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 regularly 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,

frozen, lyophilized and ground to a fine powder with a ball mill before analyses. For analyses, all sampled of each compartments were pooled. An aliquot of each powder (1 mg) was transferred into tin capsules (Elemental Microanalysis, UK, 6 x 4 mm, ref. D1006, BN/139877). 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

147 labelling. The total weight of each compartment analysed (i.e. leaves, twigs, trunk
148 phloem and xylem, and fine roots) was extrapolated from that of six equivalent trees
149 (same size and same diameter) grown on the same site under the same conditions.
150 Those trees were felled as follows: two in October of the first labelling year (2009);
151 two in the following May (2010); and two the following February (2011). Total leaf
152 biomass was corrected according to the LMA. All data were expressed as proportion
153 of recovered ^{15}nitrogen (PRN) in a specific compartment using the following
154 calculation Eq. (1):

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

155

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

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

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163

164 **3. Results**

165 For each labelling, the two trees analysed displayed similar patterns of total
166 recovered ^{15}N in each compartment (data not shown) and ^{15}N partitioning throughout
167 the experiment. Consequently, results was expressed as the mean of both trees (L_1 :
168 1+2; L_2 : 3+4, L_3 : 5+6).

169

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)

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 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

195 the rhizospheric soil and 32.5% from the microbial biomass (Fig. 2d). During the
196 following 40 days (until October 28, 2009), the PRN from the soil decreased to 8.5%
197 in the rhizospheric soil and 9.5% in the microbial biomass (Fig. 2d). On the same
198 date, 6% of the ^{15}N was recovered from the fine roots (Fig. 2c). Less than 1% of the
199 ^{15}N was recovered from the phloem, xylem and twigs (Fig. 2a, b). In December (day
200 84) the PRN from the soil was similar to that of the previous date and 4% of the ^{15}N
201 was recovered from the fine roots (Fig. 2c, d).
202
203 **3.2. ^{15}N partitioning within plant-soil system before and after budburst**
204 Almost one year after the first labelling (L_1), and before budburst (April 8, 2010, 318
205 days after labelling), 7.5% of the ^{15}N were recovered in the sampled compartments.
206 Thereafter, recovery remained stable at around 12% until September (460 days after
207 labelling, Table 1).
208 On April 8, 2010, i.e. 318 days after L_1, 11.5% of the recovered ^{15}N was found in
209 fine roots (Fig.1 c). Twigs contained 4.5% of recovered ^{15}N (Fig. 1a), while phloem
210 contained 4% (Fig. 1b). Less than 0.5% of ^{15}N was recovered from the rhizospheric
211 soil and microbial biomass (Fig. 1d).
212 Eight days after budburst (April 28, i.e. 337 days after L_1), 25% of the recovered ^{15}N
213 was observed in new leaves. By May 19, this had decreased to 17% (Fig. 1a). On
214 April 28, twigs contained 3.5% of the recovered ^{15}N (Fig.1 a), phloem 4% (Fig. 1b)
215 and fine roots 10% (Fig.1c). From then until September (i.e. 460 days after
216 labelling), the PRN from leaves remained relatively stable (22%), whereas it largely
217 decreased in fine roots (0.35%) (Fig. 1a, b, c). Less than 0.2% of the total ^{15}N
218 recovered over the season was from the rhizospheric soil and microbial biomass (Fig.
219 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 51.5%, 40 days after soil ^{15}N labelling. This recovery decreased to a mean of 19% during the rest of the season.

4.2. N dynamics in soil-tree systems during the first leafy season

Following the first labelling procedure, the ^{15}N was quickly incorporated into leaves; more than 90% of the ^{15}N applied was accounted for in leaves one day after labelling. Thereafter this portion decreased continuously along the season. The unaccounted for fraction of the ^{15}N had presumably been transferred to other

294 compartments, including those which were not sampled, i.e. branches and coarse
295 roots.
296 This important foliar N remobilisation was observed to continue in leaf-labelled trees
297 until yellowing, i.e. the end of September. Data currently available on woody plants
298 show that nitrogen is mainly re-translocated from leaves to storage sites during the
299 autumn (Coleman and Chen, 1993;Cooke and Weih, 2005;Dong et al., 2002;Taylor,
300 1967), due to the predominant role of leaf senescence in the constitution of N stores.
301 Leaf senescence leads to the breakdown of leaf proteins, the transfer of their nitrogen
302 to the perennial plant parts and the formation of N storage compounds (vegetative
303 storage proteins and amino acids) (Dong et al., 2000;Tromp, 1983). In this study, a
304 noticeable increase of percentage of recovered ^{15}N in fine roots was observed on
305 September 30 (16%). This compartment could be defined as a storage compartment
306 in young sessile oaks. Such an observation has been already reported for oaks of the
307 same pole stand (Gilson et al., 2014), and similar findings were reported for field-
308 grown adult peach trees by Tagliavini et al (1997), being typical of other young
309 deciduous trees (Millard and Proe, 1991;Salaün et al., 2005;Tromp and Ovaa,
310 1979;Wendler and Millard, 1996). On this date (end of September), branches and
311 coarse roots could also have contributed significantly to N storage, as previously
312 described (Bazot et al., 2013).
313 At the same time, root uptake can also contribute directly to storage, as proposed by
314 Millard (1996). Indeed, 49 days after labelled ^{15}N had been applied to surrounding
315 soil (L_2), in September, 5.75% was recovered from the trees' fine roots. It can be
316 underlined that at the end of September, foliage ^{15}N made up 73% of the ^{15}N
317 recovered in roots, whereas soil ^{15}N uptake contributed to 27% of the ^{15}N recovered
318 in roots (eq. 2, Fig. 4). The soil N uptake in this period was mainly recovered in the

root system; there was little labelled N in the rest of the trees. This is consistent with the results of Tagliavini et al (1997) and Jordan et al (2012), who found a significant fraction of labelled N in fine root samples of peach trees supplied with ^{15}N applied on soil before fruit harvest in September.

Concomitantly with root N uptake for storage, notably in fine roots, a strong immobilization of N in microbial biomass was observed. Indeed, on October 7 (i.e. 28 days after labelling), when yellowing was well advanced, 12.5% of the applied ^{15}N was recovered in microbial biomass and 21.5% in rhizospheric soil: there was a competition for soil N between microbial N immobilization and reserve synthesis by root N uptake at that time. This is consistent with the idea that soil microorganisms are strong short term-competitors for soil N due to their high surface area to volume ratio, wide spatial distribution in the soil and rapid growth rates, compared with plants roots (Hodge et al., 2000). Thereafter, root N uptake was still efficient during late yellowing (between October 7 and October 28), since ^{15}N recovered from the fine roots slightly increased from 3.5% to 5.5%, whereas that recovered from microbial biomass decreased from 12.5% to 10%. This could be explained by 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).

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.

Soil N uptake became really effective between 8 and 30 days after budburst. Indeed, whatever the date of the soil labelling (autumn or the current spring), 30 days after budburst, a sharp decrease in ^{15}N in the microbial biomass was observed, depending on an increase of ^{15}N in fine roots and in young leaves. In June 28 (at leaf maturity), 40% of the ^{15}N recovered from leaves came from stored ^{15}N, 10% came from ^{15}N applied to soil the previous autumn, and 40% came from ^{15}N applied on soil the current March, one month before budburst (Eq. 2, Fig. 4). This pattern of contribution was maintained throughout the season. Similar findings have been reported for other species. For example, 20-30% of shoot leaf N was supplied by spring-applied fertilizer for mature pear trees (Sanchez et al., 1990) and mature almond trees (Weinbaum SA, 1984), while only 13% of a solution of nitrate-N and ammonium-N applied to soil, contributed to total leaf N of apple trees (Neilsen et al., 1997). *Sorbus aucuparia* had remobilized half the N from storage before any was 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.

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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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584 **Table**

585 Table 1 :

586 Labelling characteristics and recovery of ^{15}N administered in each labelling
587 campaign from the sampled compartments of each tree, on each sampling occasion
588 (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/JDN	% of recovered ^{15}N		DAL/JDN	% of recovered ^{15}N		DAL/JDN	% of recovered ^{15}N	
	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			
Year 1	**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						
	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
Year 2	**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

589

590

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, 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 for each category (continuous and dotted) correspond to tree 1 and tree 2.

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 and xylem, 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.

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 and xylem, 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.

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 :

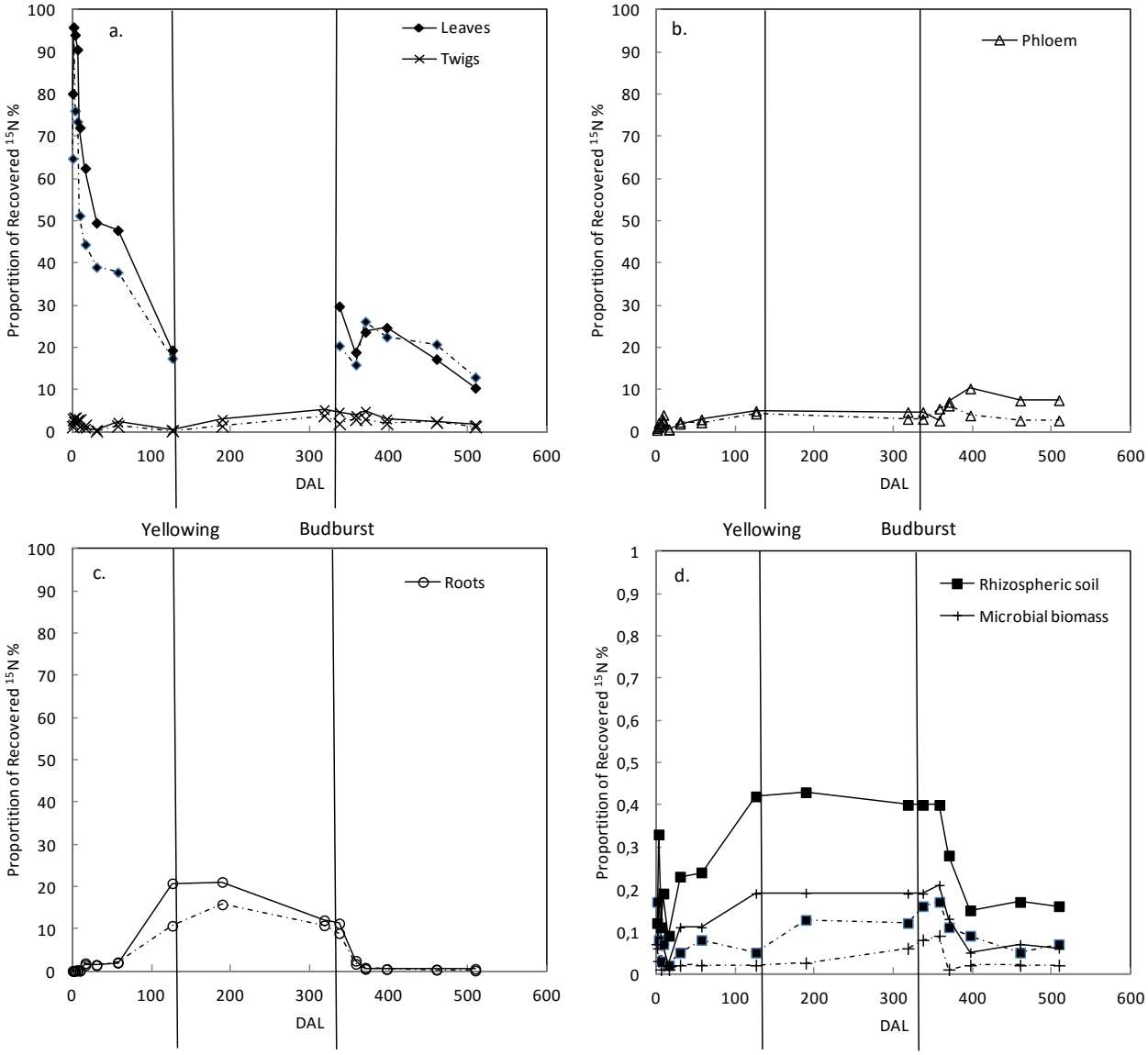

a.
Leaves
Twigs
b.
Phloem
c.
Roots
d.
Rhizospheric soil
Microbial biomass
Proportion of Recovered ^{15}N %
DAL
Yellowing
Budburst

Figure 2 :

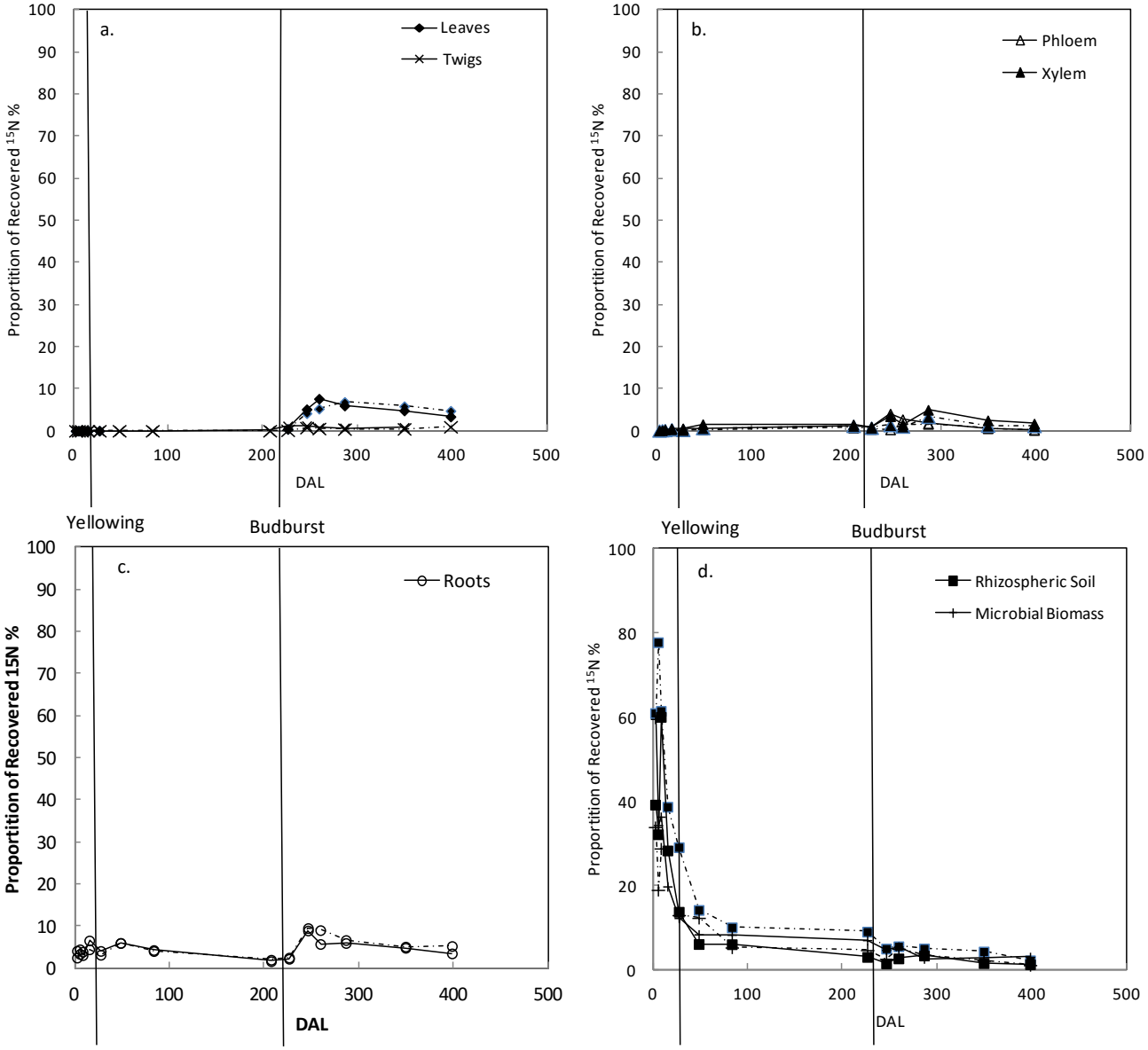

a.
Leaves
Twigs
Proportion of Recovered ^{15}N %
DAL
Yellowing
Budburst
b.
Phloem
Xylem
Proportion of Recovered ^{15}N %
DAL
Yellowing
Budburst
c.
Roots
Proportion of Recovered 15N %
DAL
d.
Rhizospheric Soil
Microbial Biomass
Proportion of Recovered ^{15}N %
DAL

Figure 3:

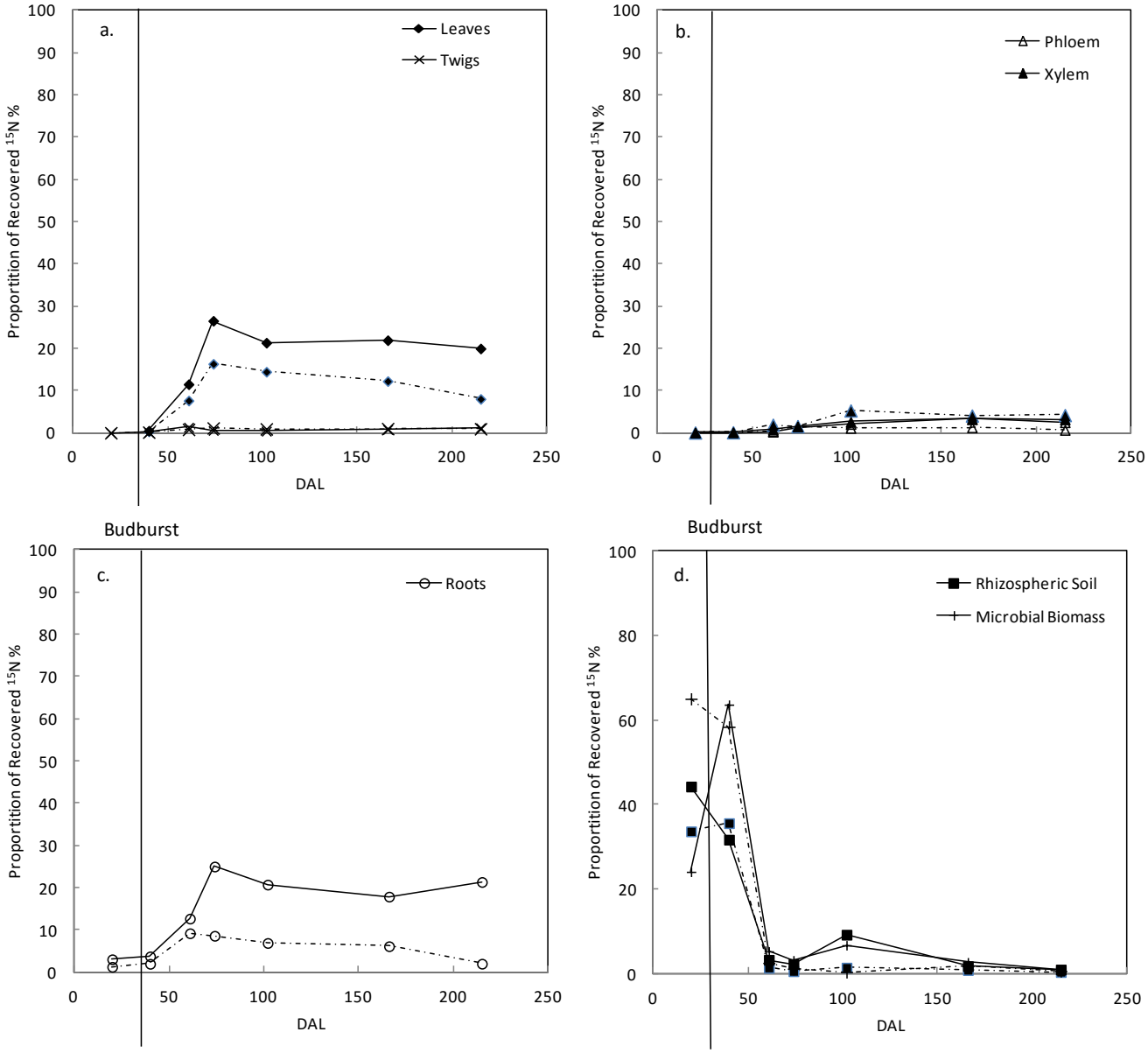
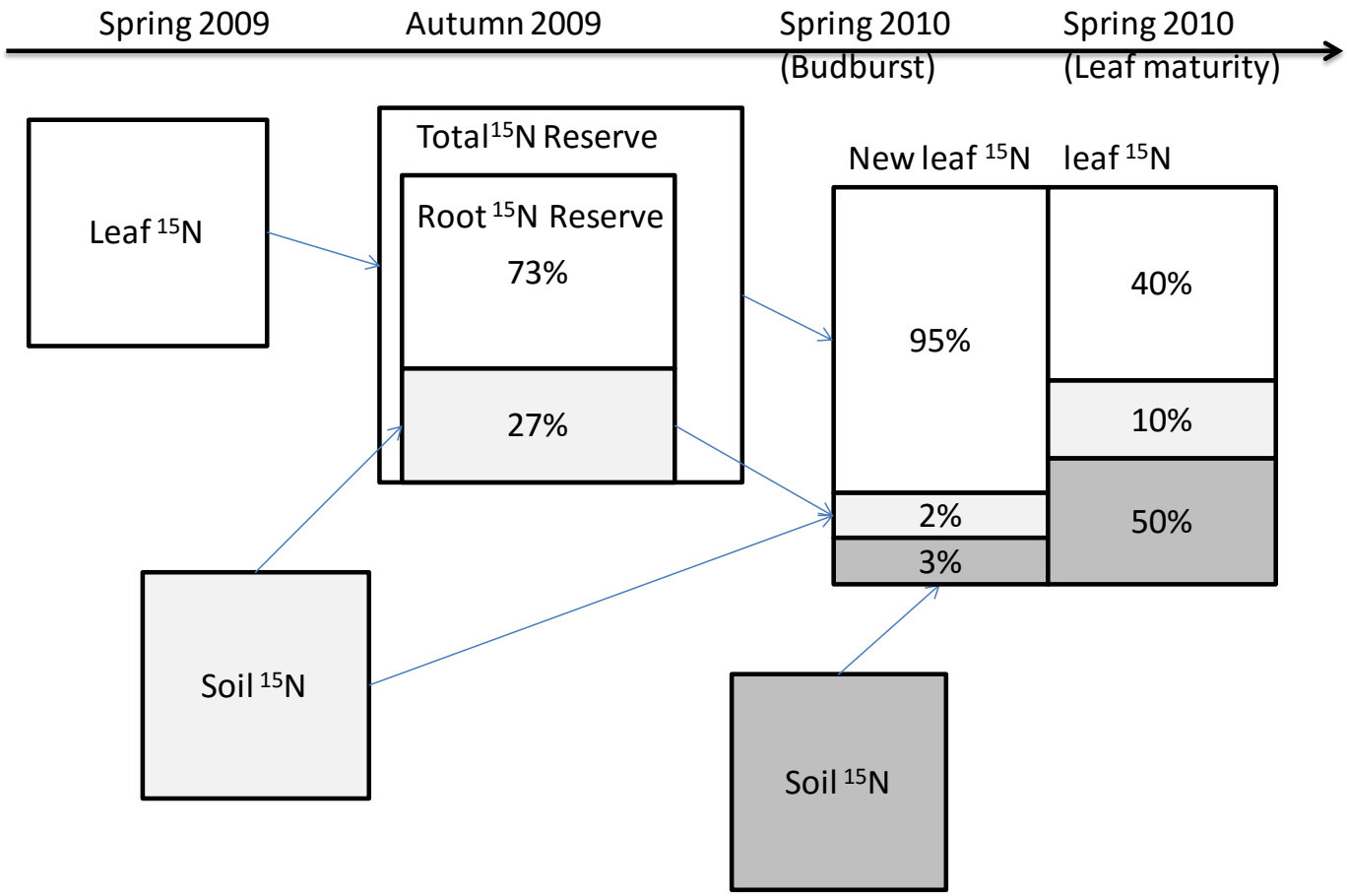

a.
Leaves
Twigs
b.
Phloem
Xylem
c.
Roots
d.
Rhizospheric Soil
Microbial Biomass
Proportion of Recovered ^{15}N %
DAL
Budburst

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