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ORIGINAL PAPER

C depletion and tree dieback in young peach trees: a possible consequence of N shortage?

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Abstract

• ***Key message*** **Bud burst disruption, carbon depletion and tree dieback in spring were experimentally linked to N shortage the previous autumn. Dieback occurred despite tree N concentrations were compatible with plant survival: their N stores being blocked in the roots and woody axes.**

• ***Context*** Tree dieback is generally linked to hydraulic failure or carbon (C) starvation but seldom to poor nitrogen (N) resources.

• ***Aim*** We provide here an experimental evidence linking autumn N shortage, C depletion and tree dieback in spring.

• ***Methods*** Young peach trees were either N deprived or fertilised in autumn, and then fed in excess in spring. Spring supplies were ^{15}N-labelled. The effects of the deprivation on tree development, N uptake and C status were then assessed by coupling in situ measurements of shoot development with organ biochemical and isotopic determinations.

• ***Results*** All deprived trees died within 3 months after burst. Bud burst was severely disrupted, and vegetative growth limited to the expansion of a few leaves. The dead trees absorbed between 39 and 117 mg ^{15}N in spring, and their roots and axes contained 758 mg more nitrogen than the fertilised trees, suggesting that they did not mobilise their N reserves in spring. They also had lower non-structural carbohydrate concentrations (<3.9 % DW) than the fertilised trees (>15.4 % DW), which were below the threshold accepted for plant survival.

• ***Conclusion*** Two possible causes of total non-structural carbon (or TNC) depletion are discussed: insufficient storage due to advanced leaf senescence or increase in the C costs regarding winter embolism recovery.

Keywords Tree dieback · N storage · C storage · Bud burst

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1 Introduction

Tree dieback has generally been linked to hydraulic failure and/or carbon (C) starvation (Sevanto et al. 2014; McDowell and Sevanto 2010; Sala et al. 2012). Numerous dedicated studies have focused on forest decline which has increased dramatically throughout the world during the past 2 decades as a consequence of global change (Allen et al. 2010). The consensus view has been that higher temperatures coupled with frequent and severe drought events decreased carbon assimilation and increased tree respiration (Granier et al. 2007; Vickers et al. 2012), thus compromising plant survival. The risk of tree death after an extreme climatic event is furthermore amplified by poor soil resources (Rozas and Sampedro 2013). However, although nitrogen (N) is a major constituent of plants, its specific role in tree dieback has, to our knowledge, never been explored.

This point nevertheless deserves consideration given the interdependency of C and N acquisition in plants. An N deficiency causes leaf yellowing and blighting (Taiz and Zeiger 2010), decreases leaf N concentrations and, in turn, photosynthesis (Cao et al. 2007). Additionally, N uptake is proportional to root respiration (Bloom et al. 1992; Reich et al. 1998) and is dependent on the carbohydrate supply to the roots (Jordan et al. 1998).

Winter and spring are critical periods for the survival of stressed deciduous trees (Galvez et al. 2013; Breda et al. 2006) which are reliant on their C and N reserves to ensure

their maintenance (Sauter and Vancleve 1994), the development of cold hardiness (Charrier and Ameglio 2011) and their first growth flush (Stassen et al. 1981a, b; Millard and Neilsen 1989). A deficit of N storage may be partially compensated for by the restoration of N uptake before bud burst; however, the associated C costs are prohibitive (Thitithanakul et al. 2012; Jordan et al. 2014). Autumn storage is therefore of crucial importance to tree perenniality.

Orchard trees are likely more susceptible to poor nutrient conditions than forest trees because of (i) the large quantities of biomass lost each year due to fruit production and pruning (El-Jendoubi et al. 2013) and of (ii) their wide spacing which limits light competition and favours crown expansion. Therefore, they usually display higher growth rates than timber species and, until recently, have benefited from significant water and nutrient supplies. However, orchard management techniques have evolved, favouring low input strategies in response to increasing ecological concerns and constraints, such as limited access to water and the implementation of restrictive fertilisation guidelines, particularly in Western Europe. Moderate stresses are now commonly applied to the risk of imposing suboptimal conditions for tree development.

Low N supplies may therefore limit autumn storage and thereby compromise early spring development. This theory was evaluated in young peach trees (*Prunus persica* L. Batch) during the present study, which analysed the consequences of N deprivation applied in the autumn on spring development (or dieback). For this purpose, the trees were either N deprived, N limited or N unlimited in autumn, and then fed in excess, regarding their growth needs, in spring. Furthermore, the spring N supplies were ^{15}N-labelled. The effects of autumn N deprivation on (i) tree development, (ii) N uptake and (iii) C status (namely C depletion) could then be assessed by coupling in situ measurements of shoot development with destructive harvests and biochemical and isotopic determinations in plant organs. This study focused in particular on the fate of these deprived trees. Indeed, those trees died within 3 months after bud burst and had therefore been excluded from two previous studies analysing the effects of a non-zero but limited autumn N supply on tree architecture (Jordan et al. 2009), gross growth and nutrient status (Jordan et al. 2012). Our aim here was therefore to document the link between N deprivation in autumn, C depletion and tree dieback in spring.

2 Materials and methods

Experimental design The study was carried out at the INRA Research Centre in Avignon (southern France). Forty-one-year-old peach rootstocks (*P. persica* cv. GF305) with a diameter of between 6 and 8 mm were grafted with pushing buds of peach (cv. RO52) on March 16, 1999, and then transplanted into 10-dm^3 pots filled with a 50 % vermiculite and 50 % peat mixture. The trees were left in a greenhouse for 1 month and then moved outside. During the growth period, chemical treatments were applied regularly to deter pests. Two drippers per pot, each delivering 2 dm^3 h^{-1}, supplied a nutrient solution concentrated at 1 g dm^{-3} of a commercial 14/7/27 % NPK fertiliser. The trees were irrigated for 6 min, ten times each day.

At the end of shoot elongation, 18 trees were selected for their homogeneity and divided into groups of six individuals to receive three different levels of N supply (details below) between September 13 and November 10. Six further trees were kept under automatic irrigation for subsequent evaluation of the natural abundance of ^{15}N. Leaf fall was monitored by counting the number of leaves per tree on eight occasions between September 30 (100 % leaves) and November 8 (0 leaves).

On February 24, 2000, after soaking the roots in tap water for 3 h, the trees were transplanted into 15-dm^3 pots containing an "N-free" substrate composed of 60 % sand (Biot B4, ref 16.14.2) and 40 % pozzolana. The trees were left outside and fed until harvest with a ^{15}N-labelled solution (details below). The number of flower and vegetative buds was counted every 2 days, from February 28 to March 20 for flower buds and to March 30 for vegetative buds. The buds were included in the counts when the petals or leaf tissues became visible after separation of the bud scales. The number of expanded leaves was monitored once a week between April 11 and harvest. The small rosette leaves that had been preformed in the buds were counted separately from the larger ones inserted on the elongated axes that resulted from the plastochronal activity of apical meristems.

Nutrition and treatments Between September 13 and November 10, each group of six trees received a different level of N supply which was either null (0 N treatment), too small to ensure optimal spring development (limiting treatment), or provided in excess according to plant needs (control treatment). These three autumn treatments corresponded to a weekly supply of 0, 1.3 and 2.6 g NO_3^-plant^{-1}, respectively. Nitrate and other nutrients were supplied three times a week (on Monday, Wednesday and Friday) in a 0.3-dm^3 nutrient solution which, depending on the treatment, contained 0, 1.5 or 3 g NO_3 dm^{-3} as $Ca(NO_3)_2$. The solution also contained the following in moles per cubic metre, $MgSO_4$ 1, KCl 0.2, K_2SO_4 1.5, KH_2SO_4 0.5, Fe EDDHA (ethylenediamine-di(*o*-hydroxyphenylacetic acid)) 0.1, and in μmol m^{-3}, H_3BO_3 206.58, $MnCl_2$ 116.57, $CuSO_4$ 4.72, $ZnSO_4$ 32.41 and $MoNH_4$ 28.15. No excess solution drained from the pots. On the four remaining days of each week, field capacity was restored by automatic irrigation with tap water for ten sequences of 6 min each (corresponding to a daily supply of 4 dm^3 tree^{-1}). The number of irrigations per day was reduced

to five (29 September), then to three (15 October) and finally to 0 (2 November). No irrigation was supplied between leaf fall and spring transplantation.

On February 24, 2000, the NO_3^- concentration in the nutrient solution was labelled with 2.6 at.% ^{15}N and adjusted to 1.5 mmol dm^{-3}. The concentrations of the other nutrients were the same as in the solution used during the autumn. Each tree received 0.3 dm^3 day^{-1} from March 2 to April 2, then 0.5 dm^3 day^{-1} until April 18, 1 dm^3 day^{-1} until May 4, 1.5 dm^3 day^{-1} until May 15 and 2 dm^3 day^{-1} until tree sampling. The supply was adjusted so as to ensure that some of the solution was available to the plants throughout the day, in saucers placed under the pots.

Tree sampling Two destructive samplings of three limited and control trees were made at the end of the first growth flush, i.e. on May 29 (harvest 1) and June 13 (harvest 2), respectively. The six trees used to evaluate the natural abundance of ^{15}N were sampled on February 25.

For the 0 N trees, the harvests were adjusted to plant death. A tree was assumed to be dead when all its vegetative buds had dried. The four trees which died during bud burst (see "Results") were harvested on May 10 (harvest 1). The two remaining trees were harvested on May 29 and on June 13, respectively, and grouped for the statistical analyses as they both died during the first growth stage, i.e. during the rapid leaf expansion stage.

The trees were subsampled for biochemical analyses as follows: thin and thick roots (less than and more than 0.5 cm in diameter, respectively), rootstock trunk, main axis, secondary axis, stems of current-year shoots, leaves, and fruits or flowers. Because N is stored preferentially in the bark and non-structural C mainly accumulates in wood, the wood and bark were separated for biochemical analyses.

Biochemical analyses All samples were kept at −20 °C until freeze drying and weighing. The samples were ground in a stainless steel Dangoumeau grinder (Prolabo France) and cooled with liquid N_2. Total N concentrations and ^{15}N excess levels were determined using a Tracer-MAT continuous flow mass-spectrometer (Finnigan MAT, Hemel Hempstead, UK). ^{15}N enrichment was used to calculate the amount of labelled N taken up from the fertiliser solution in 2000, as described by Millard and Neilsen 1989.

The extractions and determinations of soluble sugar concentrations were performed as described by Gomez and Faurobert (2002): extraction in a methanol-chloroform-water medium and determination by HPLC (Sugar PaK 1 column at 80 °C and refractometer, Waters, Milford, MA). The starch concentration was determined on pellets, as described by Jordan and Habib (1996): solubilisation by autoclaving, depolymerisation and determination of the resulting glucose using the reference enzymatic method. Total non-structural carbohydrate (TNC) was assumed to be the sum of soluble sugars and starch.

The concentrations and contents of each compound thus determined in the subsamples were calculated from the dry weight (DW) and concentrations in (i) the perennial structure comprising the roots, bark and wood of the rootstock trunk, main and secondary axes, (ii) current-year organs (stems, leaves, fruits or flowers), and (iii) the whole tree.

Data analyses Randomisation (or permutation) tests (Manly 1991) performed at 5 % level were used to evaluate the effects of treatments and/or harvest dates. Empirical distributions of these variables under the null hypothesis of no treatment effect were derived from 2500 random assignments of the trees to the different treatments and harvest dates (R 2.11.0 software, www.r-project.org/). This random assignment was justified because the trees (i) had been raised under the same conditions, (ii) were equivalent in terms of size and (iii) were randomly allocated to the groups. The test statistics were the pairwise differences between the means of the variables per group. Two observed means were considered to differ significantly if their difference was within the distribution tails of the empirical distributions of these differences under the null hypothesis.

The effect of the treatments on leaf fall was analysed by comparing the number of leaves remaining on the trees at each counting date. The effects of treatment and harvest date on TNC and total N organ concentrations and contents were assessed by comparing the six possible combinations of treatments and harvest dates. For tree ^{15}N contents, the effect of harvest date was compared separately for each treatment, since the 0 N trees contained about 20 times less ^{15}N than the fertilised (limited and control) trees.

3 Results

Leaf senescence In autumn, the 0 N trees could be rapidly identified by the yellow colour of their leaves. Indeed, yellowing started earlier and was more intense in the 0 N trees than in fertilised trees. On the control trees, in particular, the leaves remained green almost up to leaf fall.

Leaf fall started in October and comprised two phases (Fig. 1). Until October 22, leaf fall remained limited and was earlier in the 0 N trees, which had lost 11 % of their leaves by October 15, while the others had only lost 4 %. This trend was reversed after October 22 once the fall rates had increased under all treatments, but the differences only became significant on

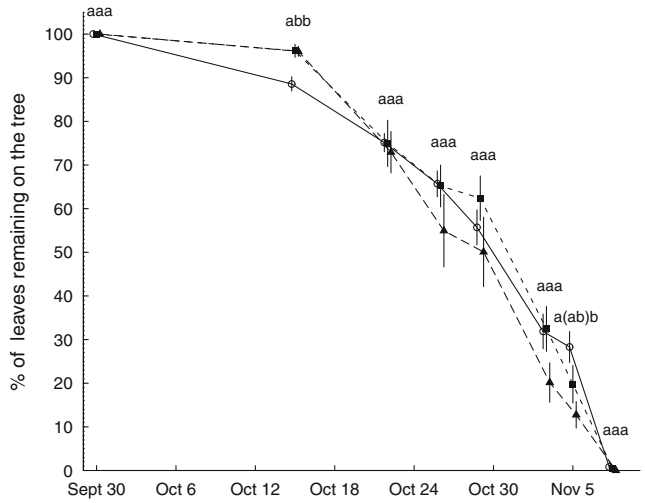

Fig. 1 Percentage of leaves remaining on the trees during leaf fall in autumn for the 0 N (*open circles*, *solid line*), limited (*full squares*, *dashed line*) and control trees (*full triangles*, *long dashed line*). *Each symbol* is the mean of six trees plotted with standard errors. The means were ranked (*a*, *ab*, *b*) from the lowest to the highest values. They are significantly different if coded with *different letters*. Statistical significance was inferred from randomisation tests based on the generation of 2500 random orders

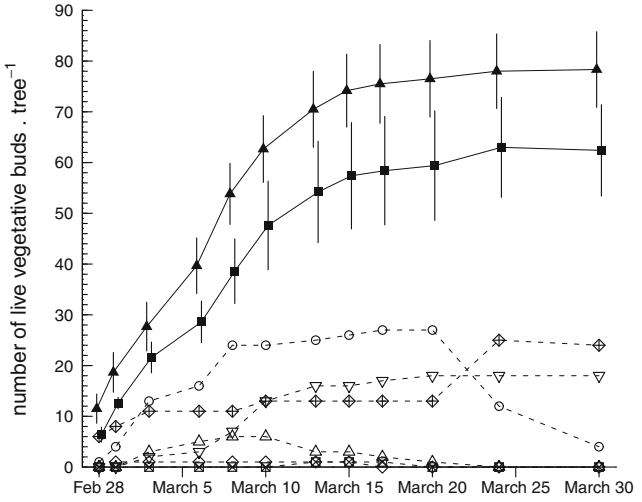

Fig. 2 Dynamic response of bud burst (number of live green vegetative buds) for the 0 N (*open symbols*, *dashed lines*), limited (*full squares*, *solid line*) and control trees (*full triangles*, *solid lines*). For the limited and control trees, *each symbol* is the mean of six trees plotted with standard errors. For the 0 N trees, *each dashed line* associated with an *open symbol* represents a single individual

November 5. At that date, the number of leaves remaining on the trees ranged from 13 % (control trees) to 28 % (0 N trees). Leaf fall was then completed rapidly, i.e. before November 8, due to a wind storm.

Flowering, bud burst and development Flower buds emerged before March 17, whatever the treatment, but the number of developing buds was very small on the 0 N trees. Indeed, two of these trees did not flower at all, two produced only one flower and the remaining two produced 25 and 26 flowers, respectively. This was much less than the numbers counted on the limited trees (60±9.3: SE or standard error) and control trees (88±8.6). Full bloom was observed at around March 20, but none of the "0 N flowers" produced a fruit.

Vegetative bud burst was achieved on March 24 and was also very low on the 0 N trees (Fig. 2). All of them developed at least one vegetative bud, but huge variations in bud number and lifespan were observed among the trees. A vegetative bud was excluded from the counts when all its leaflets completely dried out. Four trees dried and died during March, i.e. before the leaves preformed in the initial rosettes had fully expanded. Three of them had developed fewer than six buds, but the fourth developed 25 buds which was as many as the two trees that were still surviving at this stage.

These remaining 0 N trees dried out between April 19 and May 15 for the first one and between May 9 and June 7 for the second one. They had developed respectively 12 and 43 rosette leaves but no axis leaves. By contrast, the number of expanded leaves on the fertilised trees increased rapidly during April and May due to axis elongation. Indeed, on April 19, 36 % (or 100±19) and 50 % (or 201±55) of the tree leaves were neoformed, i.e. inserted on the elongated axes of the limited and control trees, respectively. These proportions had reached 53 and 63 % on May 9.

Spring N uptake Spring N uptake was restored in all trees before dieback (Table 1) and increased significantly in line with survival time. Indeed, the 0 N trees had absorbed 2.4 % (42 mg ^{15}N) of their total N content in spring even if death occurred during bud burst. This percentage reached 5.7 % (78 mg ^{15}N) in the tree which survived that stage. However, these intakes remained small when compared with the fact that the limited and control trees had absorbed more than 1000 mg ^{15}N by May 29.

Tree N status Tree N concentrations were similar in all trees whatever the treatment and harvest date (Fig. 3a), while tree N contents were proportionate to tree DW and varied by a factor

Table 1 Spring N uptake (mg ^{15}N tree^{-1}) as a function of treatment and harvest date

	0 N trees	Limited trees	Control trees
Harvest 1	42 a±8.1	1154 a±220	1370 a±67
Harvest 2	78 b±40	1349 a±323	2004 b±25

The numbers are means and standard errors of two (harvest 2, 0 N trees), three (limited and control trees) or four (harvest 1, 0 N trees) replicates. The effect of harvest date was tested for each treatment, by randomisation tests based of the generation of 2500 random orders. It was significant (5 % level) if coded with different letters. The effect of treatment was not tested due to the important difference between the 0 N and fertilised trees

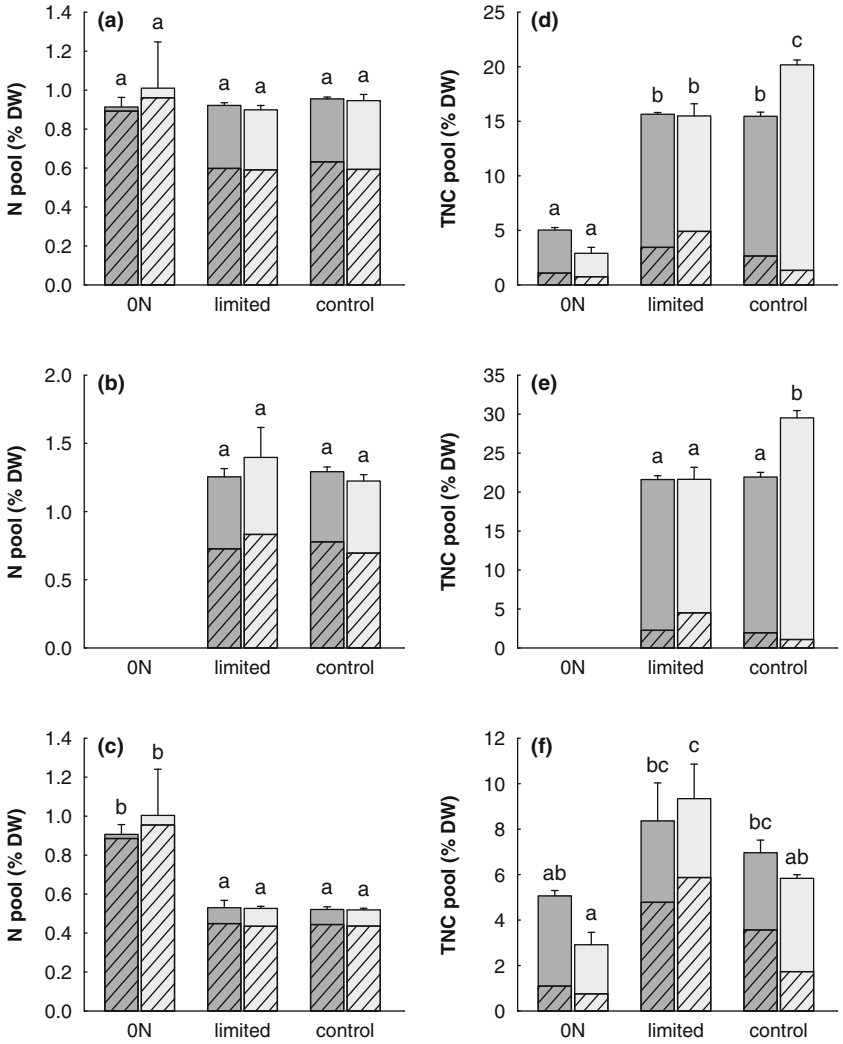

Fig. 3 N and TNC concentrations (means and standard errors in % DW) as a function of treatment and harvest date for **a**, **d** the whole trees, **b**, **e** the current-year organs, i.e. the flowers, fruits, leaves and current-year stems, and **c**, **f** the perennial structures, i.e. the roots and old axes. Harvests 1 and 2 are represented by *dark* and *pale grey bars*, respectively. Starch and ^{14}N are shown by *hatched areas*, and the remainder, ^{15}N and soluble sugars by non-hatched areas. The effect of treatment and harvest date was tested by randomisation tests based on the generation of 2500 random orders. It was significant (5 % level) if coded with *different letters*

of 3.5 (Fig. 4a). However, the 0 N trees differed markedly from their fertilised counterparts, firstly because their N pool was mainly composed of ^{14}N, which represented only between 63 and 66 % of total N in the fertilised trees. Indeed, the limited and control trees absorbed one third of their total N between bud burst and harvest.

Secondly, the current-year organs, i.e. the leafy shoots and fruits, represented less than 1 % of the tree DW on the 0 N trees, while in the fertilised trees, the new shoots and fruits accounted for between 47 and 61 % of the tree DW and contained between 69 and 78 % of the tree N content (Fig. 4a). Indeed, the fertilised trees had higher total, ^{14}N and ^{15}N concentrations in their current-year organs (Fig. 3b) than in their perennial structures (Fig. 3c).

In the perennial structures, i.e. the roots and old axes, the N concentrations and contents were significantly higher in the 0 N trees than in the fertilised ones. The differences were mainly due to ^{14}N. The fertilised trees were sampled after store emptying since the ^{14}N content of their perennial structures was low (859±122 mg ^{14}N) and independent of treatment and harvest date. By contrast, the 0 N trees contained 1617± 192 mg ^{14}N, and the difference (758 mg ^{14}N) probably reflected the N stored by the 0 N trees, which was not mobilised from perennial organs to sustain shoot and fruit growth. Indeed, in the limited and control trees, the amounts of ^{14}N incorporated into the current-year organs reached 1450 ±133 mg and 2130±135 mg, respectively.

Tree TNC status The tree TNC concentrations were threefold lower in the 0 N trees than in the fertilised ones (Fig. 3d). The differences in the TNC contents were even more marked (Fig. 4b). Indeed, the 0 N trees contained less than 9 g TNC, versus around 60 g in the fertilised trees, with one exception: control trees at harvest 2 contained 115 g TNC. Furthermore, both concentrations and contents decreased slightly over time in the 0 N trees (Table 2), passing from 5.5 to 2.3 % DW and from 8.5 to 4.0 g TNC, when the survival time increased. In the fertilised trees, by contrast, both concentrations and contents increased over time, but the differences were only

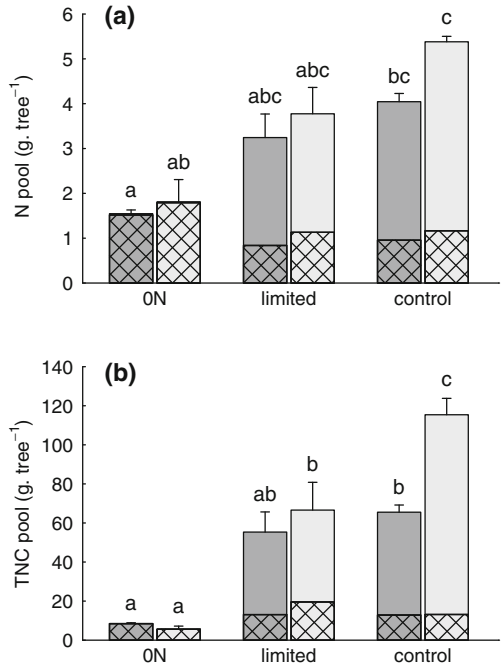

Fig. 4 Tree contents (means and standard errors in grams per tree) as a function of treatment and harvest date for **a** N and **b** TNC. Harvests 1 and 2 are represented by *dark* and *pale grey bars*, respectively. The contents of perennial organs are shown in *squared areas* and those of the current-year shoots by non-hatched areas. The effect of treatment and harvest date was tested by randomisation tests based on the generation of 2500 random orders. It was significant (5 % level) if coded with *different letters*

significant in the controls. Starch contributed 24 % to the TNC pool of the 0 N trees, while this proportion ranged from 7 % (control trees, harvest 2) to 31 % (limited trees, harvest 2) in the fertilised trees.

In the current-year organs (Fig. 3e), the variations over time of TNC concentrations resembled those observed for the whole trees, even though the mean concentrations were higher, i.e. between 22 and 30 % DW. Indeed, current-year organs contained between 70 % (limited trees, harvest 2) and 89 % (control trees, harvest 2) of the tree TNC (Fig. 4b).

Table 2 Tree total non-structural carbon (TNC) concentration in relation to the date of death for the 0 N trees

Date of death	TNC concentration (% DW)
March 3	5.2
March 21	5.5
March 26	5.0
March 30	4.4
May 15	3.4
June 7	2.6

Each line represented a single tree of the 0 N treatment, for which 100 % mortality was observed. A tree was assumed to be dead when all its vegetative buds had dried

Even though the perennial structures of the fertilised trees contained only a small proportion of the tree TNC, their TNC concentrations and contents were higher than in 0 N trees (Fig. 3f). The differences were the greatest between the 0 N and the limited trees. The perennial structures also had much lower concentrations than the current-year organs. Indeed, the concentrations were comprised between 2.9 % DW (0 N trees, harvest 2) and 9.3 % DW (limited trees, harvest 2) for TNC, and between 2.16 and 4.10 % DW for soluble sugars.

4 Discussion

C depletion as a consequence of N shortage In autumn, an N limitation reduces photosynthesis when the leaf N concentration drops below a threshold level, which is set at around 2.2 % N DW for rosaceae species (Cheng and Fuchigami 2000). This has been observed in trees that were unable to correct their low N status in the autumn through N uptake, which were therefore exporting N from their leaves to a greater extent (Cheng et al. 2002) and also earlier before abscission (Grassi et al. 2005) compared with well-nourished trees. However, an N limitation does not only restrict C acquisition but also C expenses, because root respiration is proportional to N uptake (Bloom et al. 1992; Reich et al. 1998). The final outcome on tree TNC content at leaf fall is still a matter of discussion since contrasting results have been published, sometimes on the same species (Bollmark et al. 1999; Von Fircks and Sennerby-Forsse 1998; Cheng and Fuchigami 2000; Cheng et al. 2002). It is however admitted that reducing the N supply in autumn will increase the tree TNC content, unless it affects leaf senescence, which was probably the case in our study. Indeed, the 0 N trees were characterised by rapid leaf yellowing and high leaf fall rate.

During winter and early spring, TNC expenses may also increase because of the necessary adaptation of the 0 N trees to N shortage. Several C costly mechanisms have been identified in the literature. Firstly, an N storage deficit can boost N uptake around bud burst, but this has not always been observed (Thitithanakul et al. 2012; Jordan et al. 2012, 2014), perhaps because it is solely reliant on TNC mobilisation. The respiration costs of the ^{15}N uptake of the 0 N trees could be estimated at between 0.18 and 0.88 g equivalent glucose, assuming that three carbon atoms are released per NO_3^- assimilated and transformed into asparagine (Sasakawa and LaRue 1986; Amthor 2000). Secondly, an N limitation may stimulate root growth (Millard and Neilsen 1989; Jordan et al. 2012) in order to increase the volume of prospected soil, in accordance with the theory of functional equilibrium. Newly developed fine white roots have thus been observed, but unlikely not quantified, on all dead trees at harvest. Thirdly, the C costs of restoring xylem function probably increases in line with N

deficiency because winter embolism is related to xylem osmolarity, i.e. to the concentrations in soluble C and N compounds (Breda et al. 2006; Sakr et al. 2003; Charrier and Ameglio 2011; Galvez et al. 2013). Despite the fact that peach wood porosity is diffuse, positive xylem pressure plays only a minor role in recovery from winter embolism (Ameglio et al. 2002). Xylem function must therefore be restored through the production of new functional conduits, i.e. by cambial reactivation (Ameglio et al. 2002), which requires large amounts of TNC and renders the species susceptible to dieback under low TNC storage conditions (Barbaroux et al. 2003).

Previous studies (Jordan et al. 1998, 2012) had demonstrated that TNC concentrations of around 10 % enable normal leaf out and growth in spring in the absence of N limitation, but if the tree N concentration is reduced by 50 %, young trees need to contain at least 15 % TNC if spring development is not to be penalised (Jordan et al. 2014). Indeed, TNC mobilisation in early spring can increase significantly (i.e. up to 26 g TNC; Jordan et al. 2014) in the case of N limitation. During the present study, all 0 N trees were TNC depleted, whatever the cause: Insufficient storage coupled (or not) with high C losses. Indeed, they contained less than 1.3 % starch, and their soluble sugar concentrations varied between 2.2 and 3.9 % DW.

Shoot development and dieback Although they were not fertilised in the autumn, the 0 N trees accumulated small but significant amounts of ^{14}N, probably by remobilising their leaf N prior to abscission. In spring, these N stores were blocked in the perennial structure, as a cause for, or a consequence of low bud break (see below). The fate of an axillary bud (i.e. its differentiation into a flower or a vegetative or blind bud) depends on the growth context of its parent internode (or growth unit) and is thus determined during vegetative growth (Kervella et al. 1995; Boonprakob et al. 1996), i.e. before the application of the treatments in the autumn. To our knowledge, bud burst in spring has never been investigated in terms of its relationship with N availability. However, low N storage is known to affect shoot development by (i) decreasing the proportion of rosettes that are transformed into elongated axes (Lobit et al. 2001; Grelet et al. 2003; Jordan et al. 2009), but not the number of developing buds set by a specific peach variety (Perezgonzalez 1993), and (ii) delaying spring development (Jordan et al. 2014).

The presence of significant amounts of unused ^{14}N has advocated for a possible role of TNC in the disruption of bud burst. Indeed, partial bud break, which usually precedes dieback, could be considered as a marker of TNC shortage (Breda et al. 2006; Marcais and Breda 2006). According to this theory, low TNC availability would limit bud break, thus in turn preventing the recovery of photosynthesis. Indeed, bud burst is dependent on the hexose content of the meristematic tissues (Maurel et al. 2004). Photosynthesis contributes to sustaining tree metabolism but only after full expansion of the first leaves (Bieleski and Redgwell 1985), which occurs at around fruit set, i.e. around end of March in the RO52 cultivar. It could therefore be assumed that four 0 N trees died before the photosynthesis would normally have been restored and the two remaining individuals after that stage. However, C depletion continued in April in those two trees which developed only a few rosettes leaves and were therefore unable to ensure significant levels of C acquisition.

Dieback as a possible consequence of TNC starvation Carbon starvation has been identified as a possible cause of tree mortality following severe stress such as defoliation (Landhausser and Lieffers 2012) or drought (Adams et al. 2013; Galiano et al. 2011). Plant withering can last for several years (Marcais and Breda 2006; Breda et al. 2006; Galiano et al. 2011), during which death (or recovery) depends on the plant's ability to rebuild its TNC reserves before the onset of a second stress (insect attack, frost or drought). Moreover, the mortality threshold varies according to the environment, size and global functioning of a tree, since stored C contributes to maintaining cell turgor and xylem integrity (Secchi and Zwieniecki 2011; Sala et al. 2012; Pantin et al. 2013) alongside other soluble compounds, which include calcium, potassium, amino acids and soluble proteins. In addition, some starch may be blocked in its reservoirs by partial hydraulic failure and thus not be available for plant metabolism (Sala et al. 2012; Sevanto et al. 2014). This failure may be due to an incomplete recovery from winter embolism (with radial growth being too small in early spring) or to midday embolism, which can be observed even under benign water stress conditions (Sala et al. 2012). Carbon starvation is possible even though the tree TNC content is above zero (McDowell and Sevanto 2010).

Markedly varying TNC concentrations in dead trees have been observed in the literature. According to Landhausser and Lieffers (2012) and Hartmann et al. (2013), TNC starvation was limited to the roots, thus contributing to maintaining a water pressure deficit gradient throughout the trunk. Galvez et al. (2013) found that *Populus tremuloides* and *Populus balsamifer* trees undergoing winter mortality contained 7 and 12 % TNC, respectively, and were almost completely starch-depleted. We determined a mean value of 4.3 % TNC. Hydraulic failure, which usually accompanies C starvation, may nonetheless contribute to tree death (Sevanto et al. 2014).

Although the 0 N trees in our study exhausted their starch reserves before dieback, this was not the case of the 1.5 N trees, which were also N limited. The latter thus maintained a significant level of starch in spring which penalised spring N uptake and delayed shoot growth (Jordan et al. 2009, 2012). This could probably be explained by the constitution of "safety reserves" under stress conditions (Silpi et al. 2007) which become inaccessible unless the onset of a dramatic event compromises tree survival (Vargas et al. 2009).

5 Conclusion

Our study provides an experimental evidence of the link between N shortage and TNC depletion, although we did not investigate the underlying mechanisms. TNC depletion occurred in trees whose N concentrations were compatible with plant survival and whose N stores were blocked in the roots and woody axes. Further investigation is therefore necessary in order to (i) explain the causes of TNC depletion, reduced storage or increased C expenses, and (ii) to determine whether TNC depletion led to plant death, possibly through hydraulic failure.

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