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

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). 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 studies were conducted on young trees or/and under controlled conditions. Few

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

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* L.) 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. The distance between each tree was at least 20 m.

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). All labelling campaigns were conducted on sunny days. 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 covering the whole area of the trenched plot, to avoid soil pollution with ^{15}N. The tarpaulin was sealed to the trunk at 50 cm height with Terostat-VII (Teroson, Henkel, Germany). It was remained on the soil during 2 weeks after labelling. Before removing the plastic

tarpaulin, crowns were sprayed with distillated water in order to avoid any soil contamination after the removing of the tarpaulin.

This first campaign aimed at the labelling of foliage and, 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, very few fine roots were present below 15 cm deep) of each labelled trees (1, 2, 3, 4, 5, 6) were sampled after labelling until the end of 2010 (Table 1). At each sampling date 20 leaves and 20 twigs were collected randomly throughout the crown. Sampling was always performed between 10:00 and 12:00 h UTC. The leaves were rinsed with distilled water to remove any excess ^{15}N. At each sampling date, two small disks of bark (14 mm diameter, 10 mm depth) were collected at 1.3 m height using a corer. Thereafter phloem and xylem tissues were separated by hand with a cutter blade. The leaf mass per 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. All plant tissues and soil samples were brought to the laboratory in a cooler. Plant tissues

were lyophilized and ground to a fine powder with a ball mill before analyses. At each sampling date, one aliquot of each plant powder (1 mg) was transferred into tin capsules (Elemental Microanalysis, UK, 6 x 4 mm, ref. D1006, BN/139877). At some date (Day After Labelling (DAL) 1, 126, 337, 460 for leaves and twigs of L_1; DAL 126, 337, 460 for roots of L_1; DAL 227 and 350 for leaves and twigs of L_2; DAL 49 and 350 for roots of L_2 and DAL 40 and 166 for leaves, twigs and roots of L_3), four aliquots of powder was transferred into tin caps in order to test the repeatability of the analysis. Total N concentration of plant and soil samples, was analysed by dry combustion using an N auto-analyser (Flash EA 1112 series, Thermofinnigan). ^{15}N abundance was quantified in the same 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). 2 fresh soil subsamples of 10 g were prepared. One subsample was fumigated for 24 h with chloroform vapour, while the other was not fumigated. Nitrogen extraction was performed using 50 mL of 0.5 M K_2SO_4 for 30 min under vigorous shaking. The extracts (fumigated and not fumigated) 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 except that the extraction solution was 0.03 M of K_2SO_4 in order to avoid any alteration of the mass spectrometer with the K_2SO_4 salt during ^{15}N analysis.

2.4. Calculations

All ^{15}N enrichments were corrected for the background natural abundance of this isotope, using control values determined in plants and soils just before labelling. The seasonal variations of the natural ^{15}N abundance of each compartments were also followed all long the season, those variations were very weak, consequently, it has been choose to use the ^{15}N natural abundance of the labelled trees just before labelling. The total weight of each compartment analysed (i.e. leaves, twigs, trunk phloem and xylem, and fine roots) was extrapolated from that of six equivalent trees (same size and same diameter) grown on the same site under the same conditions. Those trees were felled as follows: two in October of the first labelling year (2009); two in the following May (2010); and two the following February (2011). Total leaf biomass was corrected according to the LMA. All data were expressed as proportion of recovered ^{15}nitrogen (PRN) in a specific compartment using the following calculation Eq. (1):

$$PRN\,\% = \frac{Q^{15}N_{\,compartment}}{Max\,Q^{15}N} \; X\,100$$

where $Q^{15}N$ was the quantity of ^{15}N recovered from a compartment on a specific date, and Max $Q^{15}N$ was the maximum quantity of ^{15}N recovered from all the sampled compartments during the experiment.

The % contribution of each ^{15}N source (L_1 : leaves; L_2 : autumn soil N; L_3: spring soil N) to the ^{15}N recovered in the roots in autumn or in the leaves of the second year as determined according to the following calculation Eq. (2) :

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

3. Results

For each labelling, the two trees analysed displayed similar patterns of total recovered ^{15}N in each compartment (data not shown) and ^{15}N partitioning throughout the experiment. Moreover, the test of repeatability of the analysis revealed very few variability of the ^{15}N partitionning at a specific date in a specific compartment (Fig. 1, 2, 3). Consequently, results was expressed as the mean of both trees (L_1 : 1+2; L_2 : 3+4, L_3 : 5+6).

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). During winter (December 2, 2009; day 189) the PRN reached 18.5% in fine roots (Fig.1c).

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

194 The total balance for the administered ^{15}N demonstrated maximum recoveries within
195 the plant-soil systems three days after L_2 of 70%. By the end of October (49 days
196 after labelling), recoveries from the sampled compartments decreased to 22% of the
197 administered ^{15}N (Table 1).
198 Three days after labelling, 3% of the recovered ^{15}N was present from the fine roots
199 (Fig. 2c). Nine days after labelling (September 18, 2009), the PRN showed that the
200 majority of the ^{15}N was recovered from the soil, with 61% of the ^{15}N recovered from
201 the rhizospheric soil and 32.5% from the microbial biomass (Fig. 2d). During the
202 following 40 days (until October 28, 2009), the PRN from the soil decreased to 8.5%
203 in the rhizospheric soil and 9.5% in the microbial biomass (Fig. 2d). On the same
204 date, 6% of the ^{15}N was recovered from the fine roots (Fig. 2c). Less than 1% of the
205 ^{15}N was recovered from the phloem, xylem and twigs (Fig. 2a, b). In December (day
206 84) the PRN from the soil was similar to that of the previous date and 4% of the ^{15}N
207 was recovered from the fine roots (Fig. 2c, d).
208
209 **3.2. ^{15}N partitioning within plant-soil system before and after budburst**
210 Almost one year after the first labelling (L_1), and before budburst (April 8, 2010, 318
211 days after labelling), 7.5% of the ^{15}N were recovered in the sampled compartments.
212 Thereafter, recovery remained stable at around 12% until September (460 days after
213 labelling, Table 1).
214 On April 8, 2010, i.e. 318 days after L_1, 11.5% of the recovered ^{15}N was found in
215 fine roots (Fig.1 c). Twigs contained 4.5% of recovered ^{15}N (Fig. 1a), while phloem
216 contained 4% (Fig. 1b). Less than 0.5% of ^{15}N was recovered from the rhizospheric
217 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 decreased in fine roots (0.35%) (Fig. 1a, b, c). Less than 0.2% of the total ^{15}N recovered over the season was from the rhizospheric soil and microbial biomass (Fig. 1d).

Just before budburst following the second labelling (L_2, April 8, 2010, 208 days after labelling) 19% of the ^{15}N administered were recovered from all the analysed compartments (Table 1). Most of it was from the rhizospheric soil (14.5%, Fig. 2d). The microbial biomass contained 9.5% of the recovered ^{15}N and the fine roots 2% (Fig. 2d, c). The rest of the ^{15}N (less than 5%) was distributed between the twigs, trunk phloem and xylem (Fig. 2a, b). The same pattern was observed eight days after budburst (227 days after labelling): most of ^{15}N was recovered from soil microbial biomass and rhizospheric soil (12%, Fig. 2d); 2.25% was recovered from fine roots; 3.5% of ^{15}N was recovered from phloem and xylem; only 0.5% was recovered from new leaves (Fig. 2a).

From April 8 (208 days after labelling) to May 19 (247 days after labelling, and 30 days after budburst), the PRN decreased in soil microbial biomass and rhizospheric soil (7%), but increased in fine roots (9.5%) (Fig. 2 d, c). A noticeable increase of the PRN from leaves was also observed at this date (4.5%, Fig. 2a). Thereafter, the PRN from soil microbial biomass and fine roots decreased slightly from May 19 to June 28 (i.e. 247 to 287 days after labelling), then remained stable until the end of August (Fig. 2d, c). The PRN from leaves increased to 7% in June (Fig. 2a).

For trees whose soils were labelled in spring (L_3, March 20, 2010), the maximum recovery of the administered ^{15}N occurred 40 days later: 51.5% from the sampled compartments. Recovery decreased thereafter and stabilized at 19.5% until autumn 2010 (Table 1).

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

Isotope labelling experiments are technically challenging, and as a consequence are very scarce on trees growing in natural conditions. In this paper, field labelling campaigns were conducted on 20-year-old naturally regenerated oaks. For each campaign (only) two trees were labelled. Nevertheless the similarity of the results

between them suggests that the observed ^{15}N partitioning in soil and tree is a representative view of the functioning of such systems

During the first labelling procedure (L_1), a significant 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 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 as indicated by the ^{15}N recovered in the belowground compartments (Fig. 1d). Thereafter, the recovery of administered ^{15}N from the sampled compartments decreased to 14.5%, probably due to allocation of ^{15}N to non-harvested compartments, such as old branches, coarse roots or the inner part of the trunk. Indeed, data currently available on woody plants show that nitrogen is re-translocated from leaves to storage sites such as old branches, trunk or coarse roots (Valenzuela Nunez et al., 2011;Bazot et al., 2013). The soil $^{15}NH_4{}^{15}NO_3$ labelling (L_2) conducted in September was also effective. Indeed, the total balance for the ^{15}N applied to the soil demonstrated maximum recoveries within the plant-soil systems of 70%; 3 days after soil labelling. The rest of the ^{15}N was most probably lost by soil leaching (30% of the ^{15}N provide). Thereafter the recovery of administered ^{15}N from the harvested compartments decreased to 22%. As with the leaf-labelling experiment (L_1), this decrease was presumably due to allocation of ^{15}N to non-harvested compartments. Finally, the soil $^{15}NH_4{}^{15}NO_3$ labelling carried out the following March (L_3) was also effective, with maximum recoveries within the plant-soil systems of

292 51.5%, 40 days after soil ^{15}N labelling. This recovery decreased to a mean of 19%
293 during the rest of the season.
294
295 **4.2. N dynamics in soil-tree systems during the first leafy season**
296 Following the first labelling procedure, the ^{15}N was quickly incorporated into leaves;
297 more than 90% of the ^{15}N applied was accounted for in leaves one day after
298 labelling. Thereafter this portion decreased continuously along the season. The
299 unaccounted for fraction of the ^{15}N had presumably been transferred to other
300 compartments, including those which were not sampled, i.e. branches and coarse
301 roots.
302 This important foliar N remobilisation was observed to continue in leaf-labelled trees
303 until yellowing, i.e. the end of September. Data currently available on woody plants
304 show that nitrogen is mainly re-translocated from leaves to storage sites during the
305 autumn (Coleman and Chen, 1993;Cooke and Weih, 2005;Dong et al., 2002;Taylor,
306 1967), due to the predominant role of leaf senescence in the constitution of N stores.
307 Leaf senescence leads to the breakdown of leaf proteins, the transfer of their nitrogen
308 to the perennial plant parts and the formation of N storage compounds (vegetative
309 storage proteins and amino acids) (Dong et al., 2000;Tromp, 1983). In this study, a
310 noticeable increase of percentage of recovered ^{15}N in fine roots was observed on
311 September 30 (16%). This compartment could be defined as a storage compartment
312 in young sessile oaks. Such an observation has been already reported for oaks of the
313 same pole stand (Gilson et al., 2014), and similar findings were reported for field-
314 grown adult peach trees by Tagliavini et al (1997), being typical of other young
315 deciduous trees (Millard and Proe, 1991;Salaün et al., 2005;Tromp and Ovaa,
316 1979;Wendler and Millard, 1996). On this date (end of September), branches and

317 coarse roots could also have contributed significantly to N storage, as previously
318 described (Bazot et al., 2013).
319 At the same time, root uptake can also contribute directly to storage, as proposed by
320 Millard (1996). Indeed, 49 days after labelled ^{15}N had been applied to surrounding
321 soil (L_2), in September, 5.75% was recovered from the trees' fine roots. It can be
322 underlined that at the end of September, foliage ^{15}N made up 73% of the ^{15}N
323 recovered in roots, whereas soil ^{15}N uptake contributed to 27% of the ^{15}N recovered
324 in roots (eq. 2, Fig. 4). The soil N uptake in this period was mainly recovered in the
325 root system; there was little labelled N in the rest of the trees. This is consistent with
326 the results of Tagliavini et al (1997) and Jordan et al (2012), who found a significant
327 fraction of labelled N in fine root samples of peach trees supplied with ^{15}N applied
328 on soil before fruit harvest in September.
329 Concomitantly with root N uptake for storage, notably in fine roots, a strong
330 immobilization of N in microbial biomass was observed. Indeed, on October 7 (i.e.
331 28 days after labelling), when yellowing was well advanced, 12.5% of the applied
332 ^{15}N was recovered in microbial biomass and 21.5% in rhizospheric soil: there was a
333 competition for soil N between microbial N immobilization and reserve synthesis by
334 root N uptake at that time. This is consistent with the idea that soil microorganisms
335 are strong short term-competitors for soil N due to their high surface area to volume
336 ratio, wide spatial distribution in the soil and rapid growth rates, compared with
337 plants roots (Hodge et al., 2000). Thereafter, root N uptake was still efficient during
338 late yellowing (between October 7 and October 28), since ^{15}N recovered from the
339 fine roots slightly increased from 3.5% to 5.5%, whereas that recovered from
340 microbial biomass decreased from 12.5% to 10%. This could be explained by
341 microbial mortality and turnover, which releases N to the soil, combined with the

capacity of plants to sequester N for longer (Barnard et al., 2006;Bloor et al., 2009;Hodge et al., 2000).

After leaf fall, trees may have a significant capacity for nitrate uptake in the fine roots in midwinter (i.e. in the absence of leaves), as already shown in Japan oak (Ueda et al., 2010). However, in our case, N soil uptake was limited by low soil temperature, which affected the mineralization rate and root activity, since the ^{15}N recovered from roots slightly decrease between October 28 and December 2 (5.5% to 4%) and then declined to 1.75% between December 2 and April 8.

4.3. N dynamic in soil tree system the following spring

In April (before budburst), for trees with leaves labelled in the previous year (L_1), the most part of ^{15}N was recovered in their roots (11.5%). On the other hand, at the same date, most of the labelled N applied to soil in September (L_2) was recovered from the rhizospheric soil (14.5%). When soil (and hence spring N uptake) was labelled (L_3) at the beginning of March, a month later most of the ^{15}N was recovered from microbial biomass and rhizospheric soil (81%), but a small proportion of ^{15}N was recovered from the fine roots (1.5%). The latter demonstrated a small N uptake before budburst, as has previously been observed in Japan oak (Ueda et al., 2010). This early N uptake from the soil could be related to sessile oak's hydraulic properties. As a ring-porous species, sessile oak achieves 30% of its annual radial stem growth before leaf expansion in spring (Breda and Granier, 1996). Water flow pathways are then restored each spring before the onset of transpiration (Breda and Granier, 1996). This enables early root N uptake from soil as soon as a threshold soil temperature is reached.

Eight days after budburst, most of the ^{15}N applied to leaves (L_1) was recovered from new leaves (25.2%) and new twigs (mean of 3.5%). This clearly underlined that a significant proportion of ^{15}N used to synthesize new leaves came from ^{15}N stored during the previous autumn, as shown for *Ligustrum* (Salaün et al., 2005). Moreover, this N came from foliar N of the previous year, not from soil N uptake during the previous autumn. Indeed, trees labelled the previous autumn on soil (L_2) showed a similar partitioning of ^{15}N in leaves and twigs before budburst (208 days after labelling) and eight days after budburst (227 days after labelling), there was no mobilisation of ^{15}N for the new leaves and twigs synthesis for those trees. Less than 1% of ^{15}N taken up from soil before budburst was recovered in leaves and twigs 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 (Bazot et al., 2013). 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). Indeed roots were the main site of NO_3^- reduction (Gojon et al., 1991). 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

391 white birch (Wendler and Millard, 1996); sycamore maple (Millard and Proe, 1991);
392 Japan oak (Ueda et al., 2009); pedunculate oak (Vizoso et al., 2008); and sessile oak
393 (El Zein et al., 2011a).
394 Considering trees whose soil had been labelled in autumn (L_2), eight days after
395 budburst the proportion of recovered ^{15}N in microbial biomass decreased slightly
396 whereas it slightly increased in fine roots compared to the previous sampling date.
397 One can suppose that the increased soil temperature and the first flux of C from plant
398 to soil (rhizodeposition) stimulated microbial biomass turnover, making ^{15}N
399 available for root uptake. Very little ^{15}N was recovered from the other compartments
400 of the trees.
401 Soil N uptake became really effective between 8 and 30 days after budburst. Indeed,
402 whatever the date of the soil labelling (autumn or the current spring), 30 days after
403 budburst, a sharp decrease in ^{15}N in the microbial biomass was observed, depending
404 on an increase of ^{15}N in fine roots and in young leaves. In June 28 (at leaf maturity),
405 40% of the ^{15}N recovered from leaves came from stored ^{15}N, 10% came from ^{15}N
406 applied to soil the previous autumn, and 40% came from ^{15}N applied on soil the
407 current March, one month before budburst (Eq. 2, Fig. 4). This pattern of
408 contribution was maintained throughout the season. Similar findings have been
409 reported for other species. For example, 20-30% of shoot leaf N was supplied by
410 spring-applied fertilizer for mature pear trees (Sanchez et al., 1990) and mature
411 almond trees (Weinbaum SA, 1984), while only 13% of a solution of nitrate-N and
412 ammonium-N applied to soil, contributed to total leaf N of apple trees (Neilsen et al.,
413 1997). *Sorbus aucuparia* had remobilized half the N from storage before any was
414 taken up by the roots (Millard et al., 2001). Finally, there is a concomitant/concurrent

remobilization and uptake of N from the soil by some other species, as shown for scots pine (Millard et al., 2001) and walnut (Frak et al., 2002).

5. Conclusion

This paper completes knowledge of internal and external nitrogen cycles in a forest ecosystem. We highlighted that in autumn, N reserves are formed from N remobilized from leaves and N uptake by roots. This N is stored in roots, principally most probably in the form of amino-acids and nitrate. Those reserves, especially N coming from leaves, contributed significantly to new tissue synthesis the following spring. Nevertheless, N uptake was also observed in spring before budburst; this N was not transferred to new twigs and new leaves during the first days following budburst. N uptake from soil only contributed significantly to the synthesis of new tissues when leaves were fully expanded. Two months after budburst the relative contributions of ^{15}N originating from leaves and ^{15}N uptake from soil were 40:60, whereas they were 95:5 eight days after budburst.

Acknowledgements

Financial support was provided by Ecology Systematic Evolution lab and the French National Research Agency through the "CATS Carbon Allocation in Tree and Soil project". The authors would like to acknowledge the contributions of Jérome Ngao and Daniel Berveiller to data collection and field work, and Michèle Viel and Patricia Le Thuaut for technical assistance. We are grateful to the French National Forest Office (ONF) for allowing us to carry out these experiments.

Author contribution statement

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

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Table

Table 1 :

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

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
	DAL/JD	% of recovered ^{15}N		DAL/JD	% of recovered ^{15}N		DAL/JD	% of recovered ^{15}N	
Year 1	**1/148**	39	25	**3/255**	68	72			
	3/150	31	25	**6/258**	68	50			
	6/153	30	24	**9/261**	68	70			
	9/156	22	19	**16/268**	33	38			
	16/163	19	16	**28/280**	31	22			
	30/177	17	15	**49/301**	29	15			
	57/205	17	14	**84/336**	29	14			
	126/273	15	14						
	189/336	14	13						
Year 2	**318/98**	8	7	**208/98**	24	14	**20/98**	65	28
	337/118	11	13	**227/118**	12	10	**40/118**	63	40
	358/139	10	13	**247/139**	16	20	**61/139**	16	14
	370/152	14	14	**260/152**	22	21	**74/152**	20	25
	397/180	11	10	**287/180**	38	18	**102/180**	20	25
	460/244	13	11	**350/244**	13	12	**166/244**	18	21
	509/293	7	5	**399/293**	10	8	**215/293**	11	21

Figure captions

Figure 1 : Partitioning of recovered ^{15}N (PRN%) from the sampled compartments following the first labelling campaign, i.e. from May 26, 2009 to October 20, 2010. a. leaves ♦ and twigs ×, b. phloem Δ, c. fine roots ○, d. rhizospheric soil ■ and microbial biomass + (for those compartments the Y axis was adjusted to 1). DAL: Days after labelling. The two lines, continuous and dotted, correspond to tree 1 and tree 2. Vertical bars indicate standard errors.

Figure 2 : Partitioning of recovered ^{15}N (PRN%) from the sampled compartments following the second labelling campaign, i.e. from September 08, 2009 to October 20, 2010; a. leaves ♦ and twigs ×, b. phloem Δ, c. fine roots ○, d. rhizospheric soil ■ and microbial biomass +. DAL: Days after labelling. The two lines for each category (continuous and dotted) correspond to tree 3 and tree 4. Vertical bars indicate standard errors.

Figure 3 : Partitioning of recovered ^{15}N (PRN%) from the sampled compartments following the third labelling campaign, i.e. from April 8, 2010 to October 20, 2010; a. leaves ♦ and twigs ×, b. phloem Δ, c. fine roots ○, d. rhizospheric soil ■ and microbial biomass +. DAL: Days after labelling. The two lines for each category (continuous and dotted) correspond to tree 5 and tree 6. Vertical bars indicate standard errors.

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

Figure 1 :

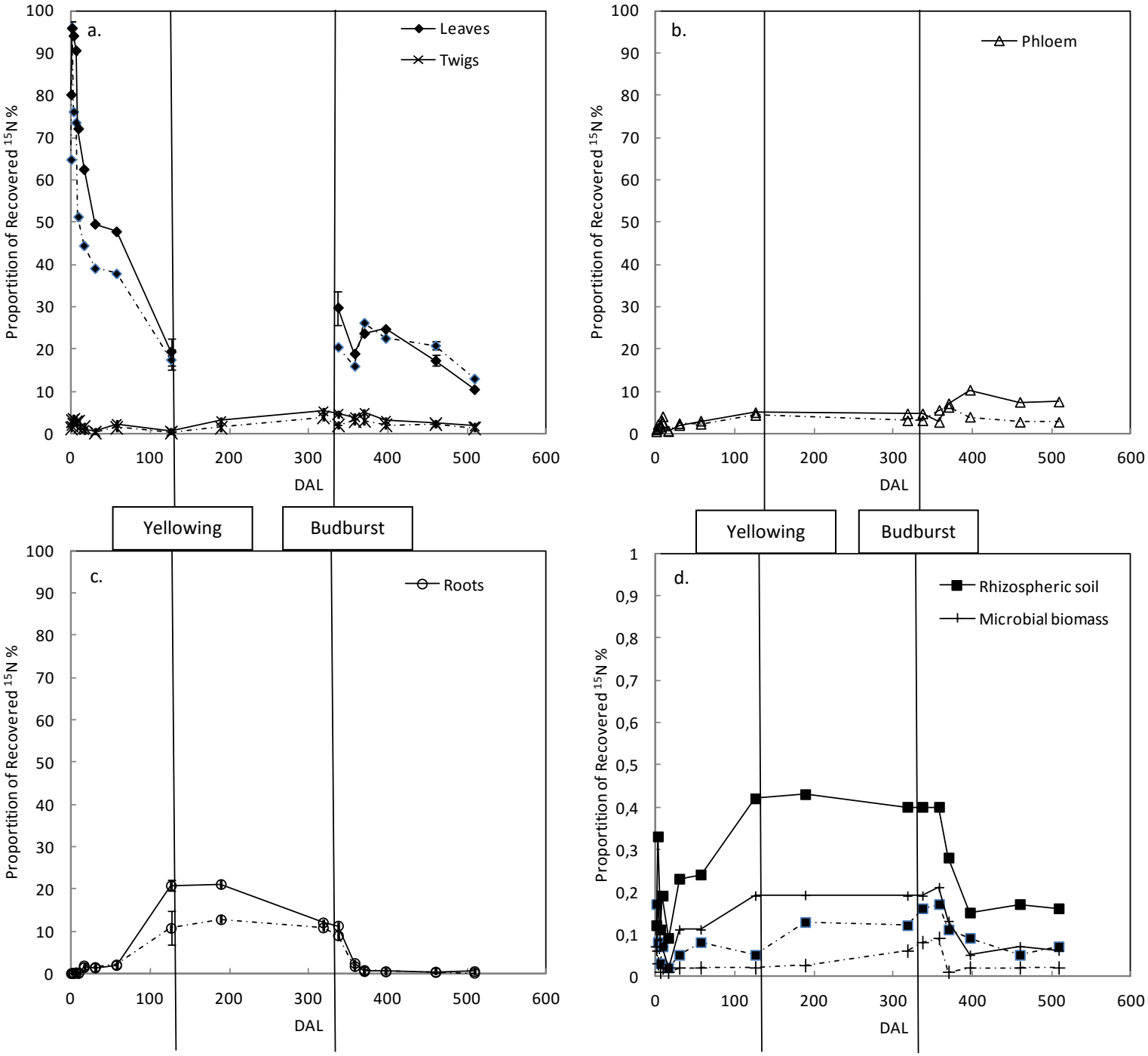

a.
Leaves
Twigs
b.
Phloem
c.
Roots
d.
Rhizospheric soil
Microbial biomass
Proportition of Recovered ^{15}N %
DAL
Yellowing
Budburst

Figure 2 :

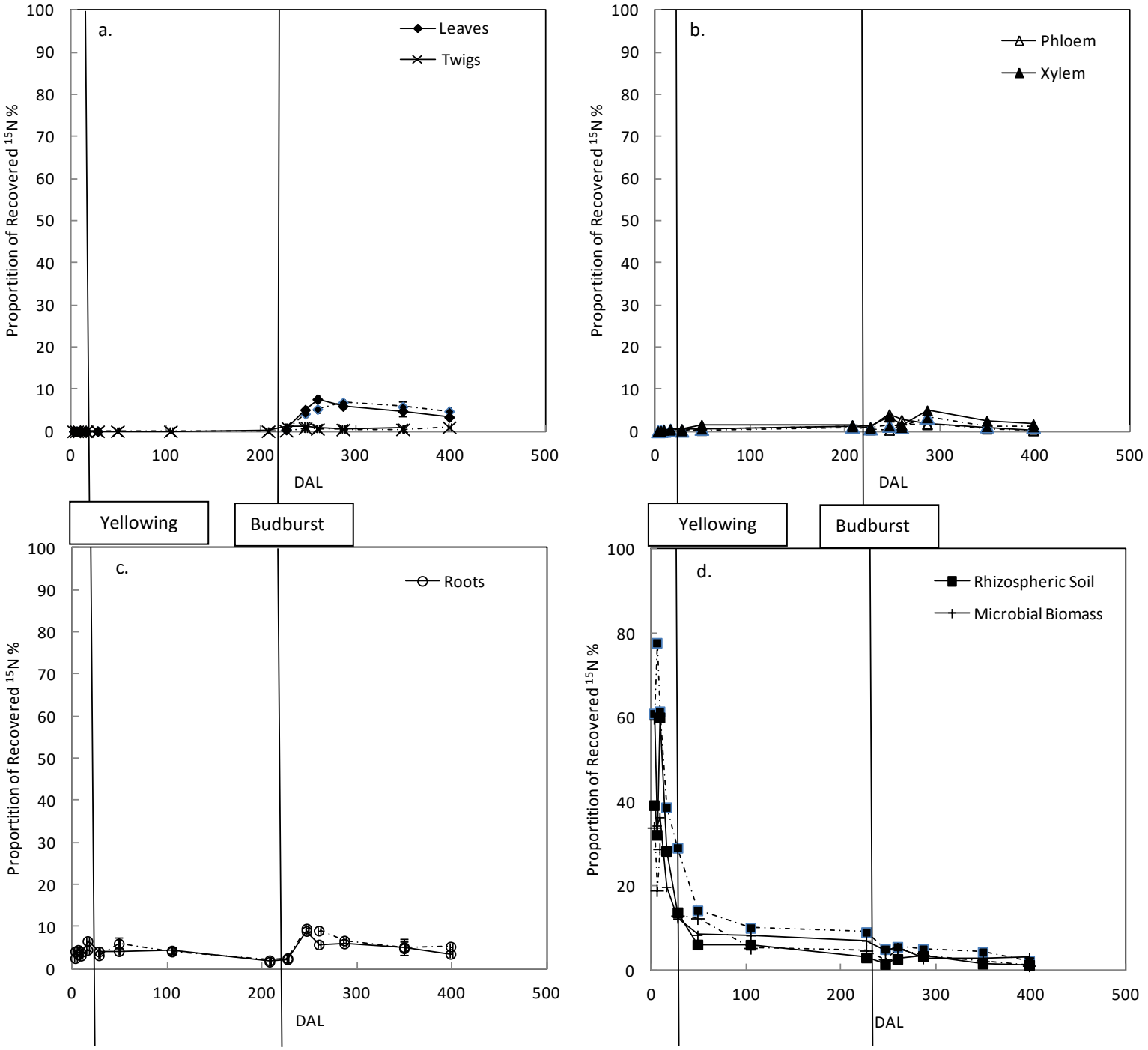

a.
Leaves
Twigs
b.
Phloem
Xylem
c.
Roots
d.
Rhizospheric Soil
Microbial Biomass
Proportition of Recovered ¹⁵N %
DAL
Yellowing
Budburst

Figure 3:

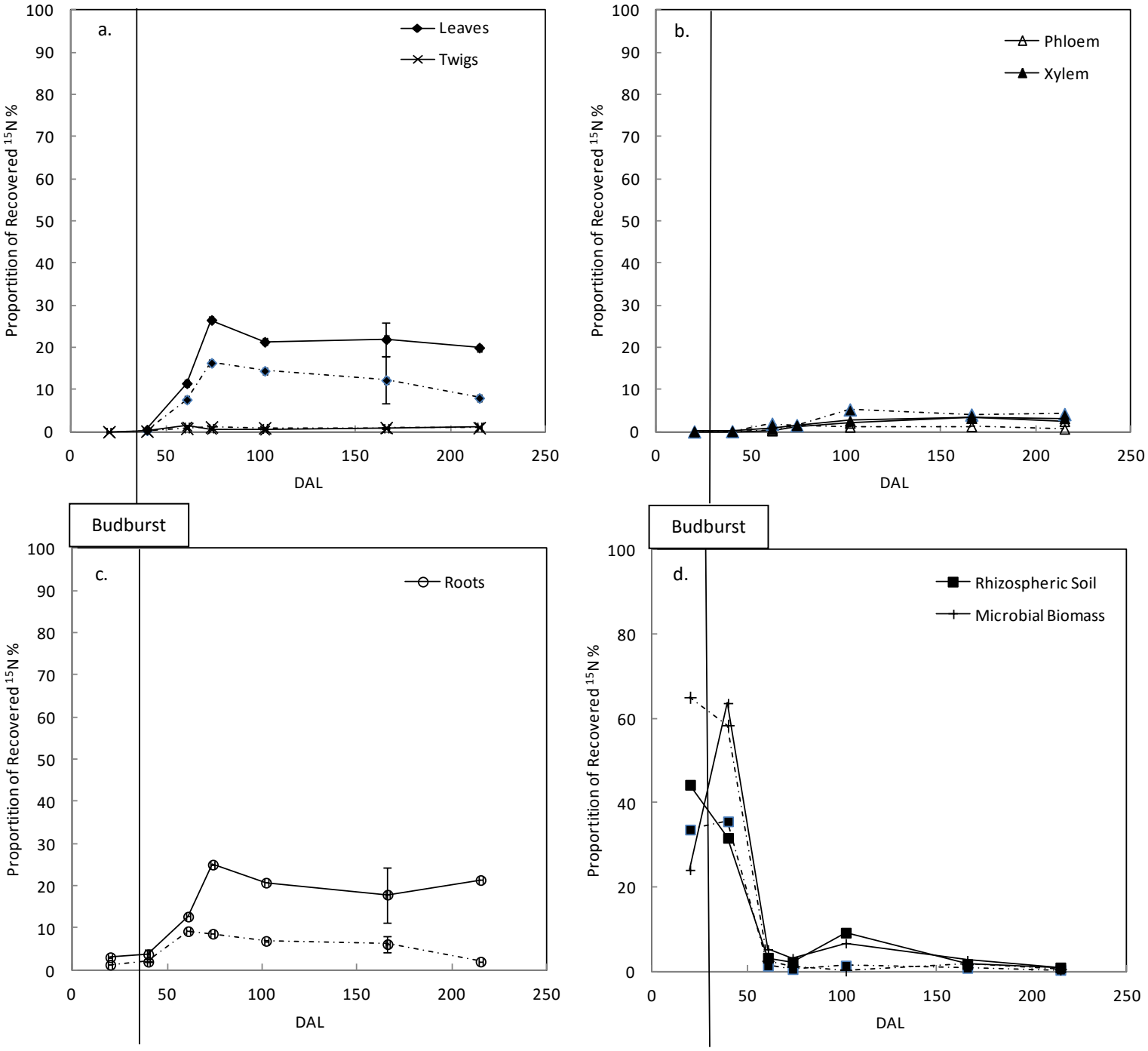
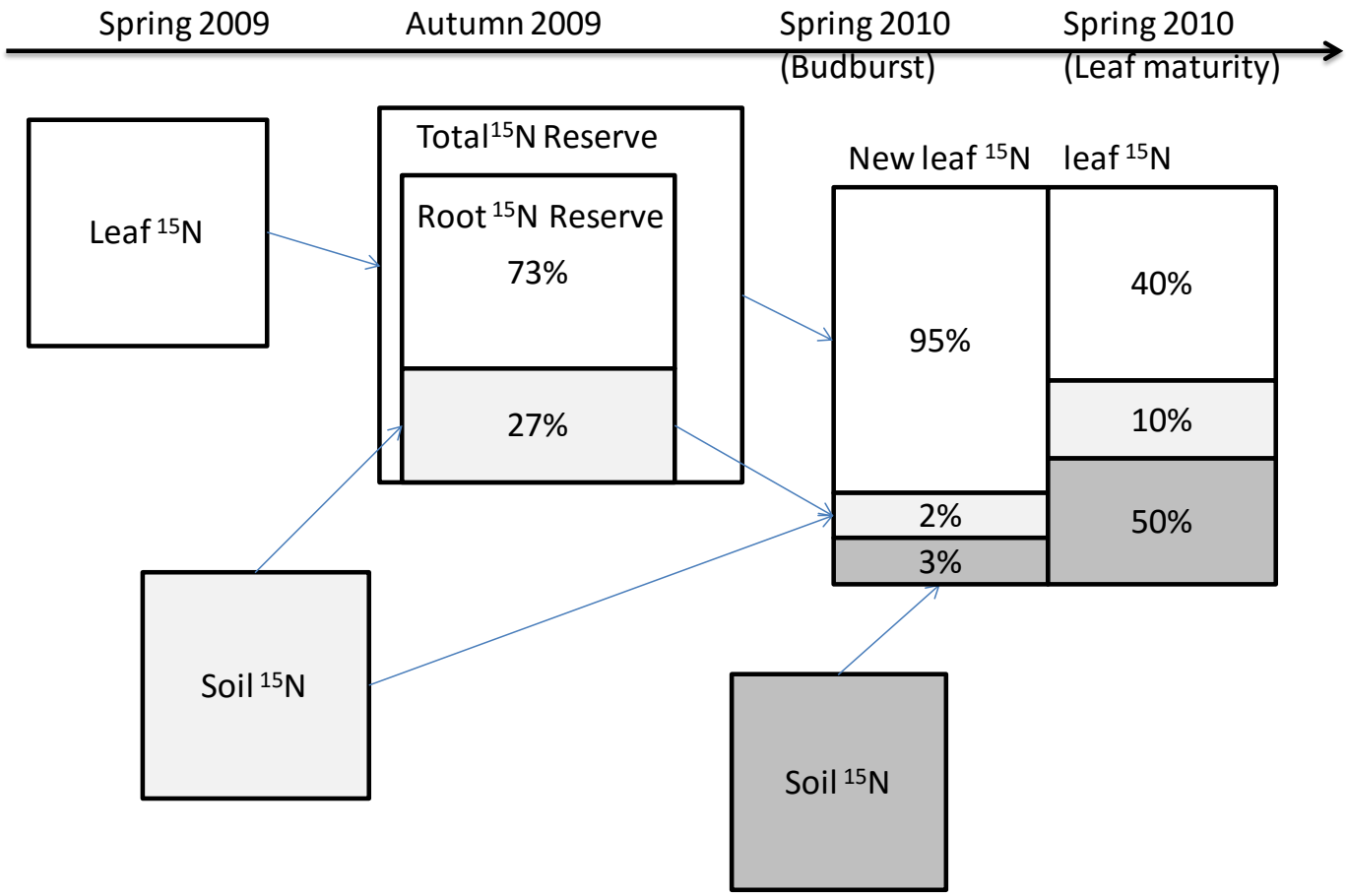

a.
Leaves
Twigs
b.
Phloem
Xylem
c.
Roots
d.
Rhizospheric Soil
Microbial Biomass
Budburst
Budburst
Proportion of Recovered ^{15}N %
DAL
0
10
20
30
40
50
60
70
80
90
100
0
50
100
150
200
250

Figure 4 :


Spring 2009
Autumn 2009
Spring 2010 (Budburst)
Spring 2010 (Leaf maturity)
Leaf ^{15}N
Total ^{15}N Reserve
Root ^{15}N Reserve
73%
27%
New leaf ^{15}N
leaf ^{15}N
95%
40%
10%
2%
50%
3%
Soil ^{15}N
Soil ^{15}N