNa/HNs and HNa/LNs treatments, respectively. On 6 March, 24 April and 13 May, the symbols (LNa/LNs: open circles, LNa/HNs: full circles, HNa/LNs: open triangles and HNa/HNs: full triangles) represent the total N uptake during this period, as calculated from the analysis of the sampled trees. The means were ranked (a), (ab) and (b) from the lowest to the highest value. They are significantly different if coded with different letters. Statistical significance was inferred from randomization tests based on the generation of 2500 random orders.
axillary buds (Table 2); rosettes and long daughter axis positions were unaffected (not shown). The LNs trees developed >60% of their ramifications on the distal segment, i.e., on the top 20 cm of long daughter axes. This percentage decreased to ~50% in HNs trees, which developed more ramifications on their basal segments, i.e., on 0–10 cm segment.

**Gross growth**

Total tree DW at harvest was never affected by treatments (Table 3), but the organ DW was altered. Thus, on 6 March, LNa trees had a higher root DW than the others. As a result, the root/shoot ratio was higher in LNa trees than in HNa trees.

On 24 April, the delayed shoot growth observed on LNa trees was confirmed by their lower leaf and stem DW when compared with HNa trees. The LNa trees also had a higher root DW, but the differences with HNa trees were reduced because root DW decreased between 6 March and 24 April in LNa trees while remaining stable in HNa trees. The DW of axes was not affected by the treatments but increased by 29 to 34% between 6 March and 24 April.

After 24 April, growth also concerned roots. Up to 13 May, root biomass increased by 0–36%, axis biomass by 17–25% and shoot biomass by 160–230%. As a result, no further difference in organ DW could be evidenced between the treatments on 13 May.

**Within-tree N allocation**

At bud burst, tree and organ N contents (Table 4) and concentrations (Table 5) were markedly affected by the autumn treatments, being lower in LNa trees. Then, between 6 March and April, root and axis N contents fell by ~40% in HNa trees but rose by 12 and 31%, respectively, in LNa/LNs and LNa/HNs trees. In LNa trees, N mainly accumulated in the roots, since axis N concentrations remained either stable (LNa/HNs) or fell (LNa/LNs). As a result, on 24 April, organ N contents were independent of treatment. Nitrogen concentrations were also higher in the leaves and stems of LNa trees, which displayed lower shoot growth than the others.

Between 24 April and 13 May, N contents increased in all organs, and on 13 May, the treatments showed no effect on

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**Table 1.** Long daughter axis ramifications: number of ramifications and of expanded leaves per grand-parent axis, i.e., parent axes of long daughter axes.

<table>
<thead>
<tr>
<th>Date</th>
<th>LNa/LNs</th>
<th>LNa/HNs</th>
<th>HNa/LNs</th>
<th>HNa/HNs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of buds</td>
<td>7 May</td>
<td>48.50 ± 9.32</td>
<td>62.12 ± 8.31</td>
<td>56.375 ± 16.85</td>
</tr>
<tr>
<td></td>
<td>13 May</td>
<td>73.83 ± 19.33</td>
<td>83.67 ± 14.63</td>
<td>52.83 ± 17.89</td>
</tr>
<tr>
<td>Number of expanded leaves</td>
<td>7 May</td>
<td>73.00 ± 13.76</td>
<td>91.62 ± 12.75</td>
<td>85.12 ± 25.85</td>
</tr>
<tr>
<td></td>
<td>13 May</td>
<td>136.66 ± 46.14</td>
<td>184.00 ± 40.81</td>
<td>94.00 ± 38.29</td>
</tr>
</tbody>
</table>

The results are the means ± standard errors of six replicates: three trees per treatment and two parent axes per tree. The means were not significantly different if coded with the same letter. Statistical significance was inferred from randomization tests based on the generation of 2500 random orders.

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**Table 2.** Relative distribution (as a percentage of the total) of the location of ramifications, i.e., total number of axillary buds along the long daughter axes.

<table>
<thead>
<tr>
<th></th>
<th>LNa/LNs</th>
<th>LNa/HNs</th>
<th>HNa/LNs</th>
<th>HNa/HNs</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–10 cm portion</td>
<td>15.3</td>
<td>14.1</td>
<td>32.5</td>
<td>23.8</td>
</tr>
<tr>
<td>10–20 cm portion</td>
<td>21.2</td>
<td>18.0</td>
<td>18.7</td>
<td>26.5</td>
</tr>
<tr>
<td>20–30 cm portion</td>
<td>63.6</td>
<td>68.0</td>
<td>48.8</td>
<td>49.7</td>
</tr>
</tbody>
</table>

χ² test

χ²: 17.4 (df = 6)

The axes were divided into three portions, and the proportions of developing buds in each segment then estimated.
Table 3. Organ and tree dry biomass (g organ⁻¹) as a function of treatment and sampling date.

<table>
<thead>
<tr>
<th></th>
<th>LNa/LNs</th>
<th>LNa/HNs</th>
<th>HNa/LNs</th>
<th>HNa/HNs</th>
</tr>
</thead>
<tbody>
<tr>
<td>a: Leaves</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 March</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 April</td>
<td>104* (4.53)</td>
<td>97.7* (2.95)</td>
<td>111* (9.40)</td>
<td>123* (5.84)</td>
</tr>
<tr>
<td>13 May</td>
<td>277* (25.8)</td>
<td>276* (8.49)</td>
<td>335* (67.9)</td>
<td>288* (57.3)</td>
</tr>
<tr>
<td>b: Stems</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 March</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 April</td>
<td>42.2* (4.51)</td>
<td>38.2* (0.71)</td>
<td>53.8* (7.06)</td>
<td>54.7* (4.08)</td>
</tr>
<tr>
<td>13 May</td>
<td>176* (13.4)</td>
<td>176* (6.12)</td>
<td>213* (35.4)</td>
<td>180* (30.0)</td>
</tr>
<tr>
<td>c: Axes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 March</td>
<td></td>
<td>154* (10.1)</td>
<td></td>
<td>145* (7.9)</td>
</tr>
<tr>
<td>24 April</td>
<td>276* (4.56)</td>
<td>316* (5.89)</td>
<td>220* (3.46)</td>
<td>214* (5.01)</td>
</tr>
<tr>
<td>13 May</td>
<td>270* (17.3)</td>
<td>302* (14.3)</td>
<td>292* (23.1)</td>
<td>251* (20.0)</td>
</tr>
<tr>
<td>d: Roots</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 March</td>
<td></td>
<td>204* (18.1)</td>
<td></td>
<td>152* (15.5)</td>
</tr>
<tr>
<td>24 April</td>
<td>189* (12.3)</td>
<td>180* (6.64)</td>
<td>153* (6.70)</td>
<td>143* (8.06)</td>
</tr>
<tr>
<td>13 May</td>
<td>203* (23.9)</td>
<td>217* (8.80)</td>
<td>209* (49.7)</td>
<td>143* (18.4)</td>
</tr>
<tr>
<td>Whole tree</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 March</td>
<td></td>
<td>358* (36.0)</td>
<td></td>
<td>296* (30.8)</td>
</tr>
<tr>
<td>24 April</td>
<td>497* (25.3)</td>
<td>474* (14.9)</td>
<td>483* (30.4)</td>
<td>486* (18.5)</td>
</tr>
<tr>
<td>13 May</td>
<td>885* (81.8)</td>
<td>914* (2.93)</td>
<td>1004* (187)</td>
<td>827* (126)</td>
</tr>
</tbody>
</table>

The numbers are means (in bold) and standard errors (within brackets) of three (24 April, 13 May) or four (6 March) replicates. The means were ranked (a), (ab) and (b) from the lowest to the highest value. They are significantly different if coded with different letters. Statistical significance was inferred from randomization tests based on the generation of 2500 random orders, performed at organ level for each sampling date.

Organ N contents. In most cases, the concentrations were lower in LNs trees than in HNs trees. The HNa/HNs trees, which were well fed throughout the autumn and spring, had the highest root and axis N concentrations, indicating that these trees accumulated N in their perennial organs.

Within-tree TNC allocation

At bud burst, LNa trees that had been limited in the autumn contained about twice as much TNC (55 vs 27 g TNC per tree) than the others. The roots were the main storage organ, and depending on the treatment contained 40 and 17 g TNC, respectively, the remainder being distributed between the axes in proportion to their biomass (Tables 4 and 5).

Between 6 March and 24 April, TNC contents decreased notably in LNa trees but increased slightly in the others. Allocation patterns were also altered, favouring growing shoots and depleting the roots and axes. Thus, on 24 April, shoots contained between 53 and 67% of total tree TNC. Allocation was proportional to shoot biomass, since the TNC concentrations were independent of the treatment, with few exceptions. The LNa/LNs trees that had undergone a double limitation displayed higher TNC concentrations in their stems and roots than others. In contrast, TNC losses were the highest in LNa/HNs trees that had the lowest TNC contents on 24 April.

Between 24 April and 13 May, TNC contents increased rapidly up to a mean value of 90 g tree⁻¹. The differences between treatments were limited to the stems and axes, in which the TNC contents and concentrations tended to be lower after N limitation in the autumn.

Discussion

Methodology

The N uptake dynamic in early spring was determined on an hourly basis, the aim being, first, to evaluate how far plants could compensate for less autumnal storage, thus increasing N uptake earliness or rates, second, to determine the stage at which N uptake became significant enough to be limited by spring N supply and, third, to relate early spring N uptake to plant TNC availability and growth. These results completed our previous ones focusing on the tree response to pre-conditioning treatment in the autumn (Jordan et al. 2011).

We measured instantaneous N uptake rates under controlled and constant N availability at the root surface. We were therefore able to observe short-term adjustments, such as the existence of two successive phases in N uptake regulation, which could not have been evidenced by budget studies performed over longer time steps. However, a slow increase appeared over time in the difference between the values of cumulative N uptake measured in situ and those calculated from tree sampling and organ N analysis. This deviation could not be attributed to leakage or a bacterial consumption of NO₃⁻ in the...
Table 4. Organ and tree N and TNC contents (g organ⁻¹, or g tree⁻¹) as a function of treatment and sampling date.

<table>
<thead>
<tr>
<th>Organ TNC content (g organ⁻¹)</th>
<th>Organ N content (g organ⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LNa/LNs</td>
<td>LNa/LNs</td>
</tr>
<tr>
<td>HNa/LNs</td>
<td>HNa/LNs</td>
</tr>
<tr>
<td>HNa/HNs</td>
<td>HNa/HNs</td>
</tr>
</tbody>
</table>

### a: Leaves
- **6 March**
  - TNC: 4.94±(0.34)
  - N: 3.89±(0.39)
- **24 April**
  - TNC: 4.60±(0.18)
  - N: 5.21±(0.20)
- **13 May**
  - TNC: 16.4±(0.72)
  - N: 10.8±(0.96)

### b: Stems
- **6 March**
  - TNC: 1.41±(0.14)
  - N: 12.2±(0.14)
- **24 April**
  - TNC: 4.24±(0.40)
  - N: 2.84±(0.04)
- **13 May**
  - TNC: 3.85±(0.09)
  - N: 2.62±(0.14)

### c: Axes
- **6 March**
  - TNC: 15.0±(0.15)
  - N: 1.02±(0.09)
- **24 April**
  - TNC: 6.66±(0.52)
  - N: 1.16±(0.04)
- **13 May**
  - TNC: 15.3±(0.29)
  - N: 1.51±(0.20)

### d: Roots
- **6 March**
  - TNC: 10.1±(0.08)
  - N: 4.39±(0.16)
- **24 April**
  - TNC: 4.39±(0.08)
  - N: 3.65±(0.14)
- **13 May**
  - TNC: 15.3±(0.19)
  - N: 3.16±(0.30)

### Whole tree
- **6 March**
  - TNC: 22.9±(0.36)
  - N: 11.1±(0.08)
- **24 April**
  - TNC: 11.9±(0.19)
  - N: 10.1±(0.09)
- **13 May**
  - TNC: 11.3±(0.06)
  - N: 7.72±(0.04)

The numbers are means (in bold) and standard errors (within brackets) of three (24 April, 13 May) or four (6 March) replicates. The means were ranked (a), (ab) and (b) from the lowest to the highest value. They are significantly different if coded with different letters. Statistical significance was inferred from randomization tests based on the generation of 2500 random orders, performed at organ level for each sampling date.

Table 5. Organ and tree N and TNC concentrations (% DW) as a function of treatment and sampling date.

<table>
<thead>
<tr>
<th>Organ N concentration (% DW)</th>
<th>TNC concentration (% DW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LNa/LNs</td>
<td>LNa/LNs</td>
</tr>
<tr>
<td>HNa/LNs</td>
<td>HNa/HNs</td>
</tr>
</tbody>
</table>

### a: Leaves
- **6 March**
  - N: 3.90±(0.23)
  - TNC: 2.48±(0.15)
- **24 April**
  - N: 4.24±(0.08)
  - TNC: 3.89±(0.53)
- **13 May**
  - N: 4.60±(0.47)
  - TNC: 3.10±(0.17)

### b: Stems
- **6 March**
  - N: 2.61±(0.14)
  - TNC: 3.20±(0.14)
- **24 April**
  - N: 2.84±(0.19)
  - TNC: 1.66±(0.19)
- **13 May**
  - N: 1.02±(0.09)
  - TNC: 1.73±(0.15)

### c: Axes
- **6 March**
  - N: 6.28±(0.09)
  - TNC: 1.33±(0.14)
- **24 April**
  - N: 0.53±(0.01)
  - TNC: 0.57±(0.01)
- **13 May**
  - N: 1.16±(0.04)
  - TNC: 1.51±(0.20)

### d: Roots
- **6 March**
  - N: 0.57±(0.01)
  - TNC: 0.52±(0.01)
- **24 April**
  - N: 1.79±(0.14)
  - TNC: 1.20±(0.03)
- **13 May**
  - N: 1.16±(0.04)
  - TNC: 1.26±(0.01)

### Whole tree
- **6 March**
  - N: 5.75±(0.09)
  - TNC: 4.39±(0.16)
- **24 April**
  - N: 4.64±(0.09)
  - TNC: 4.39±(0.08)
- **13 May**
  - N: 6.14±(0.04)
  - TNC: 6.46±(0.06)

The numbers are means (in bold) and standard errors (within brackets) of three (24 April, 13 May) or four (6 March) replicates. The means were ranked (a), (ab), (b, bc) and (c) from the lowest to the highest value. They are significantly different if coded with different letters. Statistical significance was inferred from randomization tests based on the generation of 2500 random orders, performed at organ and tree level for each sampling date.
nutrient solution because the volume and NO₃⁻ concentration of the solution remained constant for several days after the final harvest, i.e., with no more trees on the set-up and without any addition of nitrate (Adamowicz et al. 2012).

Controlling N uptake in the autumn leads to trees with variable N status and contents. The balance between storage, uptake and growth can be characterized under a non-limiting N supply. Most studies linking N cycling and growth have been performed under partial or temporary N limitation (Niederholzer et al. 2001). In the present study, and whatever the treatment, leaf N concentrations were higher than 4.2% DW in the spring and 2.7% DW in the autumn (Jordan et al. 2011). Such values were higher than the threshold values considered as being non-limiting for N uptake in the autumn (Cheng et al. 2004 in apple), plant growth (Cao et al. 2008 in birch; Sugiuira and Tateno 2011 in unshaded trees), fruit yield (He et al. 2003 in citrus) and water use efficiency (Cao et al. 2008 in birch).

Since the positions and lengths of all axes had been fixed by four successive prunings, all trees were of the same size and shape. In particular, parent axes comprised the same number of growth units, which meant that the numbers of their axillary buds that could develop (or not) in spring were similar and independent of the autumn treatment. Growth could therefore be related precisely to tree nutrient status at burst and to spring N uptake.

**Spring N uptake versus tree N status**

At the end of the observation period, the differences between treatments with respect to tree/organ N contents and concentrations were small or nil, indicating that spring N uptake compensated for low N storage. Cumulative spring N uptake was considerable, i.e., between three and six times the mean tree N contents at bud burst. Such high rates are unusual under field conditions (Dong et al. 2001, Millard et al. 2006) and could be attributed to the fact that water and nutrient supplies were unlimited (Thitithanakul et al. 2012), and maintained constant over time at the root surface (Midiene et al. 2002, Jordan et al. 2011). Indeed, N accumulation into new organs was mainly associated with spring N uptake, since the stems and leaves contained more than 70% of total tree N on 13 May, which is much higher than the N content initially present.

In spring, the N uptake kinetic varied with the treatment, but without significantly affecting cumulative spring N uptake on 13 May. It depended successively on the autumn treatment, i.e., on tree N status at bud burst, and then on the spring N supply. The shift between the two stages occurred in April, once N uptake rates exceeded 20 mmol day⁻¹ tree⁻¹. During the first stage, LNa trees that had been N limited in the autumn absorbed ~34% of the total spring N uptake, and HNa trees <23%. This observation confirmed the findings of Niederholzer et al. (2001) and Stephens et al. (2001), who saw that the restoration of spring N uptake started earlier when the amounts of cycling N were small. However, most other studies concluded that spring N uptake depended solely on spring N availability (Millard and Proe 1993, Munoz et al. 1993, Dyckmans and Flessa 2001, Grassi et al. 2003), which could be explained by the small amount represented by the ‘compensatory N uptake’ in the first stage when compared with the total N uptake in spring of LNa trees. This amount represented thus ~10% of the cumulative spring N uptake and could be masked by the relatively high coefficient of variation typical of budget studies. During the second stage, which lasted only half as long as the first, the trees absorbed at least twofold more N than during the first stage, and doubled their N contents. Such an increase in N uptake rates over time was consistent with previous findings (Lobit et al. 2001, Grassi et al. 2003, Jordan et al. 2012, Martínez-Alcantara et al. 2012).

In LNa trees that had been N limited in autumn, absorption started as soon as N was added to the nutrient solution and peaked after 2 days, decreasing slowly after that until April, i.e., until reaching the same rates as in non-limited trees. Indeed, only a few studies have provided information on the ability of trees to take up N prior to bud burst (Dong et al. 2001, Millard et al. 2006, El Zein et al. 2011, Thitithanakul et al. 2012). Once absorbed, the N remained in the roots until new shoots developed (Dong et al. 2001) and spring N uptake did not contribute to shoot growth until 36 days after bud burst (Millard et al. 2006). Moreover, in poplar (Thitithanakul et al. 2012), N uptake in early spring was only significant for a period of 10 days after N supply, the rates then declining until leaf emergence. This pattern is similar to what we observed in LNa trees. Spring N uptake in HNa trees was delayed by ~2 weeks and then increased gradually. Two hypotheses could explain this difference. First, N uptake in the HNa trees may have been limited by reservoir saturation, i.e., by the high N concentrations in the roots and bark as hypothesized by Cheng and Fuchigami (2002), and mostly driven by plant growth as observed in autumn (Jordan et al. 2011). From this point of view, N uptake might have been restored in the HNa trees once N started to be exported from the roots to sustain shoot development. Second, the N uptake in early spring was likely related to TNC contents, because both N uptake and metabolism mobilize large quantities of carbohydrate.

**Spring N uptake versus tree TNC status**

At bud burst, the carbohydrate concentration was higher in all organs of LNa trees, which was consistent with the findings of Bi et al. (2003) and Cheng et al. (2004). By 24 April, LNa trees had lost between 17 and 26 g of their TNC, while HNa trees had increased their TNC contents by at least 12%, although their shoot growth was slightly higher. The differences in TNC management with early spring N status could therefore be related to photosynthesis (see below) and to the amounts of C invested in N uptake. Indeed, about three carbons are released by respiration per NO₃⁻ assimilated and transformed into asparagine (Sasakawa and LaRue 1986 in
cowpea; Amthor 2000, by modelling), and root respiration has been found to be proportional to N uptake (Bloom et al. 1992, Reich et al. 1998). On 24 April, LNa trees still displayed the highest N uptake rates, and had absorbed ~2 g more N than HNa trees. Such differences in N uptake led to a respiration cost of 20.2 g equivalent glucose. However, this extra cost does not mean that early spring N uptake could be considered as being regulated by plant TNC status at burst. Indeed, TNC mobilization is incomplete in most cases, because trees can sequestrate large amounts of starch for several years (Millard and Grelet 2010) at the possible expense of growth (Silpi et al. 2007) and spring N uptake (Jordan et al. 2012).

Photosynthesis contributed to overall C needs before April, especially in HNa trees that accumulated TNC in early spring. Landhausser (2011) demonstrated that, in aspen, young shoots became autonomous for C within 10 days after bud break, and Biesleski and Redgwell (1985) showed that, in apricot, leaves started to export sorbitol after their full expansion. The net photosynthesis of small unshaded trees could therefore be related to leaf expansion, which started by the end of March. On 24 April, leaf areas reached 2.39 ± 0.11 and 2.56 ± 0.15 m² in LNa and HNa trees, respectively, indicating that C assimilation was likely almost similar, whatever the treatment. In HNa trees, the recovery of N uptake was concomitant with leaf expansion. In LNa trees, leaf expansion was concomitant with a further re-increase in N uptake rates after an initial peak preceding bud burst. Indeed, N uptake increased dramatically after April, confirming that spring N uptake led to a re-increase in N uptake rates after an initial peak preceding bud burst. Indeed, N uptake increased dramatically after April, confirming that spring N uptake only becomes significant after photosynthesis exceeds growth needs (Weinbaum et al. 1978, Deng et al. 1989).

Spring N uptake versus growth and development
According to previous studies (Grelet et al. 2003, Jordan et al. 2009), shoot growth adjusted to tree N status at burst, modulating axis emergence, but not axis growth. Indeed, the maximum number of buds that develop into long daughter axes is specific to each peach cultivar (Perezgonzalez 1993), and axis elongation is mainly dependent on their position within the structure (Jordan et al. 2009). In the present study, axis emergence was slightly delayed in trees that had been N limited in the autumn, which could indicate that growth and N uptake metabolisms were competing for C allocation in early spring. However, the N treatment had no effect on the number of long daughter axes, which could mean that the trees fulfilled their initial growth potential. Axis ramification, on the contrary, was linked to the spring N supply, as had been the case in previous studies (Lobit et al. 2001, Jordan et al. 2009). The differences were, however, limited to axis topology. Reducing the N supply favoured ramification in the apical segment, i.e., those having the highest N concentrations (result not shown), as has been previously observed in response to autumn treatments (Jordan et al. 2011).

The effects of N treatment on gross growth were transitory and limited to the roots and current-year shoots. On 24 April, the axis DW was not altered by N supply despite radial growth following cambium activity (Ameglio et al. 2002). Between 6 March and 24 April, the root DW of LNa trees decreased, which could likely be related to N and TNC mobilization, representing 26 and 30 g in LNa/LNs and LNa/HNs trees, respectively. Between 65 and 80% of the spring growth was achieved between 24 April and 13 May, since the growth rates increased after 24 April in line with N uptake rates and likely with net C assimilation rates. At the end of the first growth flush, organ DW and composition were similar in all trees, whatever their treatment.

Conclusion
Our results demonstrated, first, that N uptake could be restored quite early in the spring and partially compensate for low N storage, insofar as important amounts of TNC could be mobilized for that purpose. This condition probably explains: (i) why the importance of N uptake in early spring is still debated; and (ii) the delay in shoot development probably due to a shortage of C allocation to biomass synthesis. Early spring N uptake could even exceed growth needs, implying an N accumulation in the roots before shoot elongation. Since neither plant growth nor the N concentration in current-year shoots were penalized by the N treatments, N accumulation and uptake could be oversized in terms of plant needs under especially favourable conditions.

Root N concentrations remained high in trees that had not been N limited. Therefore, the complete reuse of N stores in order to sustain early spring growth, as has classically been accepted in the literature (Millard and Grelet 2010), may be questionable in these trees. From a practical point of view, using an early spring N supply to boost shoot growth may be counterproductive (i) if fertilization is not correctly scheduled in terms of plant phenology and (ii) if it is applied to previously well-fed trees.

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Conflict of interest
None declared.

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References


