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Contribution of previous year's leaf N and soil N uptake to current year's leaf growth in sessile oak

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Abstract

The origin of the N which contributes to the synthesis of N reserves of *in situ* forest trees in autumn, and to the growth of new organs the following spring, is currently poorly documented. To characterize the metabolism of various possible N sources (plant N and soil N), six distinct 20 year-old sessile oaks were ^{15}N labelled by spraying $^{15}NH_4{}^{15}NO_3$: (i) on leaves in May, to label the N pool remobilized in the autumn for synthesis of reserves; (ii) on soil in the autumn, to label the N pool taken up from soil; (iii) on soil at the beginning of the following spring, to label the N pool taken up from soil in the spring. The partitioning of ^{15}N in leaves, twigs, phloem, xylem, fine roots, rhizospheric soil and microbial biomass was followed during two growing seasons. Results showed a significant incorporation of ^{15}N in the soil-tree system; more than 30% of the administered ^{15}N was recovered. Analysis of the partitioning clearly revealed that in autumn, roots' N reserves were formed from foliage ^{15}N (73%) and to a lesser extent from soil ^{15}N (27%). The following spring, ^{15}N used for the synthesis of new leaves came first from ^{15}N stored during the previous autumn, mainly from ^{15}N reserves formed from foliage (95%). Thereafter, when leaves were fully expanded, ^{15}N uptake from soil during the previous autumn and before budburst contributed to the formation of new leaves (60%).

keywords :

Quercus petraea, N reserves, soil N, ^{15}N labelling

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1. Introduction

Tree carbon metabolism associated with photosynthesis, C allocation and remobilization of C storage is well documented (Barbaroux et al., 2003;Dickson, 1989), but tree nitrogen metabolism is less known. Nevertheless, seasonal N cycling is a determinant of plant fitness in perennials, particularly long-lived perennials such as forest trees (Cooke and Weih, 2005). The literature describes general patterns of seasonal tree nitrogen functioning as follows. In early spring, trees' nitrogen demand for growth can be satisfied either by uptake of external sources such as ammonium, nitrate and organic N available from the soil (Gessler et al., 1998a), or by remobilization of internal stores (Bazot et al., 2013;Coleman and Chen, 1993;Cooke and Weih, 2005;El Zein et al., 2011b;Gilson et al., 2014;Millard, 1996;Taylor, 1967). In many species, N remobilization for growth in spring occurs before utilization of N taken up by roots, typically during the 20–30 days before the roots actively take up N. These species include: deciduous species, such as *Quercus petraea* (El Zein et al., 2011a), *Malus domestica* (Guak et al., 2003;Neilsen et al., 2001), *Populus trichocharpa* (Millard et al., 2006), *Prunus avium* (Grassi et al., 2003), *Pyrus communis* (Tagliavini et al., 1997) and *Sorbus aucuparia* (Millard et al., 2001); marcescent/evergreen species, such as *Nothofagus fusca* (Stephens et al., 2001); and coniferous evergreens, such as *Picea sitchensis* (Millard and Proe, 1993). In a few species (e.g., *S. aucuparia*), remobilization has completely finished before any root uptake of N occurs, even if trees are supplied with an adequate supply of mineral N in the soil. In contrast, other species have been shown to begin taking up soil N through their roots concomitantly with N remobilization. These include deciduous *Juglans nigra* × *regia* (Frak et al., 2002), *Pyrus communis* (Tagliavini et al., 1997), *Betula pendula* and evergreen *Pinus sylvestris* (Millard et al., 2001). All of these

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47 studies were conducted on young trees or/and under controlled conditions. Few
48 studies have applied ^{15}N-labeled mineral fertilizer to larger, undisturbed trees
49 growing in the field (El Zein et al., 2011a), and even those only evaluated the
50 contribution of spring N uptake to leaf and twig growth, while the contribution of
51 stored N was indirectly estimated. However, in autumn, the process of N storage (N
52 translocation from leaves to sink compartments), which starts concomitantly with
53 leaf yellowing (Bazot et al., 2013), is associated with a stimulation of soil nitrogen
54 uptake (Gessler et al., 1998b;Jordan et al., 2012;Kim et al., 2009). In the present
55 study we proposed to investigate the contribution of N storage and that of N taken up
56 from soil during autumn and spring, to the development of new leaves of 20 year-old
57 sessile oaks in the field, after budburst during the following spring. Does soil N or
58 foliar N contribute most to the storage of N compounds in autumn? Does soil N or
59 stored N contribute most to the synthesis of new leaves in spring? Soil ^{15}N labelling
60 is a suitable tool to quantify autumn and spring uptake of N by roots. Labelling of
61 foliage allows quantification of N remobilized from leaves to reserve compartments.
62 During three distinct labelling campaigns, 3 x 2 distinct 20-year-old sessile oaks
63 received $^{15}NH_4{}^{15}NO_3$ applied to their foliage (May), or on adjacent soil (September
64 and March of the following year). ^{15}N partitioning in all tree-soil compartments, i.e.
65 leaves, twigs, trunk, roots, rhizospheric soil and microbial biomass, was analysed
66 regularly. The contribution of assimilated ^{15}N to storage and remobilization was
67 investigated.
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2. Materials and methods

2.1. Site description

The experiment was conducted in an area of 20-year-old naturally regenerated oak in the Barbeau forest (48°29'N, 02°47'E), 60 km southeast of Paris, France, at an elevation of 90 m on a gleyic luvisol. The average air temperature is 10.5 °C and the annual rainfall in this temperate location is 690 mm. Six 20-year-old sessile oaks (*Quercus petraea*) were selected, their height ranged between 8 to 10 m and their average diameter at breast height was 10 cm. In order to limit possible interference of root cutting with nitrogen allocation, at least five months before labelling a 0.5-0.6 m deep trench was dug around each tree, then the trench was lined with a polyethylene film and backfilled. All roots and root exudates inside this perimeter therefore originated from the isolated tree, and were contained in this trench volume. The area delimited by the trench was about 5 m^2.

2.2. ^{15}N pulse-labelling

Three labelling campaigns were carried out: the first (L_1) on the foliage at the end of May (2009/05/27); the second (L_2) on the soil at the beginning of September (2009/09/09); and the third (L_3) on the soil the following March (2010/03/20). Two oaks were labelled during each campaign: trees 1 and 2 during L_1; trees 3 and 4 during L_2; and trees 5 and 6 during L_3. 50% of buds showing leaf unfolding (Vitasse et al., 2009), occurred in those sessile oaks on April 20, 2010; this date was defined as budburst. The L_1 campaign consisted of homogenous spraying on all foliage of 5g $^{15}NH_4{}^{15}NO_3$ (98 atom %), i.e. 1.82g of ^{15}N, dissolved in 2.5 L distilled water. Prior to L_1, soil of the surrounding trenches was protected with a plastic tarpaulin to avoid soil pollution with ^{15}N. This first campaign aimed at the labelling of foliage and,

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subsequently, of the N reserves developed from remobilization of leaf N the following autumn. The L_2 campaign consisted of homogenous spraying of 5g $^{15}NH_4{}^{15}NO_3$ (98 atom %), i.e. 1.82g of ^{15}N, dissolved in 20 L distilled water on the soil of the trench plot of two other selected oak trees (3 and 4). With this procedure, N reserves developed from autumnal soil N uptake were expected to be labelled. The third and last labelling campaign, L_3, consisted of homogenous spraying of 5g $^{15}NH_4{}^{15}NO_3$ (98 atom %), i.e. 1.82g of ^{15}N, dissolved in 20 L distilled water on the soil of the trench plot of trees 5 and 6, thus labelling their spring N uptake.

2.3. Sampling and analytical methods

Leaves, twigs, trunk phloem and xylem and soil monoliths (15 cm depth) were sampled regularly after labelling until the end of 2010. The leaves were rinsed with distilled water to remove any excess ^{15}N. The leaf mass area (LMA) was measured at each sampling date. Fine roots were hand-picked from the soil monoliths, and washed with a 0.5 M $CaCl_2$ isotonic solution. Soil adhering to roots was removed with a brush and sieved at 2 mm. Total N concentration of plant and soil samples, ground in fine powder, was analysed by dry combustion using an N auto-analyser (Flash EA 1112 series, Thermofinnigan). ^{15}N abundance was quantified in plant and soil fine powder aliquots with a mass spectrometer (PDZ Europa, University of Davis, Isotopes Facility, California).

Microbial N contents of fresh soil samples were determined using the chloroform fumigation–extraction method (Vance et al., 1987). Extraction was performed using 0.5 M of K_2SO_4 for 30 min under vigorous shaking. The extracts were filtered, then analysed for N content using an N analyser (TNM-1, Shimadzu, Champs-sur-Marne, France). The microbial ^{15}N abundance was estimated using the same procedure

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119 except that the extraction solution was 0.03 M of K_2SO_4.

120 **2.4. Calculations**

121 All ^{15}N enrichments were corrected for the background natural abundance of this
122 isotope, using control values determined in plants and soils just before labelling. The
123 total weight of each compartment analysed (i.e. leaves, twigs, trunk phloem and
124 xylem, and fine roots) was extrapolated from that of six equivalent trees (same size
125 and same diameter) grown on the same site under the same conditions. Those trees
126 were felled as follows: two in October of the first labelling year (2009); two in the
127 following May (2010); and two the following February (2011). Total leaf biomass
128 was corrected according to the LMA. All data were expressed as proportion of
129 recovered ^{15}nitrogen in a specific compartment (PRN) using the following
130 calculation Eq. (1):

$$PRN\,\% = \frac{Q^{15}N_{\,compartment}}{Max\,Q^{15}N} \times 100 \quad (1)$$

131

132 where $Q^{15}N$ was the quantity of ^{15}N recovered from a compartment on a specific
133 date, and Max $Q^{15}N$ was the maximum quantity of ^{15}N recovered from all the
134 sampled compartments during the experiment.
135 The % contribution of each ^{15}N source (L_1 : leaves; L_2 : autumn soil N; L_3: spring
136 soil N) to the ^{15}N recovered in the roots in autumn or in the leaves of the second year
137 as determined according to the following calculation Eq. (2) :

$$\%\,contribution\,{}^{15}N_{L1,\,L2,\,L3} = \frac{(Q^{15}N_{\,compartment} \,/\, Max\,Q^{15}N)_{L1,\,L2,\,L3}}{\Sigma(Q^{15}N_{\,compartment} \,/\, Max\,Q^{15}N)_{L1,\,L2,\,L3}} \times 100 \quad (2)$$

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3. Results

For each labelling, the two trees analysed displayed similar patterns of ^{15}N partitioning throughout the experiment. That why results was expressed as the mean of both trees (L_1 : 1+2; L_2 : 2+3, L_3 : 3+4).

3.1. ^{15}N partitioning within the plant-soil system during the first leafy season

3.1.1. After the foliar labelling in spring (L_1, May 27, 2009)

The total balance for the administered ^{15}N demonstrated maximum recoveries of ^{15}N within the plant-soil system of 32% one day after leaf labelling. It decreased to 13.5% of the administered ^{15}N recovered in the sampled compartments at the end of September (126 days after labelling) (Table 1).

The PRN was maximum in leaves (96%, Fig. 1a) one day after L_1, then decreased continuously during the four following months (from May 27 to September 30, 2009, i.e. until the 126th day after labelling) with a mean decrease of 80% between these two dates (Fig. 1a). The same pattern was observed in twigs, where the PRN decreased from 3% on day 1 to 0.4% on day 126 (Fig. 1a).

In the trunk phloem tissue and the fine roots, the PRN stayed relatively stable or slightly increased until day 57 (July 24, 2009). They then increased until day 126 (September 30, 2009), when they reached 4.75% in the phloem and 16% in the roots (Fig. 1b, c). The PRN from the rhizospheric soil and microbial biomass was less than 1% (Fig. 1d).

3.1.2. After the first soil labelling (L_2, September 9, 2009)

The total balance for the administered ^{15}N demonstrated maximum recoveries within the plant-soil systems three days after L_2 of 70%. By the end of October (49 days

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after labelling) recoveries from the sampled compartments decreased to 22% of the administered ^{15}N (Table 1).

Three days after labelling, 3% of the recovered ^{15}N was present from the fine roots (Fig. 2c). Nine days after labelling (September 18, 2009), the PRN showed that the majority of the ^{15}N was recovered from the soil, with 61% of the ^{15}N recovered from the rhizospheric soil and 32.5% from the microbial biomass (Fig. 2d). During the following 40 days (until October 28, 2009), the PRN from the soil decreased to 8.5% in the rhizospheric soil and 9.5% in the microbial biomass (Fig. 2d). On the same date, 6% of the ^{15}N was recovered from the fine roots (Fig. 2c). Less than 1% of the ^{15}N was recovered from the phloem, xylem and twigs (Fig. 2a, b).

3.2. ^{15}N partitioning within plant-soil system before and after budburst

Almost one year after the first labelling (L_1), and before budburst (April 8, 2010, 318 days after labelling), 7.5% of the ^{15}N were recovered in the sampled compartments. Thereafter, recovery remained stable at around 12% until September (460 days after labelling, Table 1).

On April 8, 2010, i.e. 318 days after L_1, 11.5% of the recovered ^{15}N was found in fine roots (Fig.1 c). Twigs contained 4.5% of recovered ^{15}N (Fig. 1a), while phloem contained 4% (Fig. 1b). Less than 0.5% of ^{15}N was recovered from the rhizospheric soil and microbial biomass (Fig. 1d).

Eight days after budburst (April 28, i.e. 337 days after L_1), 25% of the recovered ^{15}N was observed in new leaves. By May 19, this had decreased to 17% (Fig. 1a). On April 28, twigs contained 3.5% of the recovered ^{15}N (Fig.1 a), phloem 4% (Fig. 1b) and fine roots 10% (Fig.1c). From then until September (i.e. 460 days after labelling), the PRN from leaves remained relatively stable (22%), whereas it largely

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191 decreased in fine roots (0.35%) (Fig. 1a, b, c). Less than 0.2% of the total ^{15}N
192 recovered over the season was from the rhizospheric soil and microbial biomass (Fig.
193 1d).
194 Just before budburst following the second labelling (L_2, April 8, 2010, 208 days after
195 labelling) 19% of the ^{15}N administered were recovered from all the analysed
196 compartments (Table 1). Most of it was from the rhizospheric soil (14.5%, Fig. 2d).
197 The microbial biomass contained 9.5% of the recovered ^{15}N and the fine roots 2%
198 (Fig. 2d, c). The rest of the ^{15}N (less than 5%) was distributed between the twigs,
199 trunk phloem and xylem (Fig. 2a, b). The same pattern was observed eight days after
200 budburst (227 days after labelling): most of ^{15}N was recovered from soil microbial
201 biomass and rhizospheric soil (12%, Fig. 2d); 2.25% was recovered from fine roots;
202 3.5% of ^{15}N was recovered from phloem and xylem; only 0.5% was recovered from
203 new leaves (Fig. 2a).
204 From April 8 (208 days after labelling) to May 19 (247 days after labelling, and 30
205 days after budburst), the PRN decreased in soil microbial biomass and rhizospheric
206 soil (7%), but increased in fine roots (9.5%) (Fig. 2 d, c). A noticeable increase of the
207 PRN from leaves was also observed at this date (4.5%, Fig. 2a). Thereafter, the PRN
208 from soil microbial biomass and fine roots decreased slightly from May 19 to June
209 28 (i.e. 247 to 287 days after labelling), then remained stable until the end of August
210 (Fig. 2d, c). The PRN from leaves increased to 7% in June (Fig. 2a).
211 For trees whose soils were labelled in spring (L_3, March 20, 2010), the maximum
212 recovery of the administered ^{15}N occurred 40 days later: 51.5% from the sampled
213 compartments. Recovery decreased thereafter and stabilized at 19.5% until autumn
214 2010 (Table 1).

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Twenty days after labelling and before budburst, the soil microbial biomass contained 44.5% of the recovered ^{15}N and the rhizospheric soil 39% (Fig. 3d). The remaining ^{15}N was mainly located in the roots (2% of recovered ^{15}N, Fig. 3c). 8 days after budburst, the PRN was quite similar: 61% in microbial biomass and 32% in rhizospheric soil (Fig. 3d). ^{15}N recovered from fine roots followed a pattern similar to that observed on the previous sampling occasion (Fig. 3c). However, between 8 and 30 days after budburst (from April 28 to May 19, 2010 i.e. from 40 to 61 days after labelling), the PRN in microbial biomass and in rhizospheric soil decreased sharply to 3.2% (Fig. 3d). On that date, 17% of the ^{15}N was recovered from the fine roots (Fig. 3c) and 21.2% from the leaves (Fig. 3a). The PRN from leaves remained stable until the beginning of June (74 days after labelling) (Fig. 3a). From that date until September the PRN from leaves and fine roots declined slightly (Fig. 3a, c). The PRN from microbial biomass decreased continuously throughout the season and reached 2.5% in September (day 166 after labelling) (Fig. 3d).

4. Discussion

4.1. Efficiency of labelling

During the first labelling procedure (L_1), a substantial fraction of the added $^{15}NH_4{}^{15}NO_3$ was incorporated into the leaves of the sessile oaks. A significant proportion of the ^{15}N was then allocated to the leaves: more than 90% of the ^{15}N was recovered from this compartment. The total balance for the administered ^{15}N demonstrated maximum recoveries within the plant-soil systems of 32% one day after leaf labelling. The remaining ^{15}N was probably lost by leaf leaching. However, soil protection with plastic tarpaulins avoided all contamination of soil and roots. Thereafter, the recovery of administered ^{15}N from the sampled compartments

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240 decreased to 14.5%, probably due to allocation of ^{15}N to non-harvested
241 compartments, such as old branches, coarse roots or the inner part of the trunk.
242 Nevertheless, this labelling procedure allowed us to label foliar N used in the
243 synthesis of new tissues in spring, and to follow the remobilisation of leaves' N in
244 autumn, the contribution of foliar N to N storage, and the importance of N storage to
245 the synthesis of new compartments the following spring.
246 The soil $^{15}NH_4^{15}NO_3$ labelling (L_2) conducted in September was also effective.
247 Indeed, the total balance for the ^{15}N applied to the soil demonstrated maximum
248 recoveries within the plant-soil systems of 70%; 3 days after soil labelling. The rest
249 of the ^{15}N was most probably lost by soil leaching. Thereafter the recovery of
250 administered ^{15}N from the harvested compartments decreased to 22%. As with the
251 leaf-labelling experiment (L_1), this decrease was presumably due to allocation of ^{15}N
252 to non-harvested compartments. This labelling procedure allowed us to follow the
253 contribution of autumn soil N to internal tree N storage.
254 Finally, the soil $^{15}NH_4^{15}NO_3$ labelling carried out the following March (L_3) was also
255 effective, with maximum recoveries within the plant-soil systems of 51.5%, 40 days
256 after soil ^{15}N labelling. This recovery decreased to a mean of 19% during the rest of
257 the season. This labelling allowed us to follow the contribution of soil N in spring to
258 the synthesis of new compartments from that moment until after budburst.
259
260 **4.2. N dynamics in soil-tree systems during the first growing season**
261 Following the first labelling procedure, the ^{15}N was quickly incorporated into leaves;
262 more than 90% of the ^{15}N applied was accounted for in leaves one day after
263 labelling. Thereafter this portion decreased continuously along the season. The
264 unaccounted for fraction of the ^{15}N had presumably been transferred to other

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265 compartments, including those which were not sampled, i.e. branches and coarse
266 roots.
267 This important foliar N remobilisation was observed to continue in leaf-labelled trees
268 until yellowing, i.e. the end of September. Data currently available on woody plants
269 show that nitrogen is mainly re-translocated from leaves to storage sites during the
270 autumn (Coleman and Chen, 1993;Cooke and Weih, 2005;Dong et al., 2002;Taylor,
271 1967), due to the predominant role of leaf senescence in the constitution of N stores.
272 Leaf senescence leads to the breakdown of leaf proteins, the transfer of their nitrogen
273 to the perennial plant parts and the formation of N storage compounds (vegetative
274 storage proteins and amino acids) (Dong et al., 2000;Tromp, 1983). In this study, a
275 noticeable increase of percentage of recovered ^{15}N in fine roots was observed on
276 September 30 (16%). This compartment could be defined as a storage compartment
277 in young sessile oaks. Such an observation has been already reported for oaks of the
278 same pole stand (Gilson et al., 2014), and similar findings were reported for field-
279 grown adult peach trees by Tagliavini et al (1997), being typical of other young
280 deciduous trees (Millard and Proe, 1991;Salaün et al., 2005;Tromp and Ovaa,
281 1979;Wendler and Millard, 1996). On this date (end of September), branches and
282 coarse roots could also have contributed significantly to N storage, as previously
283 described (Bazot et al., 2013).
284 At the same time, root uptake can also contribute directly to storage, as proposed by
285 Millard (1996). Indeed, 49 days after labelled ^{15}N had been applied to surrounding
286 soil (L_2), in September, 5.75% was recovered from the trees' fine roots. It can be
287 underlined that at the end of September, foliage ^{15}N made up 73% of the ^{15}N
288 recovered in roots, whereas soil ^{15}N uptake contributed to 27% of the ^{15}N recovered
289 in roots (eq. 2, Fig. 4). The soil N uptake in this period was mainly recovered in the

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290 root system; there was little labelled N in the rest of the trees. This is consistent with
291 the results of Tagliavini et al (1997) and Jordan et al (2012), who found a significant
292 fraction of labelled N in fine root samples of peach trees supplied with ^{15}N applied
293 on soil before fruit harvest in September.
294 Concomitantly with root N uptake for storage, notably in fine roots, a strong
295 immobilization of N in microbial biomass was observed. Indeed, on October 7 (i.e.
296 28 days after labelling), when yellowing was well advanced, 12.5% of the applied
297 ^{15}N was recovered in microbial biomass and 21.5% in rhizospheric soil: there was a
298 competition for soil N between microbial N immobilization and reserve synthesis by
299 root N uptake at that time. This is consistent with the idea that soil microorganisms
300 are strong short term-competitors for soil N due to their high surface area to volume
301 ratio, wide spatial distribution in the soil and rapid growth rates, compared with
302 plants roots (Hodge et al., 2000). Thereafter, root N uptake was still efficient during
303 late yellowing (between October 7 and October 28), since ^{15}N recovered from the
304 fine roots slightly increased from 3.5% to 5.5%, whereas that recovered from
305 microbial biomass decreased from 12.5% to 10%. This could be explained by
306 microbial mortality and turnover, which releases N to the soil, combined with the
307 capacity of plants to sequester N for longer (Barnard et al., 2006;Bloor et al.,
308 2009;Hodge et al., 2000).
309 After leaf fall, even though trees may have a significant capacity for nitrate uptake in
310 the fine roots in midwinter (i.e. in the absence of leaves), as already shown in Japan
311 oak (Ueda et al., 2010), in our case, N soil uptake was limited by low soil
312 temperature, which affected the mineralization rate and root activity, since the ^{15}N
313 recovered from roots declined from 5.5% to 1.75% between October 28 and April 8.
314

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315 **4.3. N dynamic in soil tree system the following spring**

316 In April (before budburst), for trees with leaves labelled in the previous year (L_1), the
317 most part of ^{15}N was recovered in their roots (11.5%). On the other hand, at the same
318 date, most of the labelled N applied to soil in September (L_2) was recovered from the
319 rhizospheric soil (14.5%). When soil (and hence spring N uptake) was labelled (L_3)
320 at the beginning of March, a month later most of the ^{15}N was recovered from
321 microbial biomass and rhizospheric soil (81%), but a small proportion of ^{15}N was
322 recovered from the fine roots (1.5%). The latter demonstrated a small N uptake
323 before budburst, as has previously been observed in Japan oak (Ueda et al., 2010).
324 This early N uptake from the soil could be related to sessile oak's hydraulic
325 properties. As a ring-porous species, sessile oak achieves 30% of its annual radial
326 stem growth before leaf expansion in spring (Breda and Granier, 1996). Water flow
327 pathways are then restored each spring before the onset of transpiration (Breda and
328 Granier, 1996). This enables early root N uptake from soil as soon as a threshold soil
329 temperature is reached.
330 Eight days after budburst, most of the ^{15}N applied to leaves (L_1) was recovered from
331 new leaves (25.2%) and new twigs (mean of 3.5%). This clearly underlined that a
332 significant proportion of ^{15}N used to synthesize new leaves came from ^{15}N stored
333 during the previous autumn, as shown for *Ligustrum* (Salaün et al., 2005). Moreover,
334 this N came from foliar N of the previous year, not from soil N uptake during the
335 previous autumn. Indeed, trees labelled the previous autumn on soil (L_2) showed a
336 similar partitioning of ^{15}N in leaves and twigs before budburst (208 days after
337 labelling) and eight days after budburst (227 days after labelling), there was no
338 mobilisation of ^{15}N for the new leaves and twigs synthesis for those trees. Less than
339 1% of ^{15}N taken up from soil before budburst was recovered in leaves and twigs

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eight days after budburst. A distinction might be made between stored N sourced from leaves and that sourced from soil, stored mainly in roots. N from leaves could be stored as amino acids in branches, trunk, and coarse roots, whereas N taken up from soil could be stored in roots as NO_3^-. This N was not converted into amino acids by Glutamine synthetase / Glutamate synthase enzymes during winter, most probably due to low enzymatic activity in roots during winter. As a consequence, the following spring, trees first remobilized easily circulating forms of N, and N stored nearer to demands. Indeed in trees, NO_3^- is hardly transported to their leaves but rather turned into amino acids in their roots (Morot-Gaudry, 1997).

Consequently, soil ^{15}N was not the main contributor to the synthesis of new twigs and new leaves during the eight first days after budburst. At this time, 95% of new leaves ^{15}N came from ^{15}N-labelled reserves, 2% from soil labelled the previous autumn, and only 3% from soil labelled in the current spring (Eq. 2, Fig. 4). Previous studies have also found that N reserves contribute significantly to leaf expansion in young trees: in white birch (Wendler and Millard, 1996); sycamore maple (Millard and Proe, 1991); Japan oak (Ueda et al., 2009); pedunculate oak (Vizoso et al., 2008); and sessile oak (El Zein et al., 2011a).

Considering trees whose soil had been labelled in autumn (L_2), eight days after budburst the proportion of recovered ^{15}N in microbial biomass decreased slightly whereas it slightly increased in fine roots compared to the previous sampling date. One can suppose that the increased soil temperature and the first flux of C from plant to soil (rhizodeposition) stimulated microbial biomass turnover, making ^{15}N available for root uptake. Very little ^{15}N was recovered from the other compartments of the trees.

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364 Soil N uptake became really effective between 8 and 30 days after budburst. Indeed,
365 whatever the date of the soil labelling (autumn or the current spring), 30 days after
366 budburst, a sharp decrease in ^{15}N in the microbial biomass was observed, depending
367 on an increase of ^{15}N in fine roots and in young leaves. In June 28 (at leaf maturity),
368 40% of the ^{15}N recovered from leaves came from stored ^{15}N, 10% came from ^{15}N
369 applied to soil the previous autumn, and 40% came from ^{15}N applied on soil the
370 current March, one month before budburst (Eq. 2, Fig. 4). This pattern of
371 contribution was maintained throughout the season. Similar findings have been
372 reported for other species. For example, 20-30% of shoot leaf N was supplied by
373 spring-applied fertilizer for mature pear trees (Sanchez et al., 1990) and mature
374 almond trees (Weinbaum SA, 1984), while only 13% of a solution of nitrate-N and
375 ammonium-N applied to soil, contributed to total leaf N of apple trees (Neilsen et al.,
376 1997). *Sorbus aucuparia* had remobilized half the N from storage before any was
377 taken up by the roots (Millard et al., 2001). Finally, there is a concomitant/concurrent
378 remobilization and uptake of N from the soil by some other species, as shown for
379 scots pine (Millard et al., 2001) and walnut (Frak et al., 2002).
380
381 **5. Conclusion**
382 This paper completes knowledge of internal and external nitrogen cycles in a forest
383 ecosystem. We highlighted that in autumn, N reserves are formed from N
384 remobilized from leaves and N uptake by roots. This N is stored in roots, principally
385 most probably in the form of amino-acids and nitrate. Those reserves, especially N
386 coming from leaves, contributed significantly to new tissue synthesis the following
387 spring. Nevertheless, N uptake was also observed in spring before budburst; this N
388 was not transferred to new twigs and new leaves during the first days following

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389 budburst. N uptake from soil only contributed significantly to the synthesis of new
390 tissues when leaves were fully expanded. Two months after budburst the relative
391 contributions of ^{15}N originating from leaves and ^{15}N uptake from soil were 40:60,
392 whereas they were 95:5 eight days after budburst.
393 It will now be interesting to investigate soil N uptake, and the competition for N
394 between tree and microorganisms (bacteria, fungi) in both autumn and spring.
395

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400 for allowing us to carry out these experiments.
401

402 **Author contribution statement**

403 SB and LB conceived and designed the experiments. SB, CF and LB conducted all
404 field and laboratory analyses. SB carried out data analysis, wrote most of the
405 manuscript and prepared the figures. CF, CD and LB contributed to the writing of the
406 manuscript.
407

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547 **Table**

548 Table 1 :

549 Labelling characteristics and recovery of ^{15}N administered in each labelling
550 campaign from the sampled compartments of each tree, on each sampling occasion
551 (DAL : Days after Labelling).

Tree		1	2	3	4	5	6
Labelling date		2009/05/27	2009/05/27	2009/09/09	2009/09/09	2010/03/20	2010/03/20
2010 Budburst date		2010/04/20 DAL 329		2010/04/20 DAL 219		2010/04/20 DAL 32	
Amount of ^{15}N sprayed (g)		1.82	1.82	1.82	1.82	1.82	1.82
	DAL	% ^{15}N		DAL	% ^{15}N	DAL	% ^{15}N
Year 1	**1**	39	25	**3**	68	72	
	3	31	25	**6**	68	50	
	6	30	24	**9**	68	70	
	9	22	19	**16**	33	38	
	16	19	16	**28**	31	22	
	30	17	15	**49**	29	15	
	57	17	14				
	126	15	14				
Year 2	**318**	8	7	**208**	24	14	**20**	65	28
	337	11	13	**227**	12	10	**40**	63	40
	358	10	13	**247**	16	20	**61**	16	14
	370	14	14	**260**	22	21	**74**	20	25
	397	11	10	**287**	38	18	**102**	20	25
	460	13	11	**350**	13	12	**166**	18	21
	509	7	5	**399**	10	8	**215**	11	21

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554 **Figure captions**

555 Figure 1 : Partitioning of recovered ^{15}N (PRN%) from the sampled compartments
556 following the first labelling campaign, i.e. from May 26, 2009 to October 20, 2010.
557 a. leaves and twigs, b. phloem, c. fine roots, d. rhizospheric soil and microbial
558 biomass. DAL: Days after labelling. The two lines for each categories (continuous
559 and dashed) correspond to the tree 1 and the tree 2.
560

561 Figure 2 : Partitioning of recovered ^{15}N (PRN%) from the sampled compartments
562 following the second labelling campaign, i.e. from September 08, 2009 to October
563 20, 2010; a. leaves and twigs, b. phloem and xylem, c. fine roots, d. rhizospheric soil
564 and microbial biomass. DAL: Days after labelling. The two lines for each categories
565 (continuous and dashed) correspond to the tree 3 and the tree 4.
566

567 Figure 3 : Partitioning of recovered ^{15}N (PRN%) from the sampled compartments
568 following the third labelling campaign, i.e. from April 8, 2010 to October 20, 2010;
569 a. leaves and twigs, b. phloem and xylem, c. fine roots, d. rhizospheric soil and
570 microbial biomass. DAL: Days after labelling. The two lines for each categories
571 (continuous and dashed) correspond to the tree 5 and the tree 6.
572

573 Figure 4 : Conceptual scheme representing percentage contributions of ^{15}N (Eq. 2)
574 from each labelling campaign (L_1: white, L_2: light grey, L_3: dark grey) in roots in the
575 autumn, and in new leaves in the season following the first labelling campaign.

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Figure 1 :

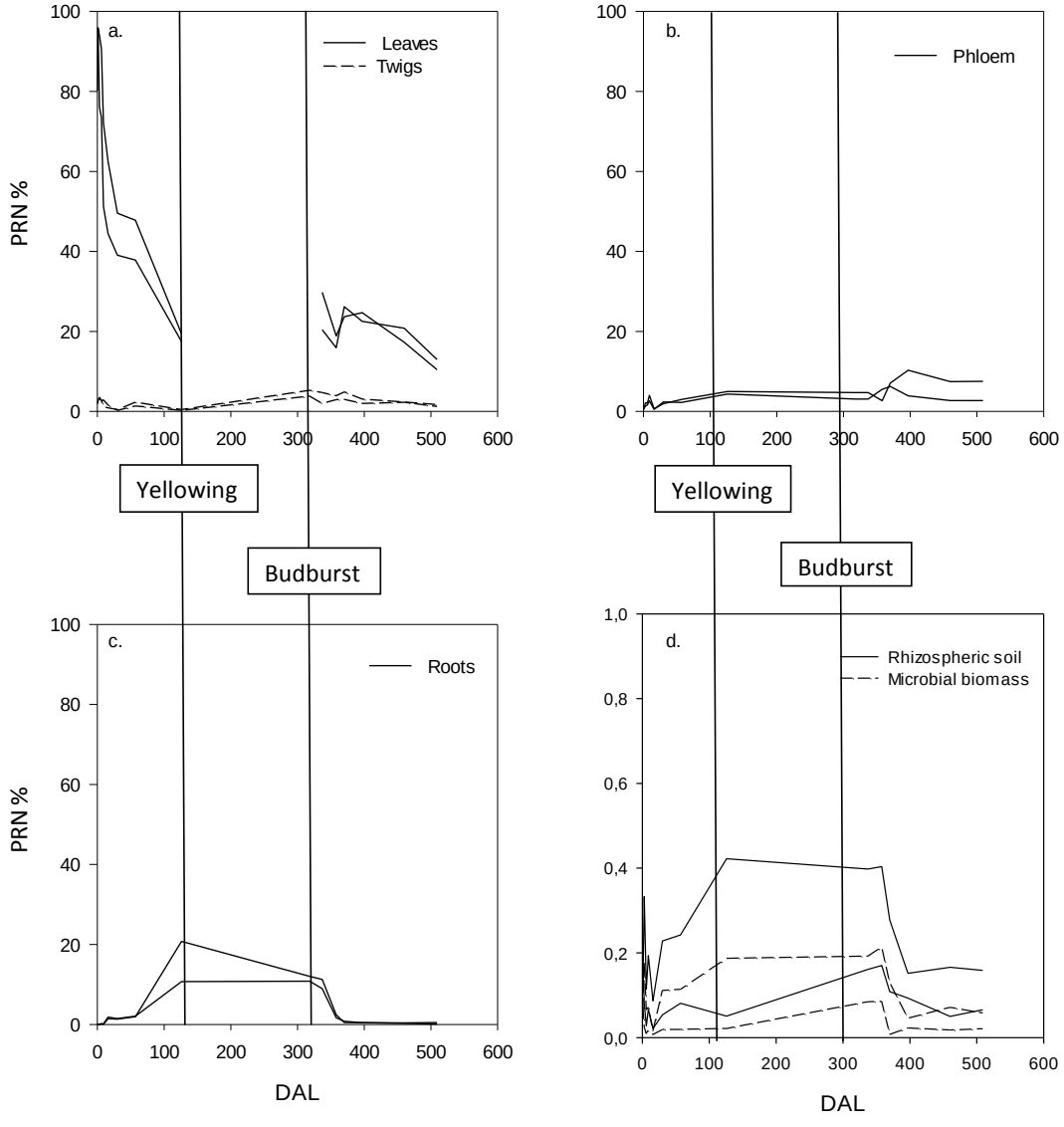

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Figure 2 :

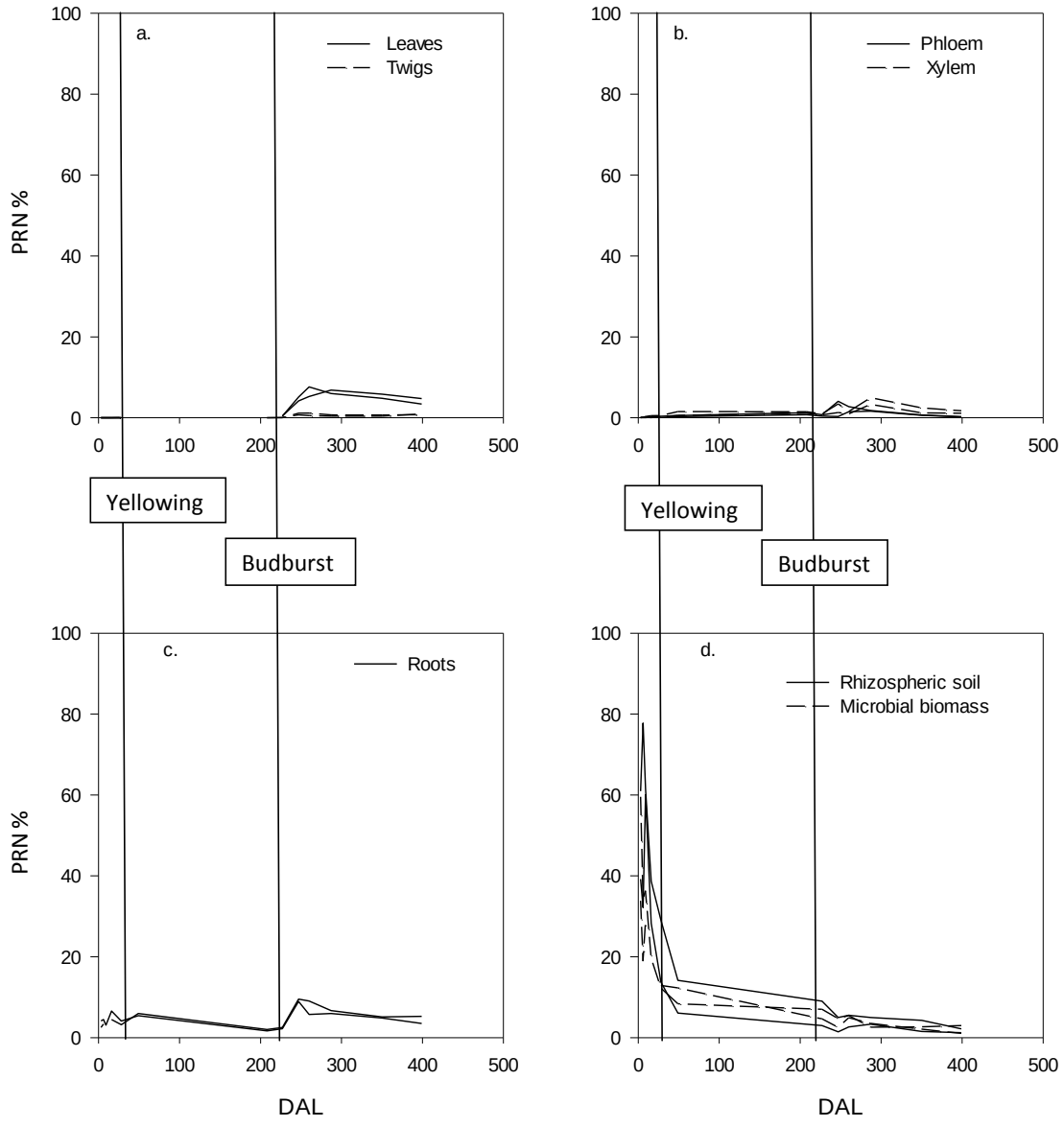

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Figure 3:

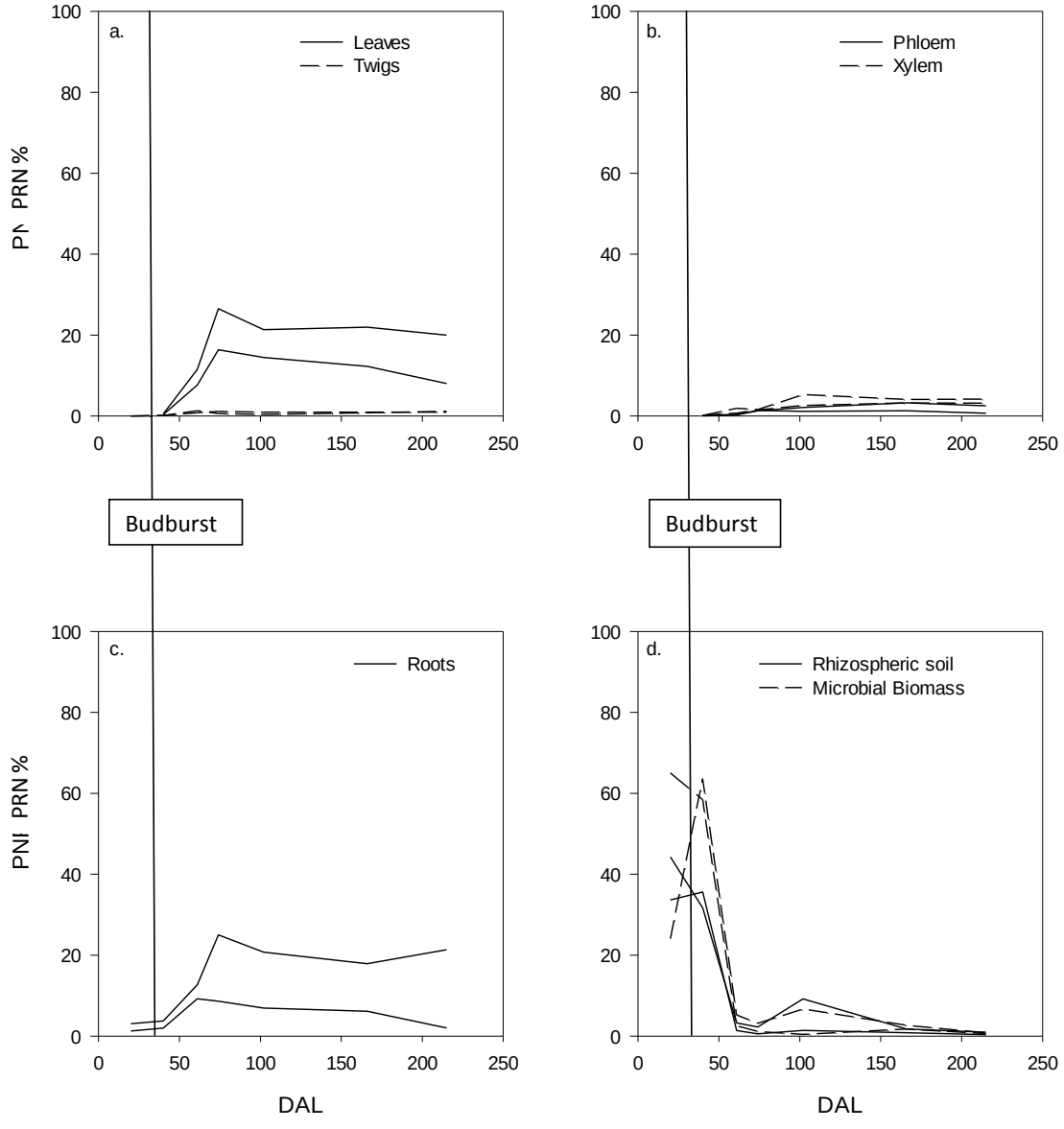

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Figure 4 :