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Autumnal nitrogen nutrition affects the C and N storage and architecture of young peach trees.

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Running head: Use of autumnal N uptake by young peach trees

Abstract: Nitrogen fertilisation is a regular practice in orchards. Its effect on tree development, N and C acquisition and allocation were evaluated simultaneously, while coupling on the same trees *in situ* measurements of N uptake and shoot development and destructive determinations of organ composition in N and Total Non structural Carbohydrates (TNC). An hydroponic set-up was designed that could grow young peach trees at constant NO₃ concentration while measuring N uptake. Forty-eight trees were raised outdoors under excessive N supply. Between October 2 and December 7, half of them were then N-limited to reduce N uptake by 75%. Organ N concentrations remained stable in the controls but were halved in N-limited trees. Growth (390 vs. 353 g DW tree⁻¹) was less affected by the treatment than N uptake (10.6 vs. 2.7 g N tree⁻¹). Growth was affected only in terms of axillary bud development, which was restricted to the median and upper crown parts. The number of buds which transformed into elongating axes (44 vs. 84 tree⁻¹) was halved, thus reducing leaf area by one third (10464 vs. 15568 cm²). Tree TNC content was not impacted. The difference in C acquisition likely balanced the C costs of N uptake. In N-limited trees, more TNC was stored as starch (73% vs. 56%), and the allocation patterns of TNC and N were altered in favour of the roots. Our results provide deeper insights into the tree integrated response to autumnal N fertilisation, focusing on an alteration of the balance between storage and growth.

Keywords: Nitrogen fertilisation, N uptake, plant architecture, N allocation, Non-structural Carbohydrate.

Abbreviations: C: Carbon, CER_{max}: leaf maximal CO₂ exchange rate, DW: Dry weight, N: Nitrogen, TNC: Total Non-structural Carbohydrate.

1 Introduction

2
3 Nitrogen fertilisation is a regular practice in orchards that affects tree development (Lobit et
4 al. 1999; Jordan et al. 2009). The effects vary with the time of application (Ampossah et al.
5 2004) and are not evenly distributed within the tree thus altering tree shape, the root to shoot
6 ratio and the balance between vegetative growth and fruit production (Cheng et al. 2002;
7 Jordan et al. 2009). Nitrogen uptake and allocation are also affected by N fertilisation
8 (Rosecrance et al. 1998, Cheng et al. 2002), and C acquisition and allocation are in turn
9 modified as a result of changes in tree growth (Kubiske et al. 1998; Veberic et al. 2002).
10 Evaluating fertilisation practices implies taking account of: (i) N acquisition, (ii) N
11 partitioning among and within tree constitutive organs (iii) N allocation between growth and
12 storage, and possibly (iv) C acquisition and allocation. Most studies to date have been limited
13 to one of these aspects and have not provided an integrated insight into the tree response to N
14 fertilisation.

15 Tree response to applied N varies with tree N status, a point that was not adequately
16 addressed by many prior experiments (Niederholtzer et al. 2001, Millard and Proe 1993) and
17 could explain inconsistencies in the literature (Bollmark et al. 1999, Niederholtzer et al. 2001,
18 Von Fircks et al. 1998). Nitrogen status altered N uptake (Cheng et al. 2002), TNC (Total
19 Non structural Carbohydrates) accumulation (Cheng et al. 2004) and the trade-off between
20 growth and storage (Sanz Perez et al. 2007; Gloser et al. 2009) which is generally assumed to
21 be mainly determined by plant phenology. In early spring, the first growth flush of tree is
22 sustained by the mobilisation of N stores (Millard 1996, Jordan et al. 2009). Thereafter, N
23 uptake from the soil becomes significant enough to sustain current growth (Lobit et al. 2001,
24 Cooke et al. 2005) over spring and summer, and to ensure perennial storage in late summer
25 and autumn (Millard 1996, Tagliavini et al. 1997, Sanz Perez et al. 2007). Thus, the synthesis
26 of storage protein in trees starts after fruit harvest (Gomez and Faurobert 2002). It is however

1 admitted that under poor nutritional conditions, trees export most of their leaf N before
2 abscission to contribute to winter storage (Millard and Proe 1993, Cheng et al. 2002), while
3 growth continues until late autumn (Kubiske et al. 1998, Kealke et al. 2005) and competes
4 with storage for C and N allocation (Cheng et al. 2002).

5 In most nutrient budget studies, growth is assessed from an increase in biomass rather
6 than tree development. Studying plant development implies focusing on the relevant
7 processes (i.e. organ initiation, emission and growth per se). Thus N nutrition modifies not
8 only tree size but also tree shape, and can therefore contribute to controlling tree architecture
9 in association with pruning. During spring and summer, N supply affects shoot growth by
10 modulating the emission of new axes (number and position within the crown) and the duration
11 of growth flushes (Lobit et al. 2001, Jordan et al. 2009, Médiène et al. 2002), but architectural
12 data are missing for autumn, when growth is supposed to decline. Indeed, late emerging axes
13 are of little importance to growers because of their small size, their position in the crown
14 outer parts and the quasi-absence of flower buds (Boonprakob et al. 1996).

15 Few studies have adequately considered the heterogeneity of the plant response to N
16 supply linked to the spatial distribution of organs within the crown. This could be of prime
17 importance for growers, who look for small sized and aerated trees to facilitate fruit harvest
18 and to optimize the penetration of light and pesticide into the crown. In early spring for
19 instance, increased N availability enhanced axis emission in crown outer parts but inhibited it
20 in centre parts (Jordan et al. 2009). The associated variation in resource allocation is usually
21 evaluated according to organ size, while age or position are seldom taken into account
22 (Munoz et al. 1993, Médiène et al. 2002). Detailing the resource gradients within the tree
23 (namely along the woody axes) should confirm the possible link suspected by Cooke et al.
24 (2005) between local resources content and axes development.

25 Experimentally, it is difficult to achieve simultaneous measurements on the major plant

processes involved in growth and storage, and particularly (i) the continuous and non-destructive monitoring of N uptake in relation to N supply, (ii) the recording of plant growth dynamics with special emphasis on shoot development, and (iii) the determination of local resource contents and their variability among and along organs. To achieve these goals, it is necessary to maintain constant N availability at the root level because periodic applications of N-fertilizer induce large variations in N concentrations at the root surface, thus altering uptake rates. To our knowledge, the instantaneous N uptake rate has never been measured on trees over a long period of time, nor has this been related to N availability at the root surface.

This paper investigates how N supply affects the autumnal development and structure of young peach trees (*Prunus persica*) and the C and N resource allocation within the tree structure. To achieve this, trees were grown hydroponically under two regimes of N supply, and instantaneous N uptake rates measured over long time periods. The development of new organs (leaves and axes), and the maintenance of existing organs were assessed to account for the effects of plant N status on shoot growth and leaf senescence (Rosecrance et al. 1998, Niederholzer et al. 2001, Cheng et al. 2002). The autumnal N supply could then be related to: (i) N uptake and plant/organ N status, (ii) the spatial distribution of C and N assimilates, each woody axis being identified and positioned within the structure, and (iii) the trade-off between storage and growth, the latter being analysed on an architectural basis.

Materials and methods

Tree preparation

The study was carried out at the INRA research centre in Avignon (southern France). Eighty 1-year-old peach rootstocks, i.e. obtained from seeds (cv "GF 305") germinated in January 2005, were planted outdoors in a hydroponic system (described below) on May 2, 2006. Each tree had a single axis of 0.8 to 1 cm in diameter. This axis was trimmed down to 25 cm in

length. The main root was cut at about 15 cm and was sparsely ramified. During the preconditioning period (May 2 – Oct. 2) three severe prunings homogenized the plant architecture and size, thereby removing on average 151.5 g dry weight (SE or standard error: 6.5) and 6.15 g N (SE: 0.29) per tree. The first pruning occurred on June 13, leaving on the main axis only 2 newly-grown secondary axes, which were trimmed down to 15 cm. This forced the axillary buds to develop into tertiary axes. On July 12, four tertiary axes (2 per secondary axis) were selected and their apices removed, once again causing new buds to develop into axes of order 4. The third pruning was performed on August 29. On each tertiary axis, two axes of order 4 were selected and cut to a length of 30 cm. On each of them, only 5 buds (i.e. 40 per tree) were allowed to develop freely into axes of order 5. Their elongation and ramification were not completed at the end of the preconditioning period, as was the case for the older axes (order ≤ 4). On October 2, 48 trees were selected for their homogeneity and maintained on the hydroponic system.

Hydroponic set-up

The hydroponic experiment comprised 40 PVC troughs (1.5 x 0.25 x 0.15 m) set outdoors in 8 rows of 5 units at interspaces of 2 m. The troughs were insulated with expanded polystyrene foam and wrapped in a white plastic sheet to prevent overheating and N pollution from rain and dust. The nutrient solutions were pumped from 4 tanks located in an underground laboratory, and injected at a constant rate of 2 L min⁻¹ into each trough, from which they drained back by gravity to their tank through PVC tubing. The recycled solution was thoroughly aerated by means of successive cascades. Each tank fed 10 troughs, i.e. 2 rows.

The temperatures, volumes, pH and nitrate concentrations of the nutrient solutions were controlled automatically and maintained at around the preset values. Analyses and corrections were performed on an hourly basis most of the time. Temperatures were maintained at 23°C ($\pm 0.5^\circ\text{C}$) until Nov 1, then decreased gradually to 14°C, imitating the soil temperature at

depth of 50 cm measured at the INRA weather station. Volumes were maintained at 500 L tank⁻¹ by the addition of deionised water using optical level detectors (LLE 102000 Honeywell, USA). Additions of sulphuric acid maintained the pH at 5.0. The nitrate concentration was measured (method described by Vercambre and Adamowicz, 1996) by automatic in-line UV absorption spectrometry (double-beam UVmc2, SAFAS Monaco). Nitrate depletion was compensated for by automatic injection (precision syringe drive PSD2, Hamilton Company, Reno, Nevada) of a stock solution containing (eq L⁻¹) 0.41 KNO₃, 0.41 Ca(NO₃)₂ and 0.18 Mg(NO₃)₂, which thus also ensured major cation repletion.

Periodic manual analyses were performed to verify the major cation concentrations (emission spectrometry) and to restore phosphate concentration (vanado-molybdate colorimetry) by the addition of a phosphate buffer (pH 5.0). The absence of leaks and denitrification were verified once the trees had been removed after the final sampling. The volume and NO₃⁻ concentrations of the nutrient solution remained constant for several days without any addition of nitrate, which confirmed the absence of leaks or of any significant bacterial consumption of NO₃⁻.

N uptake rate

The NO₃ uptake rate (U g day⁻¹ tree⁻¹) was calculated daily after C_d, V_d, n and I_t, being the respective solution [NO₃] (μmol L⁻¹), volume (L tank⁻¹), number of trees tank⁻¹ on day d at midnight, and the volume of NO₃ (μmol tank⁻¹) injected to maintain the set concentration at time t:

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

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.

Nutrition and treatments

During the preconditioning period (May 2-Oct. 2), the trees fed on a nutrient solution containing (meq L^{-1}) 1 NO_3 , 1 H_2PO_4 , 11 SO_4 , 3 K, 7 Ca and 3 Mg. Micronutrients were added (0.1 mL L^{-1} Kanieltra 6-Fe, Hydroazote, France) and completed by 16.5 mg L^{-1} EDTA-Fe. The four solution tanks were replicates.

On October 2, the 48 trees were divided in two groups. Two tanks holding the control plants were maintained on the normal nutrient solution, and two tanks were N-limited until December 2. In these tanks, the regulation of $[\text{NO}_3]$ was halted from October 2 to 5, and the nitrate concentration fell from 1.00 to 0.05 meq L^{-1} . Thereafter, the NO_3 concentration was adjusted periodically in order to maintain $U_{\text{N-limited}}/U_{\text{control}}$ at around 0.25.

In situ measurements

Growth was measured once a week throughout the treatment period on an architectural basis (Pagès et al. 1993), each axis being considered as a collection of phytomers or structural units. From this viewpoint, a phytomer comprises an internode and its upper node with the attached leaf and its axillary bud. Measurements were focused on the axes of order 5 and 6. Those of order 5, referred to as parent axes, had not reached their final length on October 2. Five of them, growing on the same axis of order 4, were selected in 18 trees and their constitutive phytomers ranked from base to top. All axes of order 6, referred to as rosettes and long daughter axes, emerged during the treatment period. The position of each developed axillary bud was defined by the rank of its father phytomer (i.e. the phytomer on which it was inserted). Transformation of the rosette into an elongated daughter axis was dated. The number of expanded leaves was also counted on each parent axis, rosette and long daughter axis.

The CO_2 exchange rate (CER) of individual leaves was determined at five dates (Oct. 12, 19, Nov. 9, 20, Dec. 1) on 6 trees per treatment. We measured CER_{max} (in $\mu\text{mol CO}_2 \text{ mol m}^{-2} \text{ s}^{-1}$), on 3 leaves per tree on sunny days around noon, to ensure light saturating conditions

(mean PPFD: 1211 $\mu\text{mol m}^2 \text{s}^{-1}$, SE: 17). The measurements were performed using a portable ADC LCA4 photosynthesis system (Hertz, UK), under ambient temperature, light and CO_2 .

Leaf fall was monitored on four trees per treatment enclosed with a 1.50 m high plastic net. Fallen leaves were collected every evening from November 2 onwards (i.e. from fall start), frozen before oven drying at 80°C for 92 hours and then weighted. Twice a week, a second sampling was performed in the morning for total N analyses. The leaves remaining on the trees were removed by hand on December 8, oven dried (80°C, 92 hours) and weighted. Leaf fall could then be computed as a % of total leaf DW.

Tree samplings

Tree N status and architecture were determined on four individuals per treatment, harvested at the beginning (Oct. 2) and at the end (Dec. 7) of the treatment period. Before sampling, lengths and diameters were measured on each older axis (order <4) and their volume calculated. At sampling, growth measurements focused on the five parent axes, annotated in situ. Their respective diameters were measured every 5 cm from base to top. Each phytomer was also characterized by its internode length. If present, the long daughter axis was described by its length, the number of its expanded leaves and its diameter at the insertion point.

The five parent axes were sub-sampled for biochemical analyses, defoliated and cut from base to top into 10 cm long pieces. Each segment had a different growth status: ceased vs. active elongation at the date of October 2 and distal parts developed afterwards. Their long daughter axes were collected separately. Supplementary tree sampling included an aliquot of roots (i.e. one main root and all its branches) washed with deionised water, and a synthetic sub-sample of older axes. The latter combined small pieces of the axes to make up a volume of c.a. 10 cm^3 , set in proportion to the respective contribution of each order (1 to 3) to the total volume of the woody structure. The axes of order 4 were collected separately. The samples were taken immediately after tree harvest, frozen in liquid nitrogen, and kept at -20°C before

freeze-drying.

Leaf area was measured using an area meter (LI 3100, Li-Cor, Lincoln, Nebraska, USA) on the leaves of three parent axes. This sample was then oven dried (80°C) and weighted to determine the specific leaf weight (SLW, g m⁻²). The rest of the tree was separated into: (i) remaining leaves, (ii) rosettes, parent and long daughter axes, (iii) elder axes, and (iv) roots. All samples were oven dried (at 80°C) before being weighted (DW, g). The total leaf area of a tree was calculated from DW and SLW.

Plant analysis

All the samples selected for biochemical analysis were ground in ball mills (MM301 and PM400/2, Retsch, Germany) cooled with liquid N₂. The total N concentration was measured on an elemental analyzer (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 non-structural carbon (TNC) pool. Tree N and TNC contents (g organ⁻¹) were calculated from organ DW, N and TNC concentrations (% DW).

Data analysis

When it was necessary to homogenize variances, the data were log or sinh transformed before computation with S-Plus software (Venables and Ripley, 1999). The axes of order 4 and those of orders 1 to 3 were grouped by calculation in a single pool of older axes (orders 1 to 4). Treatment effects on phytomer development (i.e. emission of leaves, rosettes and long daughter axes), leaf fall and subsequent N loss were evaluated by comparing the means of the two populations at each date (Student tests). Treatment effects on tree architecture were evaluated at the December sampling by comparing the number of organs (Student tests) and the positions at which the rosettes and long daughter axes appeared during treatment (chi-square: χ^2 tests). The segments of parent axes, where phytomer elongation was not completed

in October, were identified by comparing the number of phytomers longer than 2 cm at both samplings (Student tests). Axis development was considered to be independent of the tree since no difference was evidenced between the means and variances calculated for each tree receiving the same treatment. Time or treatment effects on organ DW, C and N concentrations and contents were assessed using pairwise Student tests. For C, the analyses were run separately for starch, soluble sugars, and TNC. For parent axes, we used a general linear model (GLM) with two main effects: position of the stem segment (separating the 0-10, 10-20, 20-30 cm, and upper segments) and tree category: (i) October tree sampling, (ii) control and (iii) N-limited trees of the December sampling. The effect of treatment was then detailed for each stem position.

Results

N uptake

To maintain the N uptake rates of N-limited trees to one fourth of those of control trees, the NO₃ concentration of the nutrient solution was adjusted periodically to between 0.010 and 0.025 mmol NO₃ L⁻¹ (Fig. 1). Such adjustments were compulsory because the daily NO₃ uptake rate depended on ambient climatic conditions (not shown). The resulting cumulative N uptake per tree over the treatment period (Fig. 2) calculated for each tank revealed no difference between replicates, and also that N uptake was sustained over the whole period. The validity of these autumn uptake measurements was assessed by the match between the N uptake measured during the whole period (10.85 and 2.75 g N tree⁻¹ for control and N-limited trees, resp.) and the N intake calculated from analytical measurements performed on trees sampled at the beginning and end of the treatment period (11.2 and 2.6 g N tree⁻¹ for control and N-limited trees, resp.). As expected, the autumn N uptake of N-limited trees was reduced by 76%. Their mean tree N content was also 59% lower than that of the controls.

Growth

Over the treatment period, tree DW rose from 122.6 g (SE: 5.2) to 512.7 g (SE: 32.2) and 475.8 g (SE: 34.8) in the control and N-limited trees, resp. Root DW increased by a factor of 6.3 and that of the older axes by a factor of 2.5, whatever the treatment. The autumn N supply only affected the leaves and stems of parent and long daughter axes that had not achieved their elongation at experiment start (Fig. 3a). These axes multiplied their stem DW 9.8-fold and 7.2-fold in control and N-limited trees, resp.

The differences were not related to the elongation of parent axes which increased their mean number of leaves from 18 to 28 (Fig. 4a), independently of the treatment. The leaves emerged on the youngest phytomers, located on the 30 cm-apex portion. Internode lengthening continued in the 20-30 cm portion, with the number of phytomers longer than 2 cm increasing significantly from 0.95 (SE 0.31) to 1.56 (SE 0.23) between Oct. 4 and Dec. 7. At Dec. 7, all internodes, whatever their rank, had the same length and diameter and each portion of the parent axes comprised the same number of phytomers (Fig. 3b).

The treatment basically affected the development of axillary buds along the parent axes (Fig. 4b). Indeed, axillary buds could open out and then stop developing at the rosette stage, or transform into a long daughter axis. Both the position and the final outcome of developing buds were affected. Limiting the N supply did not affect the mean number of rosettes per parent axis (5.7) but reduced the number of long daughter axes by 45% (1.1 vs. 2.1). The differences appeared on October 9 and increased over time. The growth of these long daughter axes varied also with the treatment. Limiting the N supply increased their diameter (Table 1), reduced leaf emergence (Fig. 4a), but had no effect on their final length. In N-limited trees, all long daughter axes and 48% of rosettes, developed on the 20-30 cm portion of the parent axes. They were more evenly distributed in the control trees (Table 2).

Leaf growth reflected the differences in axes growth. During the treatment period, leaf

biomass increased less in N-limited trees than in control trees (26.3 vs. 65.5 g). N-limited trees had fewer leaves (1909 vs. 2408), and leaves of smaller size (5.48 vs. 6.47 cm²) and SLW (5.68 vs. 6.34 mg cm⁻²) than controls. Leaf fall remained minor, and at the end of the treatment, the control and N-limited trees had only lost 26% and 35% of their leaf biomass resp. (Table 3). Thus the N-limited and control trees still had 10464 vs. 15568 cm² of attached leaves, resp., on December 7.

TNC and N allocation within the tree

At the tree level, there was no significant difference in TNC content at final sampling in control and N-limited trees (79 vs. 86 g). Seventy-three percent of the TNC accumulated during the treatment period.

In control trees, the root N concentration (Fig. 5a) remained constant over time. It decreased nonetheless by 12% in the leaves but increased by 28% in the older axes. More than 90% of the autumn N uptake was allocated to three compartments (Fig. 5b): the roots (41%), leaves (28%) and elongating (parent and long daughter) axes (22%). In N-limited trees, the N concentration decreased by almost half in all organs, except in older axes where the decrease was limited to 25%. The autumn N uptake was allocated only to roots (62%) and older axes (17%) with elongating axes and leaves being excluded from this distribution.

TNC concentrations increased over time in all organs, except in the leaves (Fig. 5c). In all axes, the TNC concentrations varied with treatment, being enhanced by N limitation. The low N treatment decreased the proportion of TNC in the leaves and elongating axes (18% vs. 31%), and increased it in the older axes and roots (82% vs. 69%). Therefore the higher TNC concentration in the elongating axes of N-limited trees could be attributed solely to their lesser growth when compared to control trees (Fig. 5d).

The contribution of starch to the TNC pool evolved with time and treatment, from 28% on October 4 to 56% and 73% on December 7 in control and N-limited trees, resp. The

concentration of soluble sugars (Fig. 7c) remained stable over time in the leaves and elongating axes but increased in roots and older axes of control trees by 48% and 52%, resp. The accumulation of soluble sugars (Fig. 7d) mimicked that of dry biomass, and the C accumulated in excess was mostly stored as starch. Variations in the starch contents (Fig. 7b) and concentrations (Fig. 7a) with time and treatment resembled those observed for TNC (Fig. 5c-d), with two exceptions: (i) starch never accumulated in the leaves, and (ii) the roots of N-limited trees contained 42% more starch than those of control trees.

A clear concentration gradient was also observed along the parent axes for total N ($p < 0.0001$ under GLM procedures; Fig. 6a), soluble sugars ($p < 0.0001$; Fig. 6b) but not for starch ($p = 0.59$; Fig 6c). Total N concentrations increased from base to top by a factor of 1.8, independently of sampling date and treatment. The soluble sugar concentration gradient varied with both factors ($p \leq 0.0044$). It was highest on October 2, with concentrations between the axis base and top varying by a factor of 3. On December 5, this gradient was reduced to a factor of 1.3 and 1.6 in control and N-limited trees, resp. Starch concentrations remained constant along the parent axes but increased over time and with N limitation.

Leaf senescence and its effects on individual leaf photosynthesis.

The N concentration in fallen leaves was higher in control than in N-limited trees. Prior to falling, the N was partly removed from the leaves, with about one-third of their N content being transferred to the woody axes and roots. Thus, on December 7, the N concentrations in attached and fallen leaves differed by at least 35%. The fallen leaf N concentration fell over time from 4.56% to 2.96% DW in controls and from 2.67% to 1.79% DW in N-limited trees.

The maximum leaf CO_2 exchange rate (Table 3) varied markedly from 15 (Nov. 21) to 20 (Oct. 14) $\mu\text{mol m}^{-2} \text{s}^{-1}$, but was not significantly related to N nutrition or leaf aging.

Discussion

Methodology

The effects of modifying the N supply on global tree functioning, and the spatialization of these effects within the tree structure, were evaluated using a robust experimental design. We were thus able to couple on the same trees both long term in situ measurements of N uptake and shoot development, and determinations of organ composition in N and TNC. The variability between trees was also reduced during the preconditioning period, (i) by bathing the roots in a highly homogeneous and controlled solution, in terms of its temperature and mineral composition, and (ii) by shaping the shoots with successive prunings. As a result, the coefficients of variation for organ DW, N and TNC concentrations were only 13%, 8%, and 9%, resp. Furthermore, the sampled trees were representative because their N uptake closely matched that of the whole tree population.

Uptake

The control of N nutrition restricted the daily N uptake rate of N-limited trees to 24% of that of control trees. N uptake, calculated as the sum of the daily NO₃ uptake rate, remained high during the treatment period, representing 53% and 22% of the total N absorbed since May 1st (tree installation in the experimental set-up) by the control and N-limited trees, resp. This was consistent with the data obtained by Niederholzer et al. (2001) and Cheng et al. (2002) who reported that apple trees absorb between 25% and 45% of their total N content during the two months that precede leaf fall. The discrepancy with our control peach trees could be attributed to the exceptionally mild temperatures of autumn 2006 (mean daily air temperature: 14.1°C).

Carbon acquisition was primarily controlled by leaf area not CER_{max}, which confirms the results obtained by Kubiske et al. (1998) and Cooke et al. (2005). Indeed, CER_{max} did not decline with time and treatment, despite a clear difference in specific leaf weight (SLW, 6.34 vs. 5.68 mg cm⁻² on December 7). Fifty percent (i.e. 0.35 mg cm⁻²) of that difference was

accounted for by changes to the N and TNC concentrations in N-limited leaves. The remainder was probably linked to changes in leaf anatomy, namely lamina thickness and density, as suspected by Aranda et al. (2004). Nonetheless, the mean tree TNC contents were not affected by the treatment. The difference in C acquisition likely balanced the C costs of N uptake and allocation.

Trade-off between overall growth and storage

Limiting the autumn N supply modified the trade-off between growth and storage. The acquisition of N and C resources was more affected than overall growth. As observed in poplar (Cooke et al. 2005, Sanz Perez et al. 2007), N-limited trees decreased their shoot development but not root growth, forcing the shoot to root ratio to decline. On December 7, this ratio attained 1.8 and 1.2 in the control and N-limited trees, resp. Such low values appeared to be typical of hydroponic conditions (Médiène et al. 2002), favouring the development of fine, absorbing rootlets directly inserted on the taproots.

Nitrogen storage was almost not affected in control trees, where the autumn N uptake was proportional to dry biomass accumulation, and organ N concentrations remained almost constant with time. By contrast, the N concentration decreased markedly in all organs of N-limited trees. Such a decrease has had only previously observed in late autumn in a few cases (Millard and Proe 1993). Indeed, N concentrations usually increase in late autumn since growth declines more than N uptake (Munoz et al. 1993). This was not the case in our study, likely because the N concentrations in the N-limited trees were higher than those usually found in the literature: Gomez and Faurobert (2002) measured less than 0.51% N in the DW of axes (vs. 0.75% here), while Niederholzer et al. (2001) found 2.0% N in the DW of leaves (vs. 2.6% here).

Organ TNC concentrations also increased in late autumn, whatever the treatment. However, this increase was more marked in N-limited trees than in the controls. The principal

changes affected starch, indicating that autumn TNC storage was favoured by low N conditions. By contrast, soluble sugar concentrations were not affected by time or N supply. The relationship between autumn N uptake and TNC accumulation is still debated in the literature and contrasting results have been published, sometimes relative to the same species (Bollmark et al. 1999, Von Fircks et al. 1998, Cheng et al. 2004).

C and N allocation

Reducing the N supply modified resource allocation patterns, increasing the proportions of N and TNC allocated to the roots and older axes. To our knowledge, TNC allocation patterns have generally been related more to ablation practices such as pruning (Haddad et al. 1995) than to fertilisation. According to Cheng et al. (2002), N-limited trees increased the proportion of N incorporated in their lower parts even when urea was sprayed on the leaves to replace soil fertilisation, suggesting that this is a generic plant response to low N status and not the result of a first-come first-served principle.

The amounts of N exported from the leaves prior to fall were small: 0.30 and 1.27 g N in for the N-limited and control trees, resp. Indeed, (i) the autumn leaf fall rate was low, and (ii) whatever the treatment, only 35% of leaf N was reincorporated into perennial parts. This was likely a consequence of the high N status of the trees after the preconditioning treatment. This proportion could increase to 60% in low N status apple trees (Cheng et al. 2002).

Shoot development

Limiting the autumn N supply affected shoot growth, essentially in terms of axillary bud development, i.e. the number, length and position of long daughter axes emitted during the treatment period. Thus no difference was found in the parent axes, despite the importance of their development during the treatment period, when their stem lengths increased by a factor of 1.6. At the scale of the parent axis, the treatment did not affect the proportion of developing

axillary buds, but changed their behaviour according to the position of their father phytomer on this parent axis. Under low N conditions, bud break was inhibited on phytomers that had already achieved their elongation at experiment start (located on the basal position of the parent axis, i.e. on the 0-20 cm segment). Conversely, it was promoted on phytomers that were growing during the treatment period (located on the median position of the parent axis, i.e. on the 20-30 cm segment). No difference was found regarding the phytomers emitted during the treatment period (located on the apical position of the parent axis, i.e. above 30 cm). Furthermore, bud development into short (rosette) or long daughter axes was altered. Thus the number of long daughter axes was halved, but no effect was seen on the total number of rosettes. As a consequence, the leaf number was reduced by 12%. Leaf fall was also reduced (3580 vs. 7750 cm² on December 7) and this partly compensated for the smaller leaf expansion of N-limited trees. As a result, leaf area was reduced by one third for N-limited trees, on December 7. Several other studies had already confirmed that axis emission is the main factor affected by N supply: in the spring, trees adapt their growth to the amount of cycling N, altering the number of growing axes rather than individual axis growth (Jordan et al. 2009). In the summer, both the number and length of axes vary with N supply (Lobit et al. 2001, Cooke et al. 2005). The intensity of the response depends on the branching ability of the clone, and eventually becomes non significant (Cline et al. 2006).

Different mechanisms have been proposed to explain this adaptation. Firstly, cytokinin, the production of which in the roots is correlated to N supply (Takei et al. 2001, Cline et al. 2006), may act as a long distance signal that promotes axillary bud development. Secondly, the quasi-absence of the response of low branching clones could be attributed to their increased sensitivity to auxin, a repressor of bud outgrowth (Cline et al. 2006). And thirdly, shoot N concentration may play a role because ramification is promoted by glutamine and asparagine foliar sprays (Cline et al. 2006). Moreover, Cooke et al. (2005) found that N

supply only triggers axis production when the leaf N concentration exceeds 4% DW, which was the case in our control trees. It is tempting, therefore, to relate axillary bud development to the local N concentration. Thus, under low N conditions, the N concentrations decreased almost by half in parent axes as a consequence of biomass accumulation. The decrease was greatest in the 0-20 cm segment, where ramification was inhibited. The N-limited trees were more densely branched than control trees in the median parts of the crown, their lower parts bearing only rosettes.

Conclusion

An autumn N limitation affected N uptake more than growth, and therefore markedly modified the trade-off between storage and growth. In control trees, growth remained proportional to N uptake even in late autumn, and organ N concentrations were maintained at a stable level. Reducing the N supply limited N uptake and halved the tree N concentration, but increased starch accumulation. Growth was only affected in terms of axillary bud development (i.e. the number and position of new axes), modifying tree size and shape. Our results demonstrated the high degree of phenotypic tree plasticity relative to N fertilisation. At this stage, it is difficult to conclude whether or not changing the fertilisation practice in autumn brings growers a practical benefit. Limiting autumn N supply clearly reduced the number of short shoots which have to be removed by pruning, but the lower organ N concentrations would probably restrict early spring shoot and fruit growth (Jordan et al. 2009). Additional data obtained on the remaining trees during the following spring may help evaluate further these propositions.

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Table 1 Dimensions of long daughter axes in control and N-limited peach trees. Numbers are means \pm standard errors, the total number of axes being shown into brackets. The number of expanded leaves per axis and the length (cm) of the stem were determined at the last count on all trees (Nov. 16). Axis diameter (mm) was measured on sampled trees (Dec. 7), 0.5 cm above their insertion point. Different letters between treatments indicate that mean values were significantly different (Student tests)

	Control trees	N-limited trees
Expanded leaves (number axis ⁻¹)	8.86 ^a \pm 0.23 (189)	7.93 ^b \pm 0.21 (99)
Stem length (cm)	9.90 ^a \pm 0.44 (189)	7.72 ^a \pm 0.48 (99)
Axis diameter (mm)	2.71 ^a \pm 0.07 (41)	2.23 ^b \pm 0.06 (28)

Table 2 Relative distribution (as a % of the total) of rosettes and long daughter axes along their parent axes in control and N-limited peach trees. The parent phytomers are described by their position along the stem. The treatment exerted a significant effect on long daughter axes, but not on rosettes

	Rosettes		Long daughter axes	
	Control	N-limited trees	Control	N-limited trees
0-10 cm portion	19%	25%	-	-
10-20 cm portion	33%	11%	21%	-
20-30 cm portion	32%	48%	79%	100%
30 cm-apex portion	16%	16%	-	-
Chi square test:	χ^2 : 0.806 (df = 3)		χ^2 : 0.035 (df = 3) ns	

Table 3 Maximum CO₂ exchange rate (CER_{max}, μmol m⁻² s⁻¹) of individual attached leaves and dynamics of leaf fall (as % of total leaf dry biomass). CER_{max} are means ± standard errors of 6 measurements performed at around noon under saturating light conditions. Similar letters between treatments indicate that mean values were not significantly different (Student tests). No test was made for the dynamics of leaf fall since the data consisted in a single measurement

Date	Maximum CO ₂ exchange rate (μmol m ⁻² s ⁻¹)		Leaf fall (% of total leaf dry biomass)	
	Control trees	N-limited trees	Control trees	N-limited trees
October 13	19.73 ^a ±1.28	19.38 ^a ±0.79	0 %	0 %
October 19	15.51 ^a ±0.53	15.26 ^a ±0.62	0 %	0 %
November 9	16.12 ^a ±0.94	17.61 ^a ±0.26	9.6 %	3.9 %
November 21	15.6 ^a ±0.65	15.18 ^a ±0.40	17.7 %	11.7 %
December 1	18.28 ^a ±0.76	18.54 ^a ±0.78	23.7 %	17.6 %

Figures legends

Fig 1 Nitrate concentration (mol m^{-3}) measured in the nutrient solutions of control (full lines) and N-limited (dotted lines) trees during the culture period (June 2, Dec. 7). The difference between the two replicates of each treatment was too small to be visible. Individual symbols (2564 analyses per solution) were too numerous to be plotted. The Y-axis is in log scale to show small variations in nitrate concentrations in the N-limited treatment

Fig 2 Mean cumulative N uptake (g tree^{-1}) calculated from analysis of the nutrient solutions feeding 11 control (full lines) and 11 N-limited (dotted lines) trees during the treatment period (Oct. 2 – Dec. 7). Thick and thin lines correspond to replicate solutions. On Dec. 7, the symbols (closed dots: control trees; open dots: N-limited trees.) represent the total N uptake during this period, as calculated from the analysis of four sampled trees

Fig 3 (a) Dry biomass (DW, g organ^{-1}) and (b) number of phytomers along successive sections of the parent axis on October 2 (thick line) and December 7 (dotted lines) of control (closed dots) and N-limited trees (open dots). Each symbol is the mean of four trees (a) or 20 parent axes (5 axes sampled tree^{-1}) (b) plotted with standard errors. The means coded with different letters are significantly different (Student pairwise tests)

Fig 4 Dynamic responses of the axis emission and growth of control (closed symbols) or N limited (open symbols) trees: (a) Number of leaves per parent (dots) and long daughter (triangles) axes, (b) Number of long daughter axes (dots) and rosettes (triangles) per parent axis. In (a) & (b), each symbol is the mean plotted with standard errors. The means coded with different letters are significantly different (Student pairwise tests). Measurements were taken on 90 parent axes (5 axes tree^{-1}) per treatment

Fig 5 Organ concentration (% in DW) and content (g organ^{-1}) in control (closed dots) and N limited (open dots) trees on October 2 (thick line) and December 7 (dotted lines). (a) Total N concentration, (b) Total N content, (c) TNC concentration and (d) TNC content. Each symbol is the mean of four trees plotted with standard errors. The means coded with different letters are significantly different (Student pairwise tests)

Fig 6 Concentration profile of parent axes (% in DW) between October 2 (thick line) and December 7 (dotted lines) in response to control (closed dots) and N-limited autumn supply (open dots). (a) Total N, (b) Soluble sugars and (c) Starch. Each symbol is the mean of 20 parent axes (5 axes tree^{-1}) plotted with standard errors. The means coded with different letters are significantly different (Student pairwise tests)

Fig 7 Organ carbohydrate concentrations (% in DW) and contents (g organ^{-1}) for control (closed dots) and N-limited (open dots) trees on October 2 (thick line) and December 7 (dotted lines). (a) Starch concentration, (b) Starch content, (c) Soluble sugar concentration and (d) Soluble sugar content. Each symbol is the mean of 4 trees plotted with standard errors. The means coded with different letters are significantly different (Student pairwise tests)

Figure 1

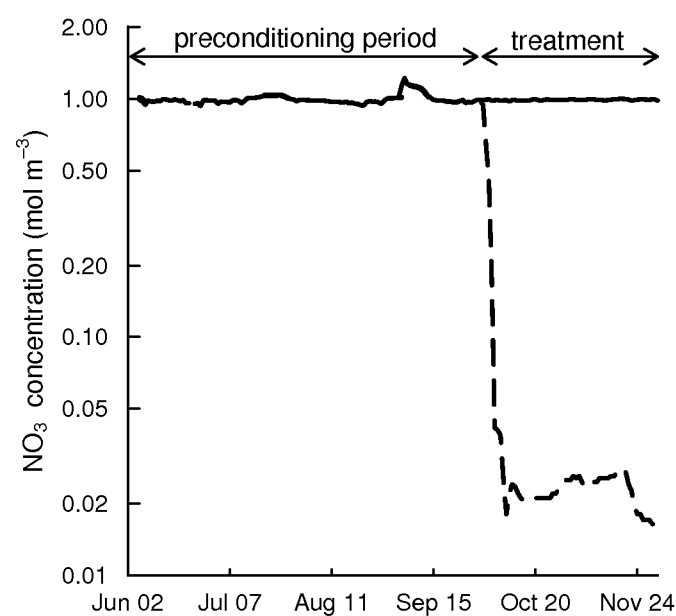


Figure 2

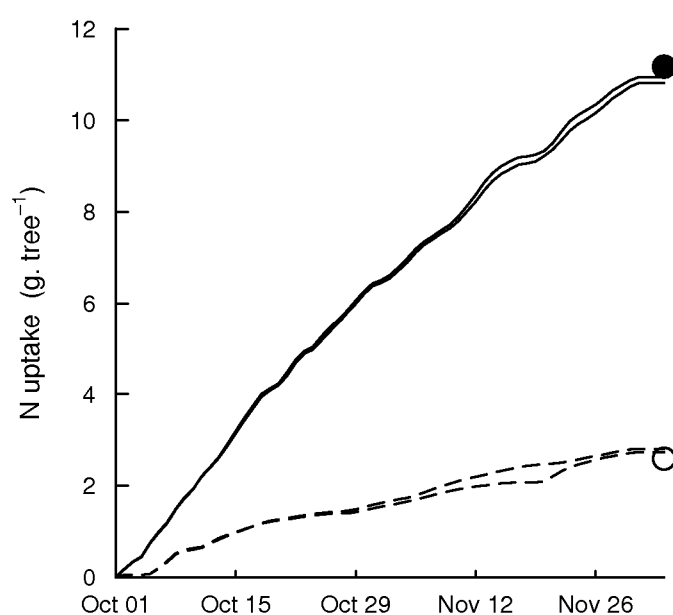


Figure 3

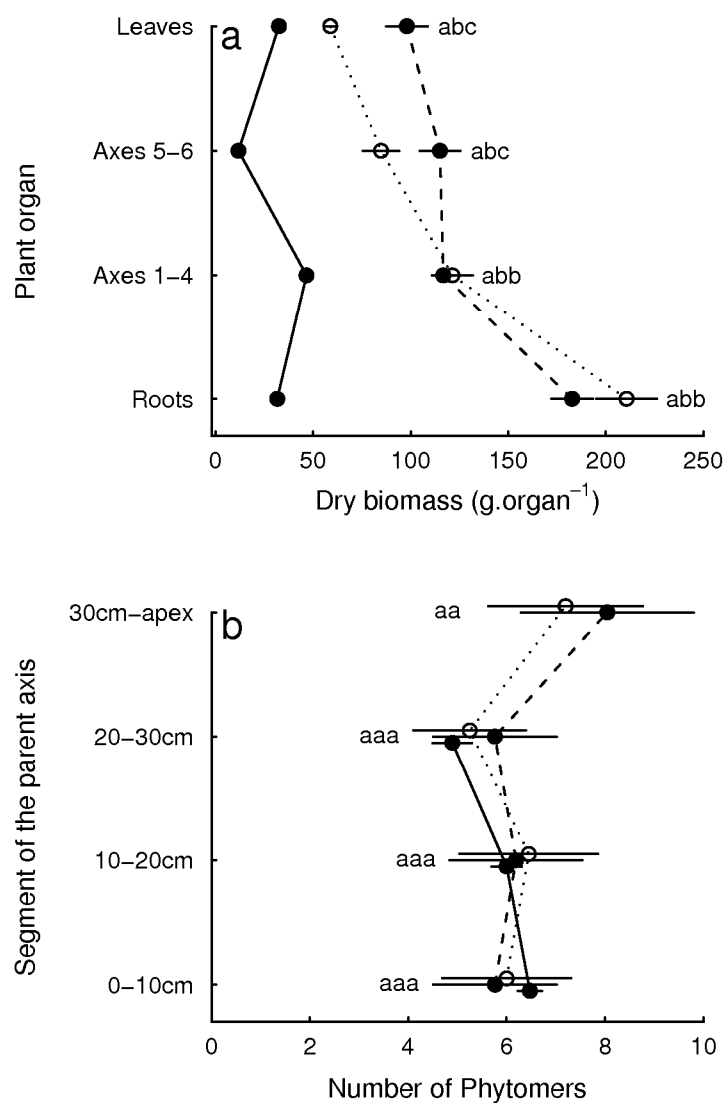
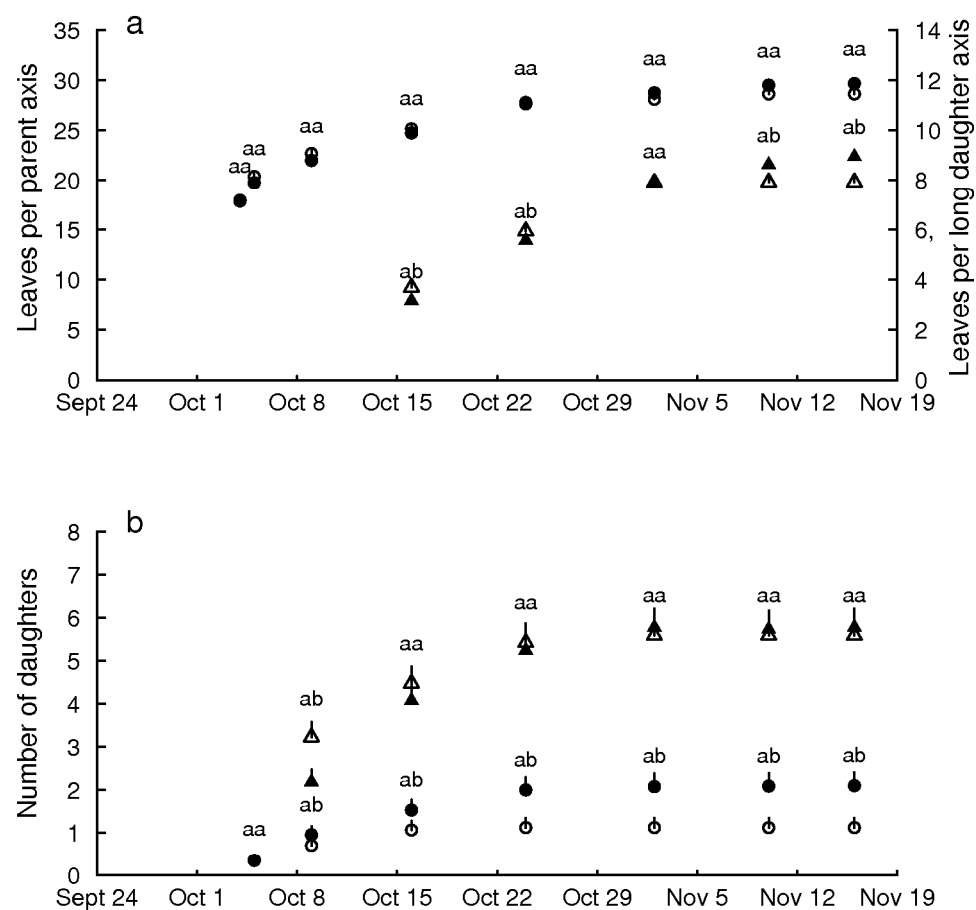
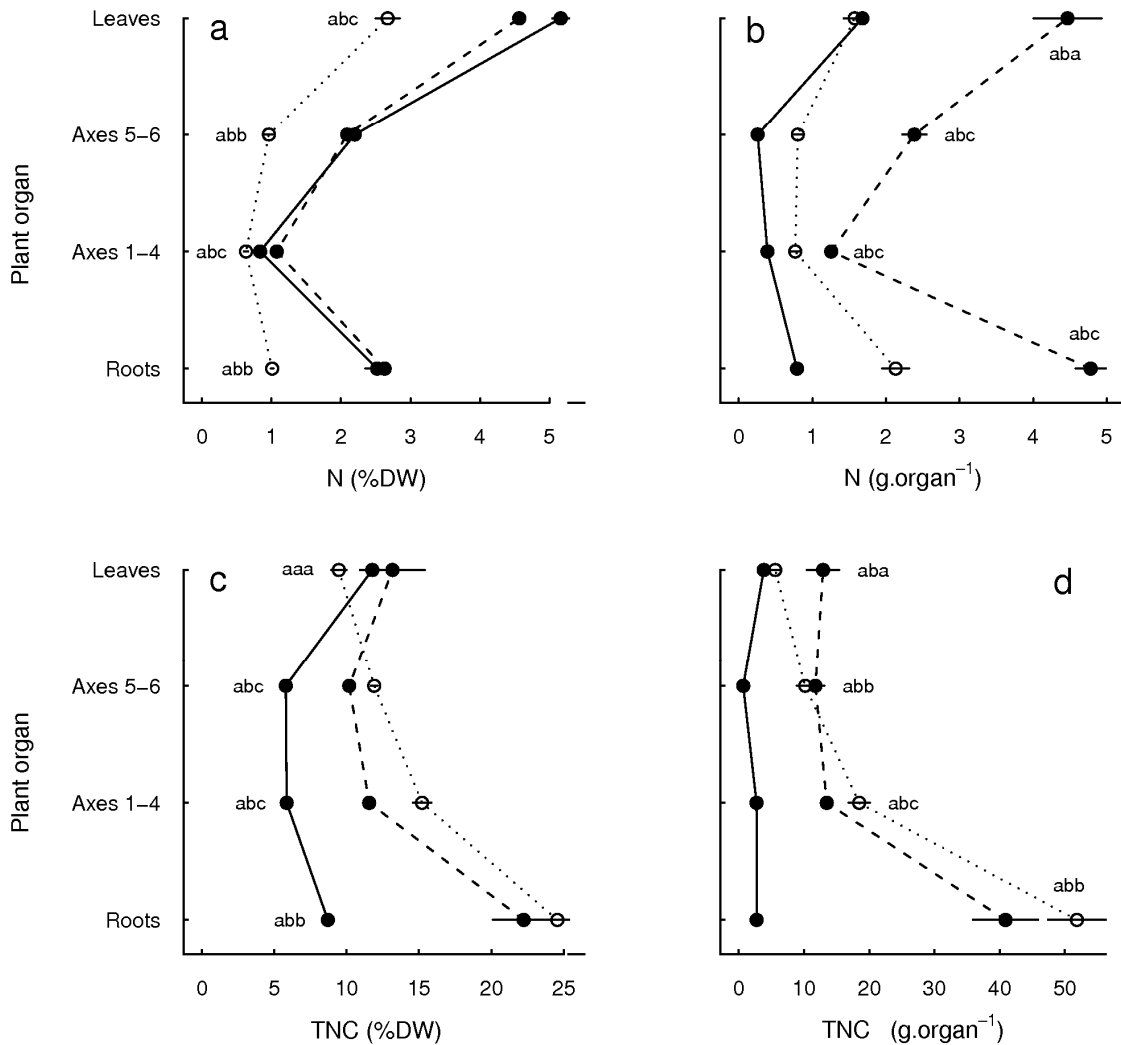


Figure 4



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Figure 5



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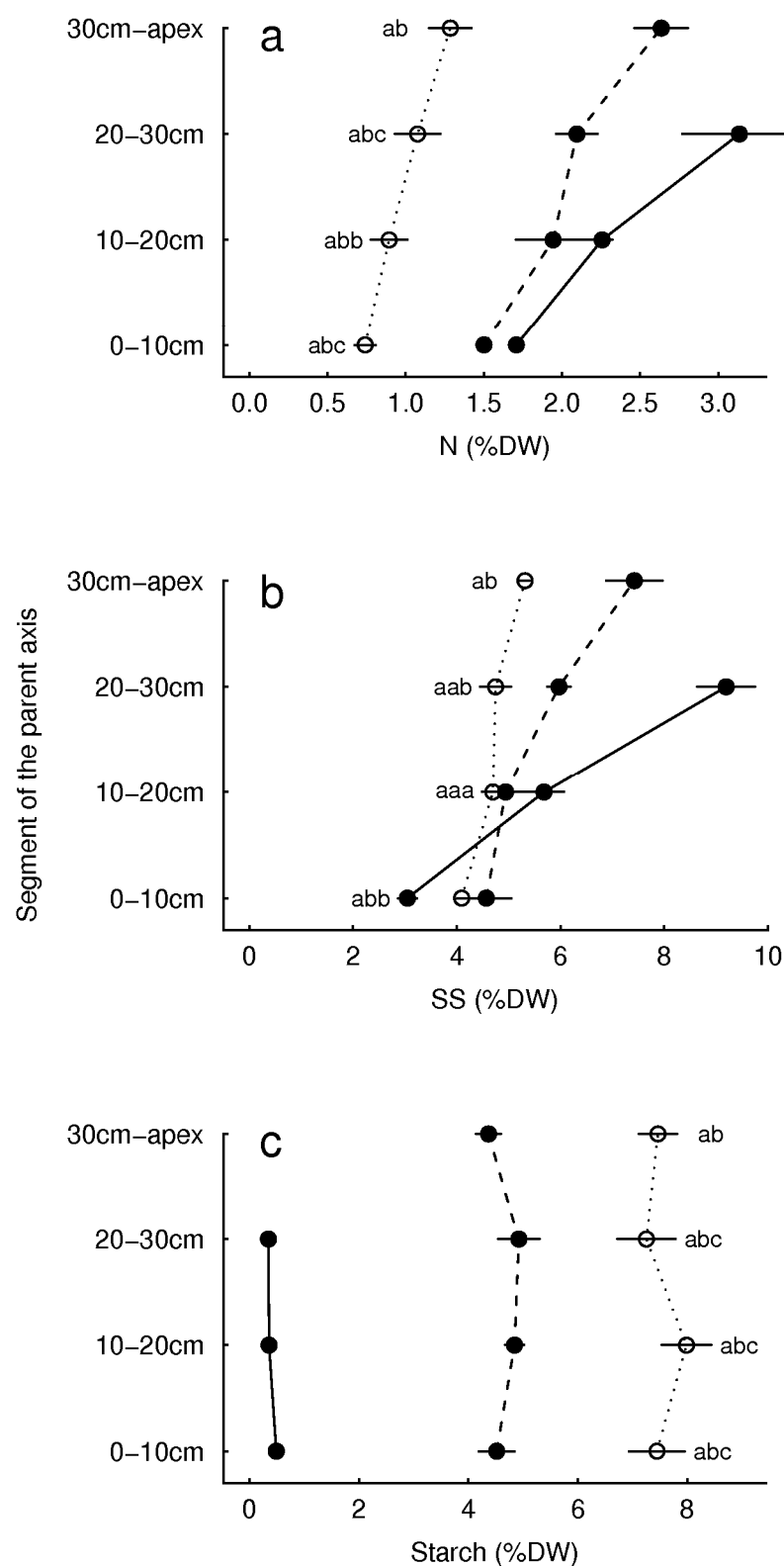
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Figure 6



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Figure 7

