Referee # 1:

General comments

1. This paper presents a study examining N sources for seasonal growth in Quercus petraea. Experimental measurements of N cycling phenology are limited and the manuscript represents a valuable addition to the literature. The author use a clever application of 15N tracers at different times to different pools to provide a solid framework to infer N cycling processes and draw conclusions regarding the origins of N used for seasonal growth in deciduous trees. However, while the theoretical underpinnings and significance of the experiments appear sound, in my opinion there are deficiencies in the methodology and presentation of results which need to be addressed so that the conclusions of the manuscript can be trusted. While isotope labelling experiments are technically challenging and expensive to perform and analyze, the ability to generalize from the experiment severely limited as i) only two tree level replicates are used for each treatment and ii) there are no proper controls for natural variation in isotope abundance over time. This former deficiency prevents any descriptive statistics or statistical analysis in the paper, while the latter means that 15N recovery is calculated using a pre-experiment baseline without any consideration as to whether background 15N content may change over time.

Response: Isotope abundance was determined all along the experiment on each sampled compartments on non labelled trees growing at the same area. The results showed very weak variations of ^{15}N natural abundance (means A% (Isotopic abundance) for leaves = 0.3644 +/- $6.24.10^{-5}$ for example). As a consequence, for all calculations, we have chosen to use the value of natural abundance just before labelling (but it could have been the mean of the temporal values).

L146-151 : 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.

2. While neither of these aspects of experimental design can easily be amended an honest discussion of these methodological shortcomings is necessary in the discussion section to understand the limitations of interpretation which arise as a result.

Response: The discussion part has been completed by the following text:

L264-269: "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. "

3. In large part this discussion repeats some information which I think could be placed in the results (overall label recovery) and omits a critical discussion of the methodology.

Response: Discussion concerning label recovery was reduced in order to limit repetition L270-293.

4. I think that these changes should also be accompanied by improving the quality of graphs and detail in the methods (see specific comments on these aspects of the paper), so the experiment can be both correctly interpreted and repeated.

On this point I find the manuscript is vague and more detail would be very useful.

Response: Materials and methods section has been completed as described below and the quality of the graphs was optimized.

5. The differences between pairs of trees are not discussed besides being referred to as 'similar' at the start of the results (l 143); while the graphs show that, indeed, the time courses of proportional 15N recovery seem similar there are no error bars representing measurement uncertainty nor clear indication of how many points are on the lines.
Response: At each sampling date 20 leaves, 20 twigs were randomly sampled on trees crown, all roots of a core were also sampled. Leaves, twigs and roots were pooled, ground in fine powder and an aliquot was analyzed (15N and %N). As consequence, it was not possible to present error bars at each sampling date. However, at few dates (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) 4 aliquots were analyzed to check the repeatability of analyzes. This type of replicate could not be done at all dates due to the excessive number of sample that would have generated. The results show a good repeatability, for these samples an average is made between the replicates, the errors bars corresponding were added on graphs (L125-129).

6. Given that there are only 15 points per series (Table 1), could these be shown on the graph to indicate periods where 15N content is inferred by a fitted line rather than a measurement?
Response: The graphs have been changed in order to visualize the sampled points all long the experiment. A winter point was added to complete the temporal patterns. Both trees were distinguished with solid lines and dotted lines.

7. Likewise, it is not clear in the methods how samples were taken, how many samples were collected, and when they were taken. 'Leaves, twigs, trunk phloem and xylem and soil monoliths were sampled regularly'. What is regularly?
Response: Table 1 presents the date (Day after labelling DAL and Julian Day JD) of sampling after labelling for each compartment and each labelled tree L112.
At each date of sampling presented on the new graphs, leaves, twigs, roots, microbial biomass, rhizospheric soil were sampled and analyzed. Winter data have been added to the graphs concerning leaves, twigs, roots, and soil compartments. In the winter, xylem and phloem tissues were not sampled in order to limit damage on the trunks.

8. Were samples taken randomly and from all trees at all dates? How were the phloem and xylem sampled? How were twigs and leaves selected?
Response: This has been detailed in the material and methods section: L110-117: "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."

9. Were multiple replicates taken at each time, allowing an uncertainty on each point to be calculated? Or is each individual point also a single measurement from a particular pool at a particular time? If so, how far can we trust the individual time series for each tree when individual measurements may not be representative of the actual mean of the pool in question?

Response: Due to technical and financial constraints we have analyzed at each date an aliquot of pooled leaves, pooled twigs or pooled roots. Nevertheless, as explain above (see point 5.), we have, at few dates, checked the repeatability of the analysis by analyzing for aliquots of a compartment L125-129.

10. I am also not sure if I follow the logic of the CFE extraction in the methodology. The commonly methodology of Vance (1987) should have a control extraction and a fumigation extraction otherwise treated identically, the difference of which is inferred to be the C or N contained in microbial biomass and liberated to the extractable pool by fumigation. Not only is no fumigation treatment mentioned (how long was it fumigated for, with what concentration of chloroform?) used for extraction (l116, 0.5M) is more than an order of magnitude than the concentration used for 'microbial 15N abundance' (l118, 0.3M). It is not clear to me if this former is a 'control' unfumigated treatment and the latter is the15 N-fumigation treatment, or if a control (unfumigated) 15N treatment was measured and is not reported. If the former, a 0.03 M solution may extract less N than 0.05 M, particularly for organic compounds (e.g. Makarov 2013, European Journal of Soil Science 46, 369-374) and estimates of microbial biomass N as the difference would be an underestimate. Also, 15N extracts from low [N]/[15N] samples such as microbial fumigation extracts are commonly concentrated using a diffusion trap method (Stark and Hart (1996). Soil Science Society of America Journal, 60, 1846–1855.). Was this performed here? If not, were15N contents high enough to be detectable on the IRMS? In my opinion, this section of the methods is weak and should either be entirely rewritten removed, along with corresponding results if the method was not robust enough for valid interpretation.

Response: All this methodology section has been completed : L134-143: "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".

11. Specific comments

L73 – how deep were the soil horizons (what would we expect to be sampled by the 15 cm corer later used?)

Response: Very few fine roots were present below 15 cm depth due to the edaphic properties of the site: gley mainly presents less than 15cm depth.

12. L74 – nitrogen deposition, if known, might be useful to include here as this study concerns N additions. High soil N availability may affect the origin of N for growth.

Response: N deposition on Fontainebleau forests was in average 8 kgN/ha/ year (Renecofor Data, National Network for Long-term FOrest ECOsystem Monitoring, 1998). More recent data estimated N through fall in Fontainebleau forest between 5 and 10 kgN/ha/ year in 2010 (Waldner et al. 2014). These quantities do not induce high N availability in soil.

13. L77 – how big were the trenched areas?
Response: It was mentioned L80: 5m² in average

14. What was the spacing of the trees?
Response: At least 20m, see L81.

15. L86 - were treatments applied in particular weather conditions? Logically, it would make sense to maximize uptake of foliar N by applying the N treatment on dry days so it is not immediately lost by being washed off the leaves.
Response: Treatments were applied on sunny days L87.

16. L90 – can you estimate how much of the sprayed N remained on the trees after application and how much was lost immediately, falling onto the plastic tarpaulin?
Response: We have not estimated this.

17. L92 – how long was the plastic tarpaulin in place? Was this long enough to prevent losses from leaf leaching (l237) from reaching the soil?
Response: L95-98: "The plastic tarpaulin 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".

18. L110 – were these grounds by hand, or in a mill? Were the samples dried, e.g. in an oven, before this?
Response: This section has been completed: L120-133 : "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)".

19. L121 – I feel that something is needed here to justify this approach rather than having a concurrent control unlabeled set of trees.
Response: L146-151: "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."

20. L143 – See general comments about this statement. Also, were these similar patterns in TOTAL recovered N, or PRN? From the manuscript it appears it was the latter but the former may also be informative.
Response: The patterns of total recovered N were also similar between both trees. L169-170.

21. L237 – N remaining on leaves could also be lost by stemflow or throughfall and washed to the base of the stem. How were the plastic tarpaulins (if in place at this time) sealed around the stem?
Response: L94-95: The tarpaulin was sealed to the trunk at 50 cm height with Terostat-VII (Teroson, Henkel, Germany).

22. L241 – Maybe this needs a little more elaboration. Allocation of15N to non-harvested components is assumed as there is not a better explanation.
Later (L270) literature begins to be cited about storage of N – this could be incorporated into here to explain where the missing 15N is going.
Response: This has been completed L281-283.

23. L271 – presumably leaf senescence is important for the constitution of N stores in deciduous plants rather than evergreen conifers, where seasonal N storage in leaves is driven by an mismatch of rates N uptake and photosynthetic C late in the growing season. L274 – Should this be evident from fig. 1b? It appears from this panel and table 1 that root N was measured 2-3 months before yellowing (DAL 57), just before the yellowing event (DAL 126) and again after budburst (DAL318). Is this enough resolution to tell whether this N was stored in fine roots at leaf senescence, or if root15N increased earlier in the growing season and subsequently declined over the winter. The two replicates do not agree over the winter period – one is fairly level and one steeply declines. Admittedly the literature suggests that this is a storage pool but I think this may be over-interpreting these particular data.
Response: A point completes the series of measure in winter. At day 189, the proportion of recovered 15N in roots is quite similar for both tree (17 and 21%) Indeed this point was not previously presented because at this sampling date (DAL 189) phloem tissue was not sampled in order to limit damage caused to the trunk. Now we clearly observe that there was an increase of 15N recovered in fine roots in autumn followed by a slight decrease during winter L191-192, L205-207.

24. L304-305 – With no indication of uncertainty, it is rather speculative to interpret differences this small as real changes!
Response: Indeed fluctuations are very small but our hypotheses were supported by previous experiment (Barnard et al., 2006; Bloor et al., 2009).

25. L309 – with no measurements over winter, is this a reasonable interpretation? Could N continue to be taken up but also be decline prior to budburst? A brief mention of a lack of change in above-ground biomass outside the growing season (if true) could help explain this.
Response: The added winter point completes the series. The proportion of 15N recovered in roots was lower in December than in October (5.5% vs. 4%) L206, which confirms the limitation of N soil uptake during winter. L344-349: “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".

26. L344 – a reference for cessation of glutamine synthetase activity would be useful.
Response: Our team has conducted analyzes of root enzyme activities in mature oaks throughout a season, the results show a reduction in GS activity in winter, these data are published in Trees structure and Function : Bazot et al., 2013, L381.

27. L393 – This final sentence is unnecessary as this suggestion for further work does not feel like a natural result of the conclusions of the manuscript.
Response: It has been removed.

28. Table 1 is very confusing. DAL for different treatments are not the same thing as the labelling occurs at different times of the year. I wonder if this can be reformatted in a way that allows for easier interpretation, perhaps by playing the data for trees 3 and 4 and 5 and 6 at positions in the table so that the real-time day of year is close to equivalent horizontally or by splitting this into three tables, one for each set of trees. Also, are the "Amount of 15N sprayed", and "Budburst" rows necessary, given that it is the same in all treatments? Budburst could instead be indicated by an entry in the table.
Response: Table 1 was simplified and Julian day numbers have been added in order to facilitate the reading of sampling days.

29. The figures need a clearer distinction between of trees. It would be nice to be able to tell which time series is from which tree. Axis titles could be the full, unabbreviated units as these are not particularly long phrases and are not standard terms which the reader can be assumed to already know. Additionally, the legends indicate that the dashed lines/ continuous lines are for the different trees, but the figure legend suggests the dashed lines are the biomass pools. This should be checked across all graphs for consistency.
Response: The graphs were corrected according to those recommendations.

30. Technical corrections
L27 – the sentence 'the literature describes is unnecessary.
Response: It has been deleted
L75 – include authority with species name
Response: It has been done
L89 – 'on' April 20, or 'by' April 20?
L106 – the 'leaf mass area' (LMA) should be 'leaf mass per area'.
Response: It has been done
L124 – is this the same six trees as measured?
Response: No, but they are similar trees grown on the same site under the same conditions with the same size.
L129 – (PRN) would be easier to interpret if it immediately follows 'proportion of recovered 15Nitrogen'
Response: It has been done
L144 – The sentence 'That why results were expressed as the mean of both trees' is

poor English and should read ‘That is why results were
Response: It has been corrected
L 147 – ‘leafy season’ - > ‘growing season’?
Response: No, Growing season (of the trunk) ended in July, whereas leaves fall at the end of September
L232 – Substantial fraction is ambiguous
Response: Replaced by '"significant"
L238 – the lack of contamination could be supported by referring to figure 1d.
Response: It has been done
L265 – this is repetitive, and along with section 4.1 could be considerably shortened. Generally, this section is repeating something that is apparent from the results.
Response: This section was reduced
L309 – this sentence is very long – could it be split up?
Response: This sentence was split.
References – numerous cases where super- or subscript is not used in reference list (e.g. line 412 ‘15N’)
Response: It has been corrected
Figure legends: remove ‘the’ from ‘the tree 1’ and ‘the tree 2’.
Response: It has been done
Figure 1d – the scale on this figure is different than the other graphs. This makes interpretation difficult. Could this be adjusted or measured in the legend?
Response: We have specified this different scale in the legend of the figure.

Referee #2 :
General comments
1. The paper represents a significant contribution to the elucidation of N flows in trees. There is no new concept or method in this study but the use of three different 15N-labelling periods (spring year n-1, autumn year n-1 and spring year n) and two labelling techniques (soil and leaf labelling) allows a good description of the contribution of leaves to the constitution of winter reserves and the contribution of N reserves to spring growth. It is clearly shown that the main contributor to the synthesis of new leaves is N stored during previous autumn. It is also shown that soil micro-organisms are good competitors for soil 15N but a significant part of the N is returned to the tree because of microbial turnover. This is a well-written paper, and a well-though out analysis. In my opinion, the subject and the core-content of the ms are appropriate and relevant to Biogeosciences. The findings are reliable because the methods developed are appropriate. I have just a problem concerning xylem and phloem measurements. Nothing is mentioned concerning phloem and xylem sampling and how the contribution of these pools to 15N partitioning is estimated.
Response: 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.”L115-118.

2. Also, for obvious technical reasons, 15N allocated to coarse roots and trunk is not taken in account in this study. It is known that these organs represent a substantial pool of N reserves and this should be discussed.

Response: Discussion was complete : L281-283 : “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).”

3. Specific points Abstract Line 12 is this proportion (30 %) true for all labelling periods?
Response: Yes, for L_1, 32% of administered ^{15}N was recovered L179; for L_2, 70% of administered ^{15}N L195, and for L_3, 51.5% of administered ^{15}N was recovered L244.

4. Material and methods Sampling. One can understand that the authors used only two replicates for each labelling for technical reasons even if it is difficult to generalize from six trees. However, the authors should be much more accurate concerning the samplings (number of sampling per tree, soil, phloem and xylem sampling) to improve this section and strengthen the validity of the conclusion.
Response: The sampling procedure was completed:
L110-118: “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."
L120-133: “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). 15N abundance was quantified in the same plant and soil fine powder aliquots with a mass spectrometer (PDZ Europa, University of Davis, Isotopes Facility, California)”.

5. Results Line 144-145 there is no verb in this sentence.
Response: The sentence was rewritten

6. I presume also there is a mistake, L2: 3+4 instead of 2+3 and L3: 5+6 instead of 3+4.
Response: It was corrected

7. Discussion Line 343-348. The authors should be much more careful here. I really do not know why the authors mention the Glutamine synthetase/Glutamate synthase pathway as no results shown in the paper concern amino acid metabolism. I presume this hypothesis is based on published literature which is not mentioned.

Response: Our team has conducted analyzes of root enzyme activities in mature oaks throughout a season, the results show a reduction in activity GS GOCAT in winter, these data are published in Trees structure and Function : Bazot et al., 2013 L381.

8. Also, the Morot-Gaudry reference is not in the reference list, and I am not sure it concerns tree physiology. I suspect there are more appropriate references concerning tree N assimilation.

Response: Morot Gaudry also presents conclusion about tree N assimilation in this book (p299). Gojon et al 1991 could be also mentioned, indeed they underlined that roots were the main site of NO3- reduction in *Prunus persica* L.

Marked-up manuscript version

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

Supprimé: The literature describes general patterns of seasonal tree nitrogen functioning as follows.

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.

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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

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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

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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.

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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):

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

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) :

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

3. Results

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¶

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).

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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)

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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)

213 The total balance for the administered ^{15}N demonstrated maximum recoveries within
214 the plant-soil systems three days after L_2 of 70%. By the end of October (49 days
215 after labelling), recoveries from the sampled compartments decreased to 22% of the
216 administered ^{15}N (Table 1).
217 Three days after labelling, 3% of the recovered ^{15}N was present from the fine roots
218 (Fig. 2c). Nine days after labelling (September 18, 2009), the PRN showed that the
219 majority of the ^{15}N was recovered from the soil, with 61% of the ^{15}N recovered from
220 the rhizospheric soil and 32.5% from the microbial biomass (Fig. 2d). During the
221 following 40 days (until October 28, 2009), the PRN from the soil decreased to 8.5%
222 in the rhizospheric soil and 9.5% in the microbial biomass (Fig. 2d). On the same
223 date, 6% of the ^{15}N was recovered from the fine roots (Fig. 2c). Less than 1% of the
224 ^{15}N was recovered from the phloem, xylem and twigs (Fig. 2a, b). In December (day
225 84) the PRN from the soil was similar to that of the previous date and 4% of the ^{15}N
226 was recovered from the fine roots (Fig. 2c, d).
227
228 **3.2. ^{15}N partitioning within plant-soil system before and after budburst**
229 Almost one year after the first labelling (L_1), and before budburst (April 8, 2010, 318
230 days after labelling), 7.5% of the ^{15}N were recovered in the sampled compartments.
231 Thereafter, recovery remained stable at around 12% until September (460 days after
232 labelling, Table 1).
233 On April 8, 2010, i.e. 318 days after L_1, 11.5% of the recovered ^{15}N was found in
234 fine roots (Fig.1 c). Twigs contained 4.5% of recovered ^{15}N (Fig. 1a), while phloem
235 contained 4% (Fig. 1b). Less than 0.5% of ^{15}N was recovered from the rhizospheric
236 soil and microbial biomass (Fig. 1d).

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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).

263 For trees whose soils were labelled in spring (L_3, March 20, 2010), the maximum
264 recovery of the administered ^{15}N occurred 40 days later: 51.5% from the sampled
265 compartments. Recovery decreased thereafter and stabilized at 19.5% until autumn
266 2010 (Table 1).
267 Twenty days after labelling and before budburst, the soil microbial biomass
268 contained 44.5% of the recovered ^{15}N and the rhizospheric soil 39% (Fig. 3d). The
269 remaining ^{15}N was mainly located in the roots (2% of recovered ^{15}N, Fig. 3c). 8 days
270 after budburst, the PRN was quite similar: 61% in microbial biomass and 32% in
271 rhizospheric soil (Fig. 3d). ^{15}N recovered from fine roots followed a pattern similar
272 to that observed on the previous sampling occasion (Fig. 3c). However, between 8
273 and 30 days after budburst (from April 28 to May 19, 2010 i.e. from 40 to 61 days
274 after labelling), the PRN in microbial biomass and in rhizospheric soil decreased
275 sharply to 3.2% (Fig. 3d). On that date, 17% of the ^{15}N was recovered from the fine
276 roots (Fig. 3c) and 21.2% from the leaves (Fig. 3a). The PRN from leaves remained
277 stable until the beginning of June (74 days after labelling) (Fig. 3a). From that date
278 until September the PRN from leaves and fine roots declined slightly (Fig. 3a, c).
279 The PRN from microbial biomass decreased continuously throughout the season and
280 reached 2.5% in September (day 166 after labelling) (Fig. 3d).
281
282 **4. Discussion**
283 **4.1. Efficiency of labelling**
284 Isotope labelling experiments are technically challenging, and as a consequence are
285 very scarce on trees growing in natural conditions. In this paper, field labelling
286 campaigns were conducted on 20-year-old naturally regenerated oaks. For each
287 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

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51.5%, 40 days after soil ^{15}N labelling. This recovery decreased to a mean of 19% during the rest of the season.

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

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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 compartments, including those which were not sampled, i.e. branches and coarse roots.

This important foliar N remobilisation was observed to continue in leaf-labelled trees until yellowing, i.e. the end of September. Data currently available on woody plants show that nitrogen is mainly re-translocated from leaves to storage sites during the autumn (Coleman and Chen, 1993;Cooke and Weih, 2005;Dong et al., 2002;Taylor, 1967), due to the predominant role of leaf senescence in the constitution of N stores. Leaf senescence leads to the breakdown of leaf proteins, the transfer of their nitrogen to the perennial plant parts and the formation of N storage compounds (vegetative storage proteins and amino acids) (Dong et al., 2000;Tromp, 1983). In this study, a noticeable increase of percentage of recovered ^{15}N in fine roots was observed on September 30 (16%). This compartment could be defined as a storage compartment in young sessile oaks. Such an observation has been already reported for oaks of the same pole stand (Gilson et al., 2014), and similar findings were reported for field-grown adult peach trees by Tagliavini et al (1997), being typical of other young deciduous trees (Millard and Proe, 1991;Salaün et al., 2005;Tromp and Ovaa, 1979;Wendler and Millard, 1996). On this date (end of September), branches and

357 coarse roots could also have contributed significantly to N storage, as previously
358 described (Bazot et al., 2013).
359 At the same time, root uptake can also contribute directly to storage, as proposed by
360 Millard (1996). Indeed, 49 days after labelled ^{15}N had been applied to surrounding
361 soil (L_2), in September, 5.75% was recovered from the trees' fine roots. It can be
362 underlined that at the end of September, foliage ^{15}N made up 73% of the ^{15}N
363 recovered in roots, whereas soil ^{15}N uptake contributed to 27% of the ^{15}N recovered
364 in roots (eq. 2, Fig. 4). The soil N uptake in this period was mainly recovered in the
365 root system; there was little labelled N in the rest of the trees. This is consistent with
366 the results of Tagliavini et al (1997) and Jordan et al (2012), who found a significant
367 fraction of labelled N in fine root samples of peach trees supplied with ^{15}N applied
368 on soil before fruit harvest in September.
369 Concomitantly with root N uptake for storage, notably in fine roots, a strong
370 immobilization of N in microbial biomass was observed. Indeed, on October 7 (i.e.
371 28 days after labelling), when yellowing was well advanced, 12.5% of the applied
372 ^{15}N was recovered in microbial biomass and 21.5% in rhizospheric soil: there was a
373 competition for soil N between microbial N immobilization and reserve synthesis by
374 root N uptake at that time. This is consistent with the idea that soil microorganisms
375 are strong short term-competitors for soil N due to their high surface area to volume
376 ratio, wide spatial distribution in the soil and rapid growth rates, compared with
377 plants roots (Hodge et al., 2000). Thereafter, root N uptake was still efficient during
378 late yellowing (between October 7 and October 28), since ^{15}N recovered from the
379 fine roots slightly increased from 3.5% to 5.5%, whereas that recovered from
380 microbial biomass decreased from 12.5% to 10%. This could be explained by
381 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.

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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

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438 white birch (Wendler and Millard, 1996); sycamore maple (Millard and Proe, 1991);
439 Japan oak (Ueda et al., 2009); pedunculate oak (Vizoso et al., 2008); and sessile oak
440 (El Zein et al., 2011a).
441 Considering trees whose soil had been labelled in autumn (L_2), eight days after
442 budburst the proportion of recovered ^{15}N in microbial biomass decreased slightly
443 whereas it slightly increased in fine roots compared to the previous sampling date.
444 One can suppose that the increased soil temperature and the first flux of C from plant
445 to soil (rhizodeposition) stimulated microbial biomass turnover, making ^{15}N
446 available for root uptake. Very little ^{15}N was recovered from the other compartments
447 of the trees.
448 Soil N uptake became really effective between 8 and 30 days after budburst. Indeed,
449 whatever the date of the soil labelling (autumn or the current spring), 30 days after
450 budburst, a sharp decrease in ^{15}N in the microbial biomass was observed, depending
451 on an increase of ^{15}N in fine roots and in young leaves. In June 28 (at leaf maturity),
452 40% of the ^{15}N recovered from leaves came from stored ^{15}N, 10% came from ^{15}N
453 applied to soil the previous autumn, and 40% came from ^{15}N applied on soil the
454 current March, one month before budburst (Eq. 2, Fig. 4). This pattern of
455 contribution was maintained throughout the season. Similar findings have been
456 reported for other species. For example, 20-30% of shoot leaf N was supplied by
457 spring-applied fertilizer for mature pear trees (Sanchez et al., 1990) and mature
458 almond trees (Weinbaum SA, 1984), while only 13% of a solution of nitrate-N and
459 ammonium-N applied to soil, contributed to total leaf N of apple trees (Neilsen et al.,
460 1997). *Sorbus aucuparia* had remobilized half the N from storage before any was
461 taken up by the roots (Millard et al., 2001). Finally, there is a concomitant/concurrent

462 remobilization and uptake of N from the soil by some other species, as shown for
463 scots pine (Millard et al., 2001) and walnut (Frak et al., 2002).
464
465 **5. Conclusion**
466 This paper completes knowledge of internal and external nitrogen cycles in a forest
467 ecosystem. We highlighted that in autumn, N reserves are formed from N
468 remobilized from leaves and N uptake by roots. This N is stored in roots, principally
469 most probably in the form of amino-acids and nitrate. Those reserves, especially N
470 coming from leaves, contributed significantly to new tissue synthesis the following
471 spring. Nevertheless, N uptake was also observed in spring before budburst; this N
472 was not transferred to new twigs and new leaves during the first days following
473 budburst. N uptake from soil only contributed significantly to the synthesis of new
474 tissues when leaves were fully expanded. Two months after budburst the relative
475 contributions of ^{15}N originating from leaves and ^{15}N uptake from soil were 40:60,
476 whereas they were 95:5 eight days after budburst.
477
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485
486 **Author contribution statement**

Supprimé: It will now be interesting to investigate soil N uptake, and the competition for N between tree and microorganisms (bacteria, fungi) in both autumn and spring.¶

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	
	337/118	11	13	**227/118**	12	10	**40/118**	63	
	358/139	10	13	**247/139**	16	20	**61/139**	16	
	370/152	14	14	**260/152**	22	21	**74/152**	20	
	397/180	11	10	**287/180**	38	18	**102/180**	20	
	460/244	13	11	**350/244**	13	12	**166/244**	18	
	509/293	7	5	**399/293**	10	8	**215/293**	11	21

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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.

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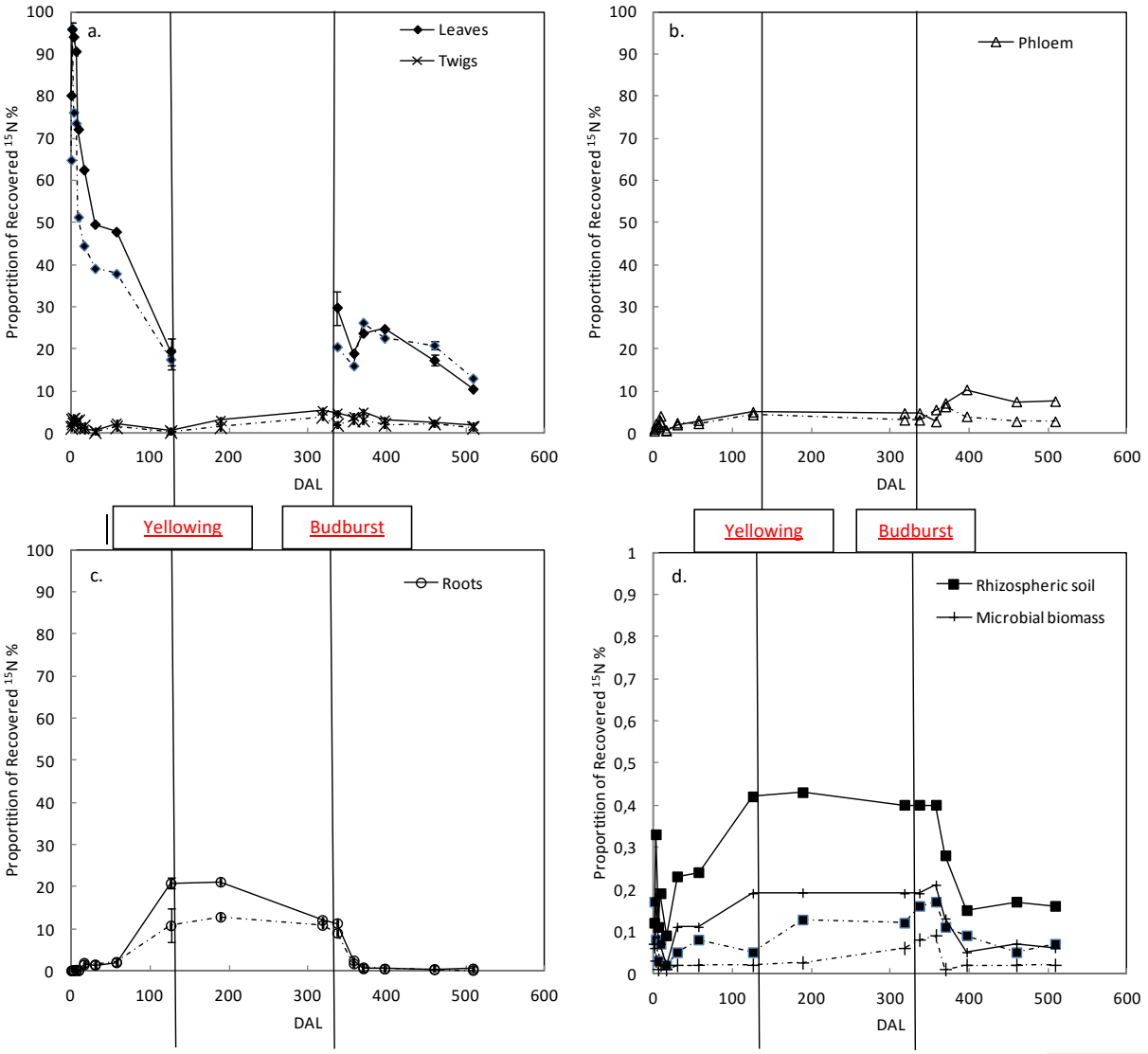

Figure 2 :

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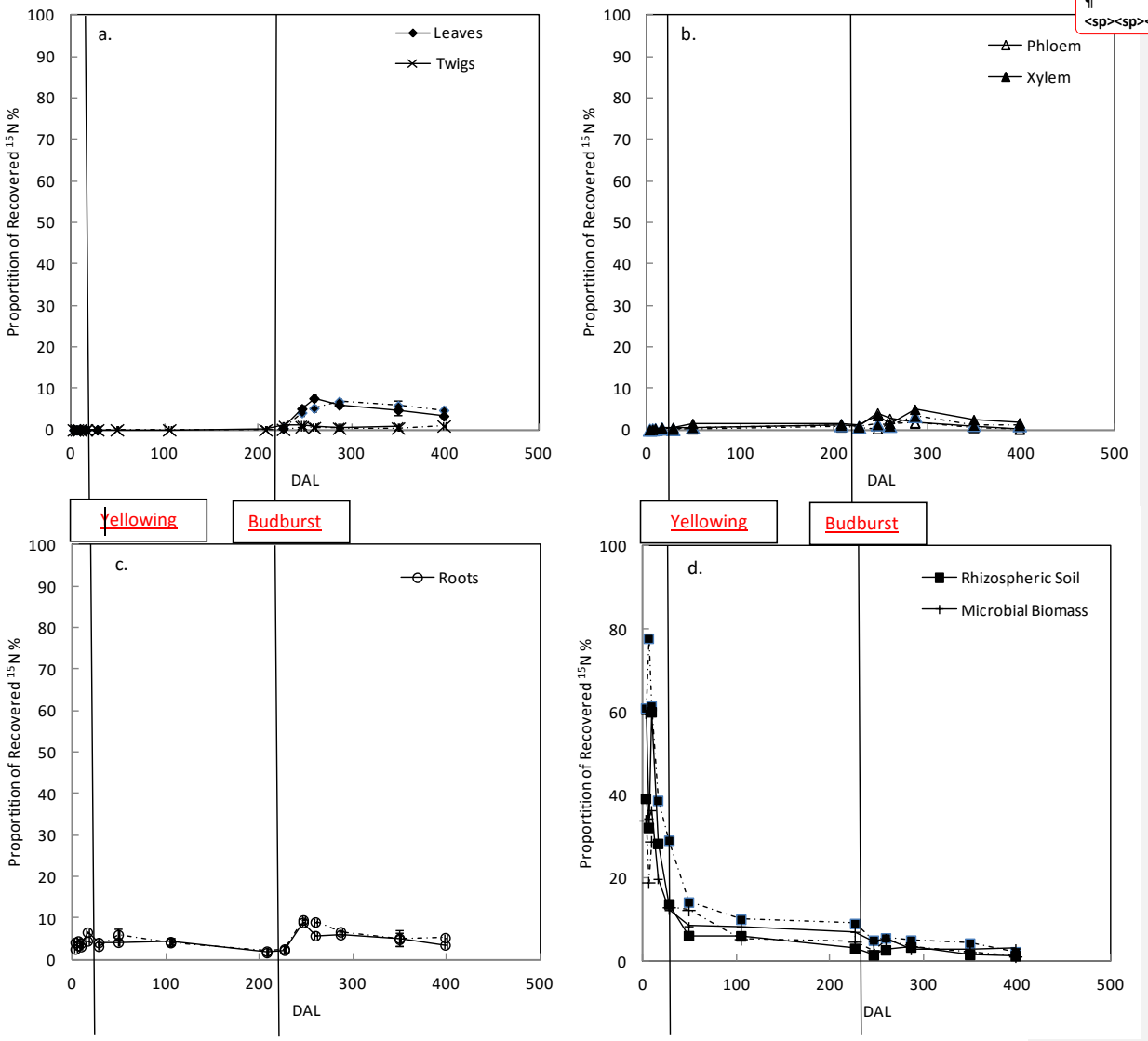

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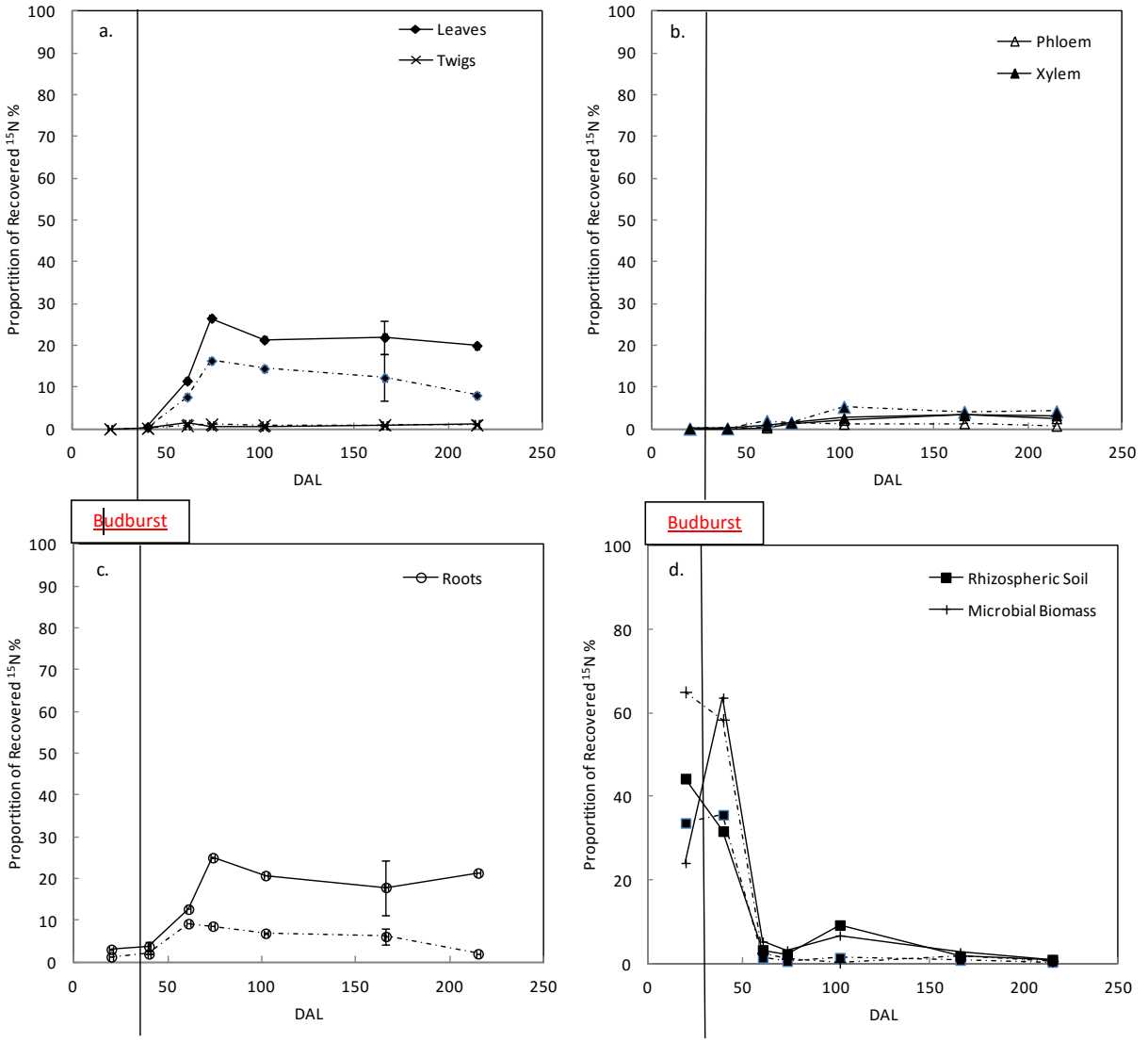

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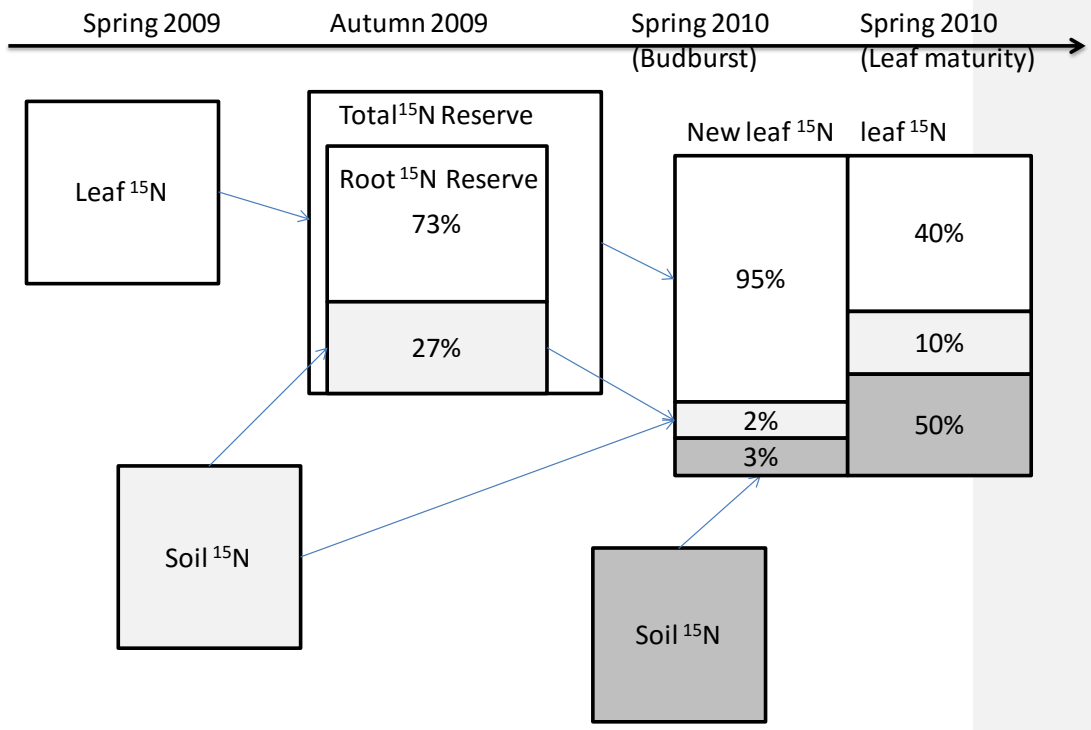
Spring 2009
Autumn 2009
Spring 2010
(Budburst)
Spring 2010
(Leaf maturity)
Leaf ^{15}N
Total ^{15}N Reserve
Root ^{15}N Reserve
73%
27%
Soil ^{15}N
New leaf ^{15}N
95%
2%
3%
leaf ^{15}N
40%
10%
50%
Soil ^{15}N