

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

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33C depletion and tree dieback in young peach trees: a possible consequence of N shortage?C

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35**Key message:** Bud burst disruption, carbon depletion and tree dieback in spring was experimentally
36linked to N shortage the previous autumn. Dieback occurred despite tree N concentrations were
37compatible with plant survival: their N stores being blocked in the roots and woody axes.

38

39**Summary:**

40**Context:** Tree dieback is generally linked to hydraulic failure or carbon (C) starvation, but seldom to poor
41nitrogen (N) resources.

42**Aim:** We provide here an experimental evidence linking autumn N shortage, C depletion and tree dieback
43in spring.

44**Methods:** Young peach trees were either N deprived or fertilized in autumn, then fed in excess in spring.
45Spring supplies were ¹⁵N-labelled. The effects of the deprivation on tree development, N uptake and C
46status was then assessed by coupling *in situ* measurements of shoot development with organ biochemical
47and isotopic determinations.

48**Results:** All deprived trees died within three months after burst. Bud burst was severely disrupted, and
49vegetative growth limited to the expansion of a few leaves. The dead trees absorbed between 39 and 117
50mg ¹⁵N in spring, and their roots and axes contained 758 mg more nitrogen than the fertilized trees,
51suggesting that they did not mobilize their N reserves in spring. They also had lower non-structural
52carbohydrate concentrations (<3.9% DW) than the fertilized trees (>15.4% DW), which were below the
53threshold accepted for plant survival.

54**Conclusion:** Two possible causes of total non-structural carbon (or TNC) depletion are discussed:
55insufficient storage due to advanced leaf senescence or increase in the C costs regarding winter embolism
56recovery.

57**Introduction:**

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

68 This point nevertheless deserves consideration given the interdependency of C and N
69 acquisition in plants. An N deficiency causes leaf yellowing and blighting (Taiz and Zeiger
70 2010), decreases leaf N concentrations and in turn, photosynthesis (Cao et al. 2007).
71 Additionally, N uptake is proportional to root respiration (Bloom et al. 1992; Reich et al. 1998)
72 and is dependent on the carbohydrate supply to the roots (Jordan et al. 1998).

73 Winter and spring are critical periods for the survival of stressed deciduous trees (Galvez
74 et al. 2013; Bréda et al. 2006) which are reliant on their C and N reserves to ensure their
75 maintenance (Sauter and van Cleve 1994), the development of cold hardiness (Charrier and
76 Ameglio 2011) and their first growth flush (Stassen et al. 1981a and b; Millard and Neilsen
77 1989). A deficit of N storage may be partially compensated for by the restoration of N uptake
78 before bud burst, however the associated C costs are prohibitive (Thitihanakul et al. 2012; Jordan
79 et al. 2014). Autumn storage is therefore of crucial importance to tree perenniality.

80 Orchard trees are likely more susceptible to poor nutrient conditions than forest trees

81because of (i) the large quantities of biomass lost each year due to fruit production and pruning
82(El-Jendoubi et al. 2013), and of (ii) their wide spacing which limits light competition and
83favours crown expansion. Therefore, they usually display higher growth rates than timber species
84and, until recently have benefited from significant water and nutrient supplies. However, orchard
85management techniques have evolved, favouring low input strategies in response to increasing
86ecological concerns and constraints, such as limited access to water and the implementation of
87restrictive fertilisation guidelines, particularly in Western Europe. Moderate stresses are now
88commonly applied to the risk of imposing suboptimal conditions for tree development.

89 Low N supplies may therefore limit autumn storage and thereby compromise early spring
90development. This theory was evaluated in young peach trees (*Prunus persica* L. Batch) during
91the present study, which analysed the consequences of N deprivation applied in the autumn on
92spring development (or dieback). For this purpose, the trees were either N deprived, N limited or
93N unlimited in autumn, and then fed in excess, regarding their growth needs, in spring.
94Furthermore, the spring N supplies were ¹⁵N labelled. The effects of autumn N deprivation on (i)
95tree development, (ii) N uptake and (iii) C status (namely C depletion) could then be assessed by
96coupling *in situ* measurements of shoot development with destructive harvests and biochemical
97and isotopic determinations in plant organs. This study focused in particular on the fate of these
98deprived trees. Indeed, those trees died within three months after bud burst and had therefore
99been excluded from two previous studies analysing the effects of a non-zero but limited autumn
100N supply on tree architecture (Jordan et al. 2009), gross growth and nutrient status (Jordan et al.
1012012). Our aim here was therefore to document the link between N deprivation in autumn, C
102depletion and tree dieback in spring.

103

104**Materials and Methods**

105 *Experimental design.* The study was carried out at the INRA Research Centre in Avignon
106 (southern France). Forty one-year-old peach rootstocks (*Prunus persica* cv. GF305) with a
107 diameter of between 6 mm and 8 mm were grafted with pushing buds of peach (cv. RO52) on
108 March 16, 1999 and then transplanted into 10 dm³ pots filled with a 50% vermiculite and 50%
109 peat mixture. The trees were left in a greenhouse for 1 month and then moved outside. During the
110 growth period, chemical treatments were applied regularly to deter pests. Two drippers per pot,
111 each delivering 2 dm³ h⁻¹, supplied a nutrient solution concentrated at 1 g dm⁻³ of a commercial
112 14/7/27% NPK fertiliser. The trees were irrigated for 6 minutes, ten times each day.

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

119 On February 24, 2000, after soaking the roots in tap water for 3 hours, the trees were
120 transplanted into 15 dm³ pots containing an “N-free” substrate composed of 60% sand (Biot B4,
121 ref 16.14.2) and 40% pozzolana. The trees were left outside and fed until harvest with a ¹⁵N-
122 labelled solution (details below). The number of flower and vegetative buds were counted every
123 two days; from February 28 to March 20 for flower buds and to March 30 for vegetative buds.
124 The buds were included in the counts when the petals or leaf tissues became visible after
125 separation of the bud scales. The number of expanded leaves were monitored once a week
126 between April 11 and harvest. The small rosette leaves that had been preformed in the buds were
127 counted separately from the larger ones inserted on the elongated axes that resulted from the
128 plastochronal activity of apical meristems.

129

130 *Nutrition and treatments.* Between September 13 and November 10 each group of six trees
131 received a different level of N supply which was either null (0N treatment), too small to ensure
132 optimal spring development (limiting treatment), or provided in excess according to plant needs
133 (control treatment). These three autumn treatments corresponded to a weekly supply of 0, 1.3 and
134 2.6 g NO₃⁻ plant⁻¹, respectively. Nitrate and other nutrients were supplied three times a week (on
135 Monday, Wednesday and Friday) in a 0.3 dm³ nutrient solution which, depending on the
136 treatment, contained 0, 1.5, or 3 g NO₃ dm⁻³ as Ca(NO₃)₂. The solution also contained the
137 following in mol m⁻³: MgSO₄ 1; KCl 0.2; K₂SO₄ 1.5; KH₂SO₄ 0.5; Fe EDDHA (Ethylenediamine-
138 di(o-hydroxyphenylacetic Acid)) 0.1; and in μmol m⁻³: H₃BO₃ 206.58; MnCl₂ 116.57; CuSO₄
139 4.72; ZnSO₄ 32.41; MoNH₄ 28.15. No excess solution drained from the pots. On the four
140 remaining days of each week, field capacity was restored by automatic irrigation with tap water
141 for ten sequences of 6 minutes each (corresponding to a daily supply of 4 dm³ tree⁻¹). The number
142 of irrigations per day was reduced to five (29 September), then to three (15 October) and finally
143 to 0 (2 November). No irrigation was supplied between leaf fall and spring transplantation.

144 On February 24, 2000, the NO₃⁻ concentration in the nutrient solution was labelled with
145 2.6 atom % ¹⁵N and adjusted to 1.5 mmol dm⁻³. The concentrations of the other nutrients were the
146 same as in the solution used during the autumn. Each tree received 0.3 dm³ day⁻¹ from March 2 to
147 April 2, then 0.5 dm³ day⁻¹ until April 18, 1 dm³ day⁻¹ until May 4, 1.5 dm³ day⁻¹ until May 15 and
148 2 dm³ day⁻¹ until tree sampling. The supply was adjusted so as to ensure that some of the solution
149 was available to the plants throughout the day, in saucers placed under the pots.

150

151 *Tree sampling.* Two destructive samplings of three limited and control trees were made at the end

152of the first growth flush, i.e. on May 29 (Harvest 1) and June 13 (Harvest 2), respectively. The six
153trees used to evaluate the natural abundance of ^{15}N were sampled on February 25.

154 For the 0N trees, the harvests were adjusted to plant death. A tree was assumed to be dead
155when all its vegetative buds had dried. The four trees which died during bud burst (see Results)
156were harvested on May 10 (Harvest 1). The two remaining trees were harvested on May 29 and
157on June 13, respectively, and grouped for the statistical analyses as they both died during the first
158growth stage, i.e. during the rapid leaf expansion stage.

159 The trees were sub-sampled for biochemical analyses as follows: thin and thick roots (less
160than and more than 0.5 cm in diameter, respectively), rootstock trunk, main axis, secondary axis,
161stems of current year shoots, leaves and fruits or flowers. Because N is stored preferentially in the
162bark, and non-structural C mainly accumulates in wood, the wood and bark were separated for
163biochemical analyses.

164

165*Biochemical analyses.* All samples were kept at -20°C until freeze drying and weighing. The
166samples were ground in a stainless steel Dangoumeau grinder (Prolabo France) and cooled with
167liquid N_2 . Total N concentrations and ^{15}N excess levels were determined using a Tracer-MAT
168continuous flow mass-spectrometer (Finnigan MAT, Hemel Hempstead, UK). ^{15}N enrichment
169was used to calculate the amount of labelled N taken up from the fertilizer solution in 2000, as
170described by Millard and Neilsen (1989).

171 The extractions and determinations of soluble sugar concentrations were performed as
172described by Gomez et al. (2002): extraction in a methanol-chloroform-water medium and
173determination by HPLC (Sugar PaK 1 column at 80°C and refractometer, Waters, Milford, MA).
174The starch concentration was determined on pellets, as described by Jordan and Habib (1996):
175solubilisation by autoclaving, depolymerisation and determination of the resulting glucose using

176the reference enzymatic method. Total Non-structural Carbohydrate (TNC) was assumed to be
177the sum of soluble sugars and starch.

178 The concentrations and contents of each compound thus determined in the sub-samples
179were calculated from the DW (Dry Weight) and concentrations in (i) the perennial structure
180comprising the roots, bark and wood of the rootstock trunk, main and secondary axes, (ii) current
181year organs: stems, leaves, fruits or flowers, and (iii) the whole tree.

182

183*Data analyses.* Randomisation (or permutation) tests (Manly 1991) performed at 5% level were
184used to evaluate the effects of treatments and/or harvest dates. Empirical distributions of these
185variables under the null hypothesis of no treatment effect were derived from 2500 random
186assignments of the trees to the different treatments and harvest dates (R 2.11.0 software, [www.r-](http://www.r-project.org/)
187[project.org/](http://www.r-project.org/)). This random assignment was justified because the trees (i) had been raised under
188the same conditions, (ii) were equivalent in terms of size, and (iii) were randomly allocated to the
189groups. The test statistics were the pairwise differences between the means of the variables per
190group. Two observed means were considered to differ significantly if their difference was within
191the distribution tails of the empirical distributions of these differences under the null hypothesis.

192 The effect of the treatments on leaf fall was analysed by comparing the number of leaves
193remaining on the trees at each counting date. The effects of treatment and harvest date on TNC
194and total N organ concentrations and contents were assessed by comparing the six possible
195combinations of treatments and harvest dates. For tree ¹⁵N contents, the effect of harvest date was
196compared separately for each treatment, since the 0N trees contained about 20 times less ¹⁵N than
197the fertilized (limited and control) trees.

198

199**Results:**

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

203 Leaf fall started in October and comprised two phases (Fig 1). Until October 22, leaf fall
204 remained limited and was earlier in the 0N trees, which had lost 11% of their leaves by October
205 15 while the others had only lost 4%. This trend was reversed after October 22 once the fall rates
206 had increased under all treatments, but the differences only became significant on November 5.
207 At that date, the number of leaves remaining on the trees ranged from 13% (control trees) to 28%
208 (0N trees). Leaf fall was then completed rapidly, i.e. before November 8, due to a wind storm.

209

210 *Flowering, bud burst and development.* Flower buds emerged before March 17 whatever the
211 treatment, but the number of developing buds was very small on the 0N trees. Indeed, two of
212 these trees did not flower at all, two produced only one flower, and the remaining two produced
213 25 and 26 flowers, respectively. This was much less than the numbers counted on the limited
214 trees (60 ± 9.3 : SE or Standard Error) and control trees (88 ± 8.6). Full bloom was observed at
215 around March 20, but none of the “0N flowers” produced a fruit.

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

223 These remaining 0N trees dried out between April 19 and May 15 for the first one, and

224between May 9 and June 7 for the second one. They had developed respectively 12 and 43 rosette
225leaves, but no axis leaves. By contrast, the number of expanded leaves on the fertilized trees
226increased rapidly during April and May due to axis elongation. Indeed, on April 19, 36% (or 100
227 ± 19) and 50% (or 201 ± 55) of the tree leaves were neoformed, i.e. inserted on the elongated axes
228of the limited and control trees, respectively. These proportions had reached 53% and 63% on
229May 9.

230

231*Spring N uptake.* Spring N uptake was restored in all trees before dieback (Table 1) and increased
232significantly in line with survival time. Indeed, the 0N trees had absorbed 2.4%, (42 mg ^{15}N) of
233their total N content in spring even if death occurred during bud burst. This percentage reached
2345.7% (78 mg ^{15}N) in the tree which survived that stage. However, these intakes remained small
235when compared with the fact that the limited and control trees had absorbed more than 1000 mg
236 ^{15}N by May 29.

237

238*Tree N status.* Tree N concentrations were similar in all trees whatever the treatment and harvest
239date (Fig 3a), while tree N contents were proportionate to tree DW and varied by a factor of 3.5
240(Fig 4a). However, the 0N trees differed markedly from their fertilized counterparts, firstly
241because their N pool was mainly composed of ^{14}N , which represented only between 63% and
24266% of total N in the fertilized trees. Indeed, the limited and control trees absorbed one-third of
243their total N between bud burst and harvest.

244 Secondly, the current year organs, i.e. the leafy shoots and fruits, represented less than 1%
245of the tree DW on the 0N trees, while in the fertilized trees, the new shoots and fruits accounted
246for between 47% and 61% of the tree DW and contained between 69% and 78% of the tree N
247content (Fig 4a). Indeed, the fertilized trees had higher total, ^{14}N and ^{15}N concentrations in their

248current year organs (Fig 3b) than in their perennial structures (Fig 3c).

249 In the perennial structures, i.e. the roots and old axes, the N concentrations and contents
250were significantly higher in the 0N trees than in the fertilized ones. The differences were mainly
251due to ^{14}N . The fertilized trees were sampled after store emptying since the ^{14}N content of their
252perennial structures was low ($859 \pm 122 \text{ mg } ^{14}\text{N}$) and independent of treatment and harvest date.
253By contrast, the 0N trees contained $1617 \pm 192 \text{ mg } ^{14}\text{N}$, and the difference ($758 \text{ mg } ^{14}\text{N}$) probably
254reflected the N stored by the 0N trees, which was not mobilised from perennial organs to sustain
255shoot and fruit growth. Indeed, in the limited and control trees, the amounts of ^{14}N incorporated
256into the current year organs reached $1450 \pm 133 \text{ mg}$ and $2130 \pm 135 \text{ mg}$, respectively.

257

258*Tree TNC status.* The tree TNC concentrations were three-fold lower in the 0N trees than in the
259fertilised ones (Fig 3d). The differences in the TNC contents were even more marked (Fig 4b).
260Indeed, the 0N trees contained less than 9 g TNC, versus around 60 g in the fertilized trees, with
261one exception: control trees at harvest 2 contained 115 g TNC. Furthermore, both concentrations
262and contents decreased slightly over time in the 0N trees (Table 2), passing from 5.5% to 2.3%
263DW and from 8.5 to 4.0 g TNC, when the survival time increased. In the fertilized trees, by
264contrast, both concentrations and contents increased over time, but the differences were only
265significant in the controls. Starch contributed 24% to the TNC pool of the 0N trees, while this
266proportion ranged from 7% (control trees, harvest 2) to 31% (limited trees, harvest 2) in the
267fertilized trees.

268 In the current year organs (Fig 3e), the variations over time of TNC concentrations
269resembled those observed for the whole trees, even though the mean concentrations were higher,
270i.e. between 22% and 30% DW. Indeed, current year organs contained between 70% (limited
271trees, harvest 2) and 89% (control trees, harvest 2) of the tree TNC (Fig. 4b).

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

278

279 Discussion

280 *C depletion as a consequence of N shortage.* In autumn, an N limitation reduces photosynthesis
281 when the leaf N concentration drops below a threshold level, which is set at around 2.2% N DW
282 for rosaceae species (Cheng and Fuchigami 2000). This has been observed in trees that were
283 unable to correct their low N status in the autumn through N uptake, which were therefore
284 exporting N from their leaves to a greater extent (Cheng et al. 2002) and also earlier before
285 abscission (Grassi et al. 2005) compared with well-nourished trees. However, an N limitation
286 does not only restrict C acquisition but also C expenses, because root respiration is proportional
287 to N uptake (Bloom et al. 1992; Reich et al. 1998). The final outcome on tree TNC content at leaf
288 fall is still a matter of discussion since contrasting results have been published, sometimes on the
289 same species (Bollmark et al. 1999; Von Fricks et al. 1998; Cheng and Fuchigami 2000; Cheng et
290 al. 2002). It is however admitted that reducing the N supply in autumn will increase the tree TNC
291 content, unless it affects leaf senescence, which was probably the case in our study. Indeed the
292 0N trees were characterized by rapid leaf yellowing and high leaf fall rate.

293 During winter and early spring, TNC expenses may also increase because of the necessary
294 adaptation of the 0N trees to N shortage. Several C costly mechanisms have been identified in the
295 literature. Firstly, an N storage deficit can boost N uptake around bud burst, but this has not

296always been observed (Thithikanakul et al. 2012; Jordan et al. 2012 and 2014), perhaps because it
297is solely reliant on TNC mobilisation. The respiration costs of the ^{15}N uptake of the 0N trees
298could be estimated at between 0.18 and 0.88g equivalent glucose, assuming that three carbon
299atoms are released per NO_3^- assimilated and transformed into asparagine (Sasakawa and LaRue
3001986; Amthor 2000). Secondly, an N limitation may stimulate root growth (Millard and Nielsen
3011989; Jordan et al. 2012) in order to increase the volume of prospected soil, in accordance with
302the theory of functional equilibrium. Newly developed fine white roots have thus been observed,
303but unlikely not quantified, on all dead trees at harvest. Thirdly, the C costs of restoring xylem
304function probably increases in line with N deficiency because winter embolism is related to
305xylem osmolarity, i.e. to the concentrations in soluble C and N compounds (Bréda et al. 2006;
306Sakr et al. 2003; Charrier and Ameglio 2011; Galvez et al. 2013). Despite the fact that peach
307wood porosity is diffuse, positive xylem pressure plays only a minor role in recovery from winter
308embolism (Ameglio et al. 2002). Xylem function must therefore be restored through the
309production of new functional conduits, i.e. by cambial reactivation (Ameglio et al. 2002), which
310requires large amounts of TNC and renders the species susceptible to dieback under low TNC
311storage conditions (Barbaroux et al. 2003).

312 Previous studies (Jordan et al. 1998 and 2012) had demonstrated that TNC concentrations
313of around 10% enable normal leaf out and growth in spring in the absence of N limitation, but if
314the tree N concentration is reduced by 50%, young trees need to contain at least 15% TNC if
315spring development is not to be penalised (Jordan et al. 2014). Indeed, TNC mobilisation in early
316spring can increase significantly (i.e. up to 26g TNC; Jordan et al. 2014) in the case of N
317limitation. During the present study, all 0N trees were TNC depleted, whatever the cause:
318insufficient storage coupled (or not) with high C losses. Indeed, they contained less than 1.3%
319starch, and their soluble sugar concentrations varied between 2.2% and 3.9% DW.

321 *Shoot development and dieback.* Although they were not fertilized in the autumn, the 0N trees
322 accumulated small but significant amounts of ^{14}N , probably by remobilizing their leaf N prior to
323 abscission. In spring, these N stores were blocked in the perennial structure, as a cause for, or a
324 consequence of low bud break (see below). The fate of an axillary bud (i.e. its differentiation into
325 a flower or a vegetative or blind bud) depends on the growth context of its parent internode (or
326 growth unit) and is thus determined during vegetative growth (Kervella et al. 1995; Boonprakob
327 et al. 1996), i.e. before the application of the treatments in the autumn. To our knowledge, bud
328 burst in spring has never been investigated in terms of its relationship with N availability.
329 However, low N storage is known to affect shoot development by (i) decreasing the proportion of
330 rosettes that are transformed into elongated axes (Lobit et al. 2001; Grelet et al. 2003; Jordan et
331 al. 2009), but not the number of developing buds set by a specific peach variety (Perezgonzalez
332 1993), and (ii) delaying spring development (Jordan et al. 2014).

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

344significant levels of C acquisition.

345

346*Dieback as a possible consequence of TNC starvation.* Carbon starvation has been identified as a
347possible cause of tree mortality following severe stress such as defoliation (Landhauser and
348Lieffers 2012) or drought (Adams et al., 2013; Galiano et al., 2011). Plant withering can last for
349several years (Marcais and Bréda 2006; Bréda et al. 2006; Galiano et al. 2011), during which
350death (or recovery) depends on the plant's ability to rebuild its TNC reserves before the onset of a
351second stress (insect attack, frost or drought). Moreover, the mortality threshold varies according
352to the environment, size and global functioning of a tree, since stored C contributes to
353maintaining cell turgor and xylem integrity (Secchi et al. 2011; Sala et al. 2012; Pantin et al.
3542013) alongside other soluble compounds, which include calcium, potassium, amino acids and
355soluble proteins. In addition, some starch may be blocked in its reservoirs by partial hydraulic
356failure, and thus not be available for plant metabolism (Sala et al. 2012; Sevanto et al. 2014). This
357failure may be due to an incomplete recovery from winter embolism (with radial growth being
358too small in early spring) or to midday embolism, which can be observed even under benign
359water stress conditions (Sala et al. 2012). Carbon starvation is possible even though the tree TNC
360content is above zero (McDowell and Sevanto 2010).

361 Markedly varying TNC concentrations in dead trees have been observed in the literature.
362According to Landhäuser and Lieffers (2012) and Hartman et al. (2013), TNC starvation was
363limited to the roots, thus contributing to maintaining a water pressure deficit gradient throughout
364the trunk. Galvez et al. (2013) found that *Populus tremuloides* and *Populus balsamifer* trees
365undergoing winter mortality contained 7% and 12% TNC, respectively, and were almost
366completely starch-depleted. We determined a mean value of 4.3% TNC. Hydraulic failure, which
367usually accompanies C starvation, may nonetheless contribute to tree death (Sevanto et al. 2014).

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

374

375

376 **Conclusion**

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

383

384

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388

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517**Table 1** Spring N uptake ($\text{mg } ^{15}\text{N tree}^{-1}$) as a function of treatment and harvest date.

518 The numbers are means and standard errors of two (harvest 2, 0N trees), three (limited and control trees) or
519four (harvest 1, 0N trees) replicates. The effect of harvest date was tested for each treatment, by randomization tests
520based of the generation of 2500 random orders. It was significant (5% level) if coded with different letters. The effect
521of treatment was not tested due to the important difference between the 0N and fertilized trees.

522

	0N 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

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532**Table 2** Tree TNC (Total non-structural carbon) concentration in relation to the date of death for
533the 0N trees. Each line represented a single tree of the 0N treatment, for which 100% mortality
534was observed. A tree was assumed to be dead when all its vegetative buds had dried.

535

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

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540 Captions to Figures:

541 **Fig 1** Percentage of leaves remaining on the trees during leaf fall in autumn for the 0N (open circles, solid
542 line), limited (full squares, dashed line) and control trees (full triangles, long dashed line). Each symbol is the
543 mean of 6 trees plotted with standard errors. The means were ranked (a), (ab), (b) from the lowest to the highest
544 values. They are significantly different if coded with different letters. Statistical significance was inferred from
545 randomization tests based on the generation of 2500 random orders.

546

547 **Fig 2** Dynamic response of bud burst (number of live green vegetative buds) for the 0N (open symbols, dashed
548 lines), limited (full squares, solid line) and control trees (full triangles, solid lines). For the limited and control
549 trees, each symbol is the mean of 6 trees plotted with standard errors. For the 0N trees, each dashed line
550 associated with an open symbol represents a single individual.

551

552 **Fig 3** N and TNC concentrations (means and standard errors in % DW) as a function of treatment and harvest
553 date for (a) and (d) the whole trees, (b) and (e) the current year organs, i.e. the flowers, fruits, leaves and
554 current year stems, and (c) and (f) the perennial structures, i.e. the roots and old axes.

555 Harvests 1 and 2 are represented by dark and pale grey bars, respectively. Starch and ^{14}N are shown by
556 hatched areas, and the remainder, ^{15}N and soluble sugars by non-hatched areas. The effect of treatment and
557 harvest date was tested by randomization tests based on the generation of 2500 random orders. It was
558 significant (5% level) if coded with different letters.

559

560 **Fig 4** Tree contents (means and standard errors in g tree^{-1}) as a function of treatment and harvest date for (a) N
561 and (d) TNC.

562 Harvests 1 and 2 are represented by dark and pale grey bars, respectively. The contents of perennial
563 organs are shown in squared areas, and those of the current year shoots by non-hatched areas. The effect of
564 treatment and harvest date was tested by randomization tests based on the generation of 2500 random orders. It
565 was significant (5% level) if coded with different letters.

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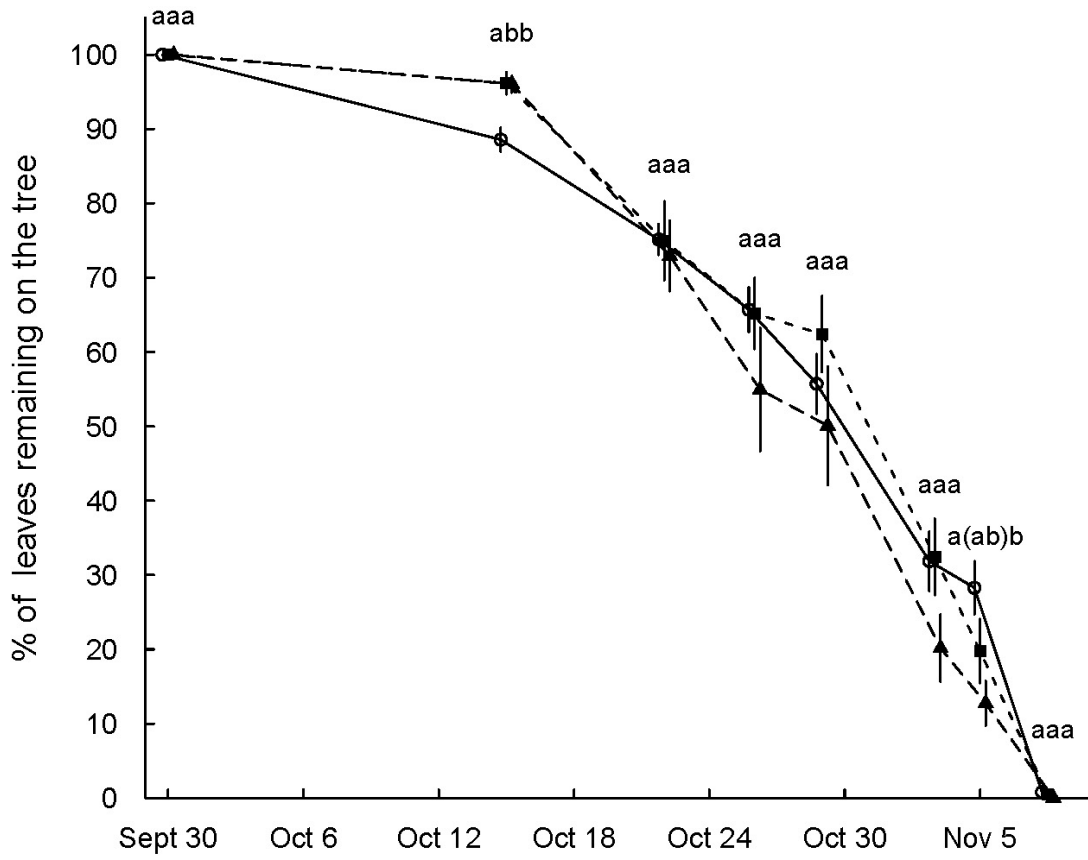


Figure 1

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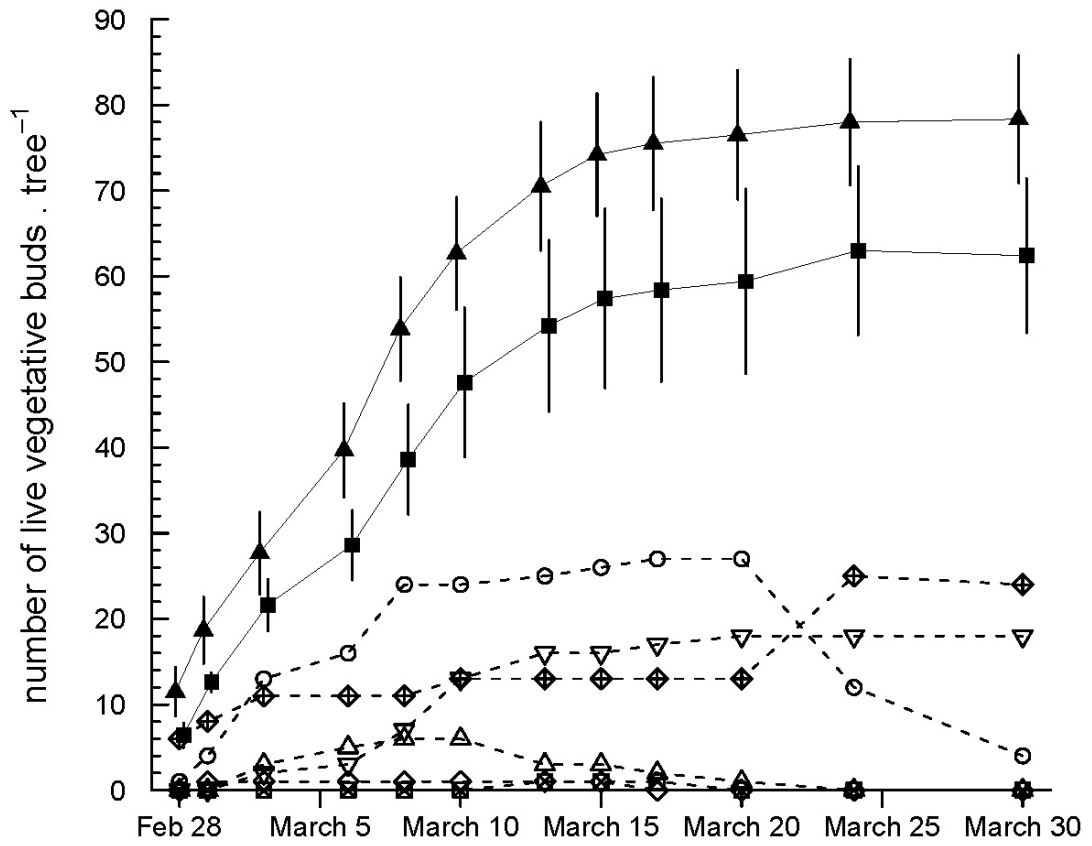


Figure 2

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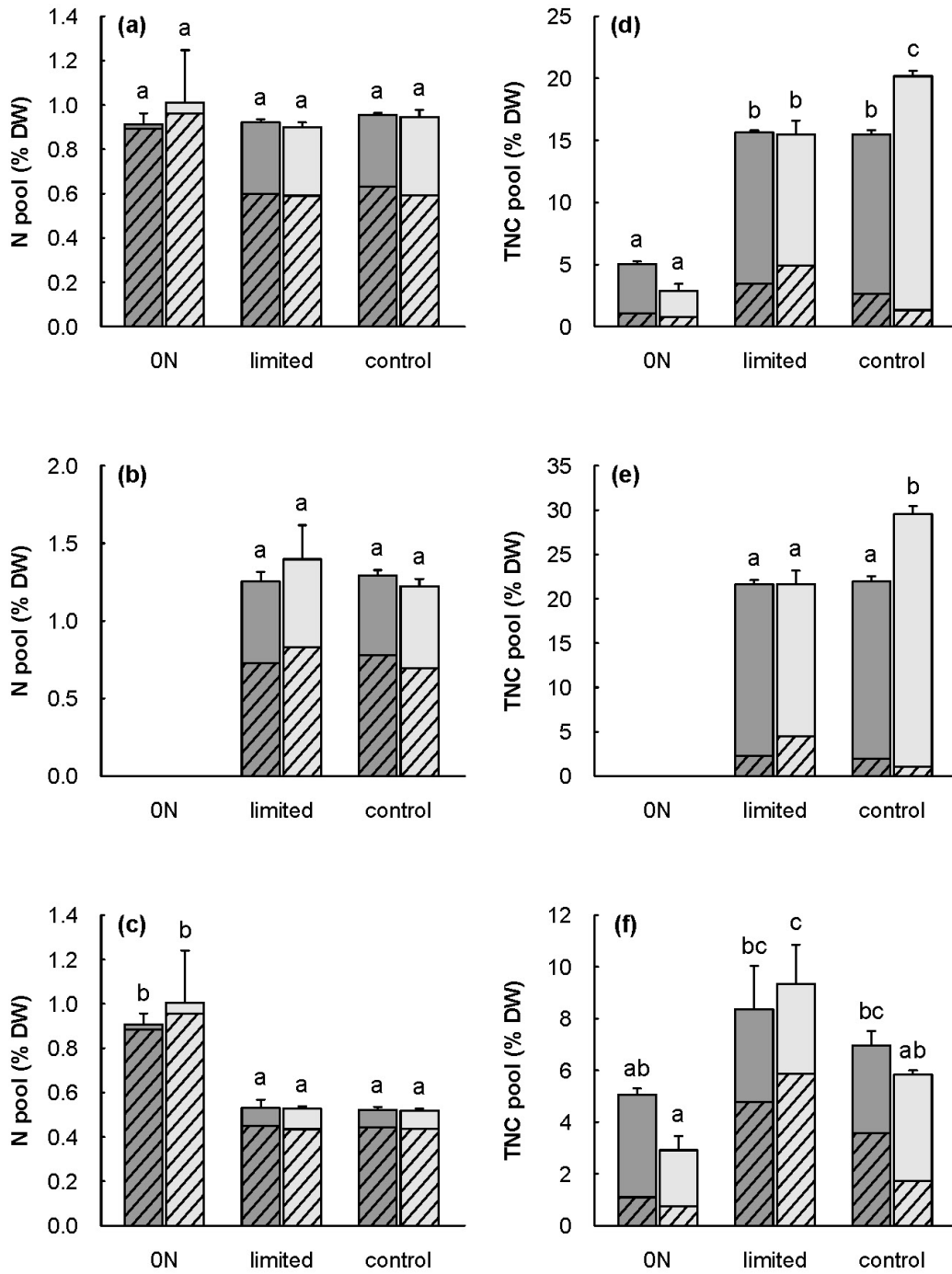


Figure 3

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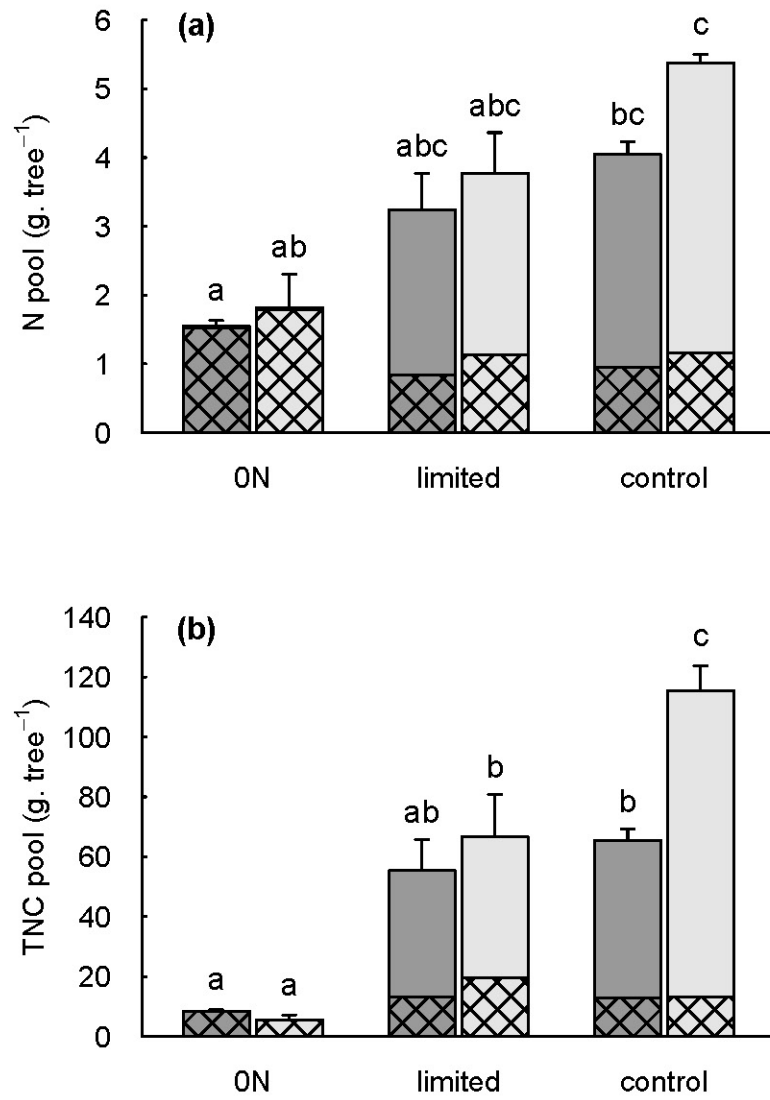


Figure 4