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# Recently absorbed nitrogen incorporates into new and old tissues: evidence from a $^{15}\text{N}$ -labelling experiment in deciduous oaks

Mubarak Mahmud · Tania L. Maxwell · Sixtine Cuff · Romain Schroeder · Stéphane Bazot · Nicolas Delpierre · Anne Marmagne · Gaëlle Vincent · Laure Barthes 

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**Abstract** We quantified the distribution of nitrogen (N), dry-matter (biomass) and of soil-applied  $^{15}\text{N}$  in tree and soil compartments in five naturally-growing 20-year-old oak trees. After applying  $^{15}\text{N}$  solution to soil at the base of the trees in spring, all the trees were felled in the fall, their root system excavated, biomass, nitrogen and  $^{15}\text{N}$  content measured in all compartments. Xylem rings-compartment contains most biomass (47%) while branches and coarse-root contains most nitrogen (29% and 14% respectively). The labelled  $^{15}\text{N}$  absorbed throughout the vegetation season, was found in all compartments except

the heartwood. The majority of recovered  $^{15}\text{N}$  was in the leaves (24%). Some often overlooked compartments (coarse root, stump, xylem and other branches) together recovered 45% of the  $^{15}\text{N}$ .  $^{15}\text{N}$  was found in all the sapwood rings, from the ring formed in the current year up to 10 year-old rings, marking the limit of the heartwood. More  $^{15}\text{N}$  was found in the younger rings compared to older rings. The  $^{15}\text{N}$  allocated to ancient rings can originate from different, non-mutually exclusive, sources: whether directly from the soil via the  $^{15}\text{N}$  uptake throughout the vegetation season and transport in the xylem sap, from the autumnal resorption of  $^{15}\text{N}$  first allocated to the leaves, or from the  $^{15}\text{N}$  mobility once allocated to the forming ring to older rings through ray parenchyma. With about 6% of the initial  $^{15}\text{N}$  retrieved in the microbial biomass at the end of the growing season, we confirmed the role of microbial biomass as forest nitrogen sink.

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## Abbreviations

DBH	Diameter at Breast Height
DOY	Day of the Year
Stump	The tree's pivot that is inside the soil
AG	Aboveground
BG	Belowground
EP	Experimental plot

## Introduction

There is an increasing need to improve the accuracy of forest biomass estimation and its relations with nitrogen fluxes for several reasons, such as the assessment of carbon pools (Grassi et al. 2017) and also for studying global biogeochemical cycles, such as nitrogen, because the amount of nutrients in trees is also related to the quantity of biomass present (Hendrick and Pregitzer 1993). Nutrients are essential for tree growth and biomass accumulation, and forest growth can be limited by soil available nutrients, such as nitrogen (Dickson 1989; LeBauer and Treseder 2008; Rennenberg et al. 2009; Norby et al. 2010; Vicca et al. 2012; Fernández-Martínez et al. 2014; Rennenberg and Dannenmann 2015; Eastman et al. 2021). This limitation is further enhanced by CO<sub>2</sub> fertilization effect (Goodale et al. 2002) which is the increased rate of photosynthesis in plant caused by increased amount of CO<sub>2</sub> in the atmosphere (Tharammal et al. 2019; Ueyama et al. 2020). Studies suggest that tree carbon allocation and nitrogen acquisition are inter-linked processes (Franklin et al. 2012). For example, Finzi et al. (2015) estimated that up to one-third of total soil N mineralization may be fuelled by C allocated to belowground tree compartment and delivered to soil via rhizodeposition. More understanding of the processes of nitrogen nutrition in trees as it drives their growth, the productivity of forest ecosystems and their capacity to limit the increase in atmospheric carbon concentration is crucial.

Oak forests represent an ecologically and economically important deciduous tree ecosystem in France (Timbal and Aussenac 1996). The ability of deciduous trees to store and internally redistribute nitrogen resources is a fundamental element of its N economy, as such Cooke and Weih (2005) divided the internal redistribution, or cycling, of N into four phases as follows: (1) primary allocation of N from N-assimilating source tissues to sink tissues during the growing season; (2) reallocation of N resources arising from metabolic recycling of N during the growing season; (3) resorption of N from senescing tissues and its transport to perennial tissues during autumn senescence; and (4) remobilization of N from perennial tissues to actively growing tissues during spring flush. It is accepted that the resumption of tree growth in spring is achieved both from the tree's internal reserves and from the

newly absorbed nitrogen from the soil (Millard and Grelet 2010). Remobilized stored N and N uptake from the soil could reach 90% and 27% respectively in growing leaves and twigs (El Zein et al. 2011a) with efficiencies that depend on environmental conditions and competition with the soil microbial biomass (Maxwell et al. 2020). It has been shown that absorbed nitrogen is mainly directed to leaves via xylem vessels (El-Zein et al. 2011a; Bazot et al. 2016). In Oak tree, which is a ring-porous species, the sap flow coming from the roots is considered to be restricted to the new, or the most recent few annual rings, principally in the large vessels of the early-wood of the current-year ring and in the narrow late vessels of the older rings (Čermák et al. 1992; Granier et al. 1994; Tognetti et al. 1996). The reallocation of nitrogen between the different parts of the tree during the growing season or the transport of nitrogen from senescent leaves into the bark, ray and axial parenchyma tissues during the autumn resorption are usually reported to be ensured through the phloem sap flow (Cooke and Weih 2005; Millard and Grelet 2010; Plavcová and Jansen 2015; Sample and Babst 2019).

Some studies of N uptake, remobilization and allocation dynamics during the growing season already exist in small trees (Millard and Grelet 2010), in adult trees (El Zein et al. 2011a; Bazot et al. 2016; Maxwell et al. 2020) and in economically important fruit trees (Legaz et al. 1995; Quartieri et al. 2002; Rocuzzo et al. 2017). However, in most of the studies, few compartments of the trees were investigated. For example, El Zein et al. 2011a, who highlighted the importance of N reserves for spring growth in 50-year-old sessile oak trees, only investigated current-year shoots (leaves, twigs and buds) and did not take into account the relative contribution of storage organs, such as roots, stump, stem and big branches. In another study, El Zein et al. 2011b compared the composition of non-structural carbon and nitrogen compounds of the inner and outer xylem rings where they highlighted the stability of non-structural nitrogen compounds concentration in the stem of sapwood during the year. Thus, the focus of their study was on the trunk xylem only.

In this work, we aimed at documenting the allocation pattern of nitrogen acquired in spring in 20-year old sessile oak trees using <sup>15</sup>N labelling experiment. We hypothesized that:

- 1) Coarse root, stump, xylem rings and other branches which are mostly neglected in the literature would be the most important woody nitrogen sinks.
- 2)  $^{15}\text{N}$  recovery in xylem rings would depend on the ring age.
- 3) The soil microbial biomass would contain  $^{15}\text{N}$  at the end of the study period. Therefore, we quantified in a 20-year old oak trees (*Quercus petraea*) the total nitrogen, the distribution pattern of recently incorporated  $^{15}\text{N}$  and the relative stocks of biomass in the different tree compartments: partitioning in leaves, branches of the current and previous years, older branches, fine roots, medium roots, coarse roots, phloem, each ring of the xylem from sapwood to heartwood and stump. Root biomass estimated by direct method (description below) was compared with biomass estimated using allometric equation. In addition to  $^{15}\text{N}$  assessment in the tree, the  $^{15}\text{N}$  in the soil microbial biomass was also quantified and incorporated into the  $^{15}\text{N}$  budget. This exhaustive assessment, taking into account the entirety of the tree compartments, has seldom been done in the literature.

## Material and methods

### Site description

The study was conducted in the Fontainebleau-Barbeau Forest, which is a naturally regenerated forest containing oak trees (*Quercus petraea* (Matt.) Lieb.), located 60 km Southeast of Paris, France (48°29' N, 02°47' E). At the time of the study, the trees were 20-years-old. In 2016, tree heights ranged between 9 and 11 m and the trunk diameter at breast height ranged from 12 to 15.7 cm. A detailed description of the site can be found in (Delpierre et al. 2016; Maxwell et al. 2020). Briefly, the site is at an altitude of 95 m, with an average temperature of 11 °C and annual rainfall of 661 mm (from 1960 to 2016, registered at the station of Meteo France of Melun located 11 km from the Fontainebleau-Barbeau Forest). The soil is a stagnic luvisol developed on a clayic bedrock (IUSS Working Group WRB 2015) with a pH ranging between 4 and 6 for the first three horizons. The soil is loamy sand for the first 20 cm and then

loamy clay for the 20–40 cm layer. A dense layer of buhrstone is located at about 60 to 90 cm in depth.

### Field preparation

#### Trenching

Five healthy, morphologically similar co-dominant oak trees were selected. On average, the distance between each tree was 20 m. In 2009, a 0.5–0.6 m deep trench was dug around each tree to create the experimental plot (EP) (average area of the EP was  $3.4 \pm 0.2 \text{ m}^2$ ). Each trench was lined with a polyethylene film and then backfilled with soil. Each EP surface remained bare and soil was undisturbed.

#### $^{15}\text{N}$ labelling

On March 1 2016 (DOY 61), the soil of the EP of each of the five trees was labelled with  $^{15}\text{N}$  by applying a homogenous spray of  $^{15}\text{N}$  labelling solution (prepared with 5 g of  $^{15}\text{NH}_4^{15}\text{NO}_3$  containing 98% atoms of  $^{15}\text{N}$  in 20 L of deionised water). Each EP was thus exposed to 1.82 g of the original  $^{15}\text{N}$ , equivalent to  $5.35 \pm 0.35 \text{ kg } ^{15}\text{N} / \text{ha}$ . The polyethylene-lining of the EP avoids the uptake of the  $^{15}\text{N}$  applied by the surrounding trees and also allows limits lateral leaching to the surrounding soil.

### Sample collection and estimation of the biomass of each compartment

On October 13 2016 (when around 70% of the leaves were yellow) five trees were felled and uprooted, their root system was excavated with a mechanical backhoe. Soil was sampled using a soil stainless steel auger bipartite (Eijkelkamp, SDEC France), with a diameter of 8 cm and a depth of 15 cm, to extract around 1 kg of fresh soil (from which soil microbial biomass was estimated) from one point per EP.

#### The leaves, branches and the trunk

The leaves, branches of the year (2016), branches of the previous year (2015) and the remaining branches (categorized as “other branches”) were collected and oven-dried. The trunk of each tree was cut into pieces of around 70 cm. One portion of the trunk, with a thickness of 5 cm taken at the DBH, was

kept in the freezer at  $-20\text{ }^{\circ}\text{C}$  for subsequent analyses of the phloem and xylem rings. All samples were oven-dried at  $60\text{ }^{\circ}\text{C}$  until the dry weight was attained (around 3 months).

### *The xylem rings and phloem*

For each tree, the size of the xylem rings was measured using a binocular magnifying glass with unit scales. Using the radius from the core to the outer edge and to the inner edge of a ring, the volume of each ring was calculated using the following allometric equation adapted from (Zianis et al. 2005) for *Quercus petraea*:

$$\text{Ring volume} = \left[ (2 * r_{\text{outer edge}})^{2.0033} - (2 * r_{\text{inner edge}})^{2.0033} \right] * H^{0.85925} * e^{-2.86353}$$

The volume is in  $\text{dm}^3$ , the radius ( $r$ ) in cm, and the height ( $H$ ) in m.

The  $r_{\text{outer}}$  edge was measured from the center of the tree to the outer edge of the considered ring. Likewise, the  $r_{\text{inner}}$  edge was measured from the center of the trunk to the inner edge of the considered ring. The height was calculated by measuring the base of the tree to the highest point, when the trees were cut down in autumn.

The volume was then multiplied by the density of the xylem ( $0.754\text{ g/cm}^3$ ) in order to have the biomass of each ring (Maxwell et al. 2020). The number of rings in trees 1, 2, and 5 were 18 while tree 4 had 22, therefore, for consistency, only 18 rings were considered in each of the trees. The total xylem biomass for each tree was obtained by summing the biomass of the 18 individual rings.

For the biomass of the phloem (which is considered as the inner bark), we used the same approach. The phloem thickness was consistently around 5 mm for each tree and the density was equal to  $0.711\text{ g/cm}^3$  (Maxwell et al. 2020). The  $r_{\text{outer}}$  edge was measured from the center of the tree to the outer edge of the phloem and the  $r_{\text{inner}}$  edge was measured from the center of the trunk to the inner edge of the phloem.

### *Fine and medium roots*

The fine roots ( $<2\text{ mm}$  in diameter) and medium roots ( $>2\text{ mm}$  &  $<1\text{ cm}$  in diameter) as defined by Finzi and Berthrong (2005), were obtained just before

the excavation in the fall. A strip of soil about 20 cm deep, 20 cm wide, and 70 cm long was sampled from a distance of 20 cm away from the trunk of each tree. This strip of soil, which represents approximately 30 kg of fresh soil, was then disaggregated and the fine roots and medium roots were taken out, rinsed with water, separated according to size (Finzi and Berthrong 2005) and placed into an oven at  $60\text{ }^{\circ}\text{C}$  to attain dry weight. The quantity of roots per tree was then extrapolated using the area of each EP.

### *Coarse roots and stump*

The coarse roots ( $>1\text{ cm}$  in diameter) and the stump were collected after the total excavation of the root system with a mechanical backhoe. The stump and the entire coarse roots were weighed. For each tree, an aliquot of around 600 g (fresh weight) of coarse roots and 300 g of stump was oven dried at  $70\text{ }^{\circ}\text{C}$  until the dry weight was attained. The total weight of coarse root and tree stump was calculated using the fresh weight to dried weight ratio of the aliquots.

### *The total root system*

The total root system biomass was estimated using two methods: (1) by summing the biomass of each root categories (fine roots, medium roots and coarse roots) called "the measured value of total root biomass" (without stump), and (2) by using the regression equation for *Quercus petraea* developed by Drexhage and Colin (2001), and the tree DBH, to calculate the "the estimated value of total root biomass". We then compared the two methods of biomass estimation (Drexhage and Colin 2001).

$$\text{Log } y_i = \beta_0 + \beta_1 \text{ log } x_i \quad (1)$$

with,

Log  $y_i$  logarithmic transformation of root biomass (kg).

log  $x_i$  log-arithmetic transformation of DBH (cm).

$\beta_0$  Intercept coefficient (-1.56).

$\beta_1$  Slope coefficient (2.44).

### Total aboveground and belowground biomasses

The aboveground biomass was considered as the sum of the biomass of the leaves, the branches of the year, branches of the previous year, the other branches, the sum of the biomass of 18 xylem rings and the phloem. The belowground biomass considered comprises the fine roots, the medium roots, coarse roots (i.e. the total root system) and the stump.

### Analyses

#### Tree sample preparation

After tree felling, five samples were taken from each compartment as pseudo-replicates (fine root, medium root, coarse root, stump, branch of the current year, branch of the previous year, other branch, leaf) from each of the five trees. Each compartment was characterized by the mean of five samples for its corresponding tree. The mean of the five trees was subsequently used to represent a given compartment. For phloem and each ring of the xylem, only one sample per tree was taken.

Roots, leaves and branches of the current year ( $n$ ) and branches of the previous year ( $n-1$ ) were pre-grounded (chopped into small pieces) using hand-operated secateurs. The stump aliquot and other branches sample were pre-grounded using a mechanical wood shredder (Timberwolf TW PTO/150H). The shredding unit of the mechanical shredder was thoroughly cleaned with air pressure between the shredding operations of each compartment to avoid sample pollution.

To isolate individual xylem rings, a 0.5 cm thick trunk cross section was cut, from which a stick of 2 mm width was removed. The sticks were then freeze-dried (Alpha 1–2 LDplus, Martin Christ). The rings were isolated using a microtome (Sledge microtomes G.S.L.1) with the limit between years being easily visible in this ring-porous species, with a manual magnifier.

Following pre-grinding, all of the samples were grounded to a fine homogenous powder using Mixer Mill MM301, Retsch for 1–6 min in 2 mL microtubes (Sarstedt) with two aluminium balls with pauses to prevent the tube from overheating. The aluminium balls were thoroughly cleaned with ethanol between each sample to avoid contamination.

### Tree sample analysis

The powder was then transferred into tin capsules (D1006, Elemental Microanalysis) using a microgram balance (LE26P, Sartorius) (2 mg for leaves; 3 mg for branches; 4 mg for the stump, other branches and roots: fine, medium and coarse; as well as for xylem and phloem). These samples were then analysed for their total N concentration by dry combustion with an N analyser (TNM-1, Shimadzu, Champs-sur-Marne, France) and for their  $^{15}\text{N}$  concentration by an isotopic mass spectrometer (FLASH 2000 HT/IRMS, Thermo Scientific, INRAE, Versailles).

#### Soil and microbial biomass

Soil microbial N was measured using the chloroform fumigation extraction method (Brookes et al. 1985). Two fresh soil subsamples of 5 g sieved at 2 mm were prepared. One subsample was fumigated for 24 h with chloroform vapour, while the other was not fumigated. Nitrogen extraction of both subsamples was performed using 20 ml of 0.5 mol L<sup>-1</sup> K<sub>2</sub>SO<sub>4</sub> for 30 min under vigorous shaking at 250 rpm for N determination. For  $^{15}\text{N}$  determination, the same procedure was followed, but with an extraction by 20 mL of 0.03 M K<sub>2</sub>SO<sub>4</sub> to avoid any interference of the mass spectrometer with the K<sub>2</sub>SO<sub>4</sub> salt during analysis. The extracts (fumigated and not fumigated, with 0.5 M or 0.03 M K<sub>2</sub>SO<sub>4</sub>) were filtered (GFC WHATMANN 1.2 µm mesh), then analysed for N content using an N analyser (TNM-1, Shimadzu, Champs-sur-Marne, France) or freeze-dried for subsequent  $^{15}\text{N}$  determination using an isotopic ratio mass spectrometer (FLASH 2000 HT/IRMS, Thermo Scientific).

The microbial biomass N was estimated as  $[(N \text{ in fumigated soil}) - (N \text{ in non-fumigated soil})] / 0.54$  (Brookes et al. 1985). The  $^{15}\text{N}$  concentrations were similarly determined from the fumigated and non-fumigated soil extracts. Values from the non-fumigated soils are presented hereafter as the 'soil extractable solution' values. Soil moisture content was determined by drying 3 replicates of around 5 g of fresh soil from each EP, until constant weight at 105 °C. The results expressed per tree (as soil N stock in g N/plot or in mg  $^{15}\text{N}$ /plot) were obtained by multiplying the concentration data (g N or mg  $^{15}\text{N}$  per gram of dry soil) by the soil density (1.1 kg/m<sup>3</sup>), and then by the volume of

soil around each tree. This was calculated by multiplying a fixed depth of 15 cm (height of the soil corer) by the specific area around each tree (3.9, 2.8, 3.9, 3.5, and 3.0 m<sup>2</sup> for trees 1,2,3,4 and 5 respectively).

## Calculations

### *Quantity of N and <sup>15</sup>N*

The amount of nitrogen present in each compartment expressed per tree, as N stock in g N / tree was found by multiplying the concentration data (g N / gram of compartment) by the estimated or measured compartment's biomass. That value was then multiplied with the percentage of <sup>15</sup>N to calculate the quantity of <sup>15</sup>N in the compartment.

The same calculation method was used for quantifying N and <sup>15</sup>N in microbial biomass and soil extractable solution.

### *Recovered <sup>15</sup>N*

The quantity of <sup>15</sup>N from the labelled solution added to the soil March 1 that was recovered at fall in the different compartments of the system in question (tree compartments, microbial biomass and extractable soil solution) was calculated using the following formula:

$$\text{Recovered}^{15}\text{N} = \text{Atom}\%^{15}\text{N excess compartment} * \text{N}\% \text{ compartment} * \text{total biomass of compartment}$$

$$\text{With Atom}\%^{15}\text{N excess compartment} = \text{Atom}\%^{15}\text{N compartment after labelling} - \text{Atom}\%^{15}\text{N compartment natural abundance}$$

The natural abundance of <sup>15</sup>N in the compartments of each tree and microbial biomass (*Atom %<sup>15</sup>N natural abundance of the compartment*) was measured prior to labelling at DOY 60 except for the leaves, in which the cited natural abundance of 0.36% was used (Deléens et al. 1997). These results were expressed in grams for nitrogen and in milligrams for <sup>15</sup>N.

To quantify the distribution of the applied <sup>15</sup>N between the compartments of the tree, results were expressed in percent (%) of the sum of the total <sup>15</sup>N recovered in those compartments. Percentage of Recovered Nitrogen (PRN) was calculated as follows:

$$\text{PRN}\% = \frac{\text{Recovered}^{15}\text{N in compartment}}{\sum \text{Recovered}^{15}\text{N}} * 100$$

## Statistical analysis

The data were analysed using the Sigmaplot program. To test for differences between compartments, a one-way Analysis of Variance (ANOVA) (n=5), was done for the biomass, nitrogen quantity and nitrogen concentration, recovered <sup>15</sup>N quantity and as well as its concentration for each compartment. In the case of non-normal residuals, a Kruskal–Wallis One Way ANOVA on ranks was completed as a non-parametric replacement for the ANOVA.

If there was a significant effect of the tree compartment on the variable measured, a Tukey post hoc test was conducted to determine which compartment was significantly different from the other. For the comparison between two means, a t-test or a Welch-test was performed depending on whether the variances were equal or not. The rejection limit was fixed to alpha=5% for all the tests used, and thus any result with a p-value inferior to 0.05 was considered to be significant.

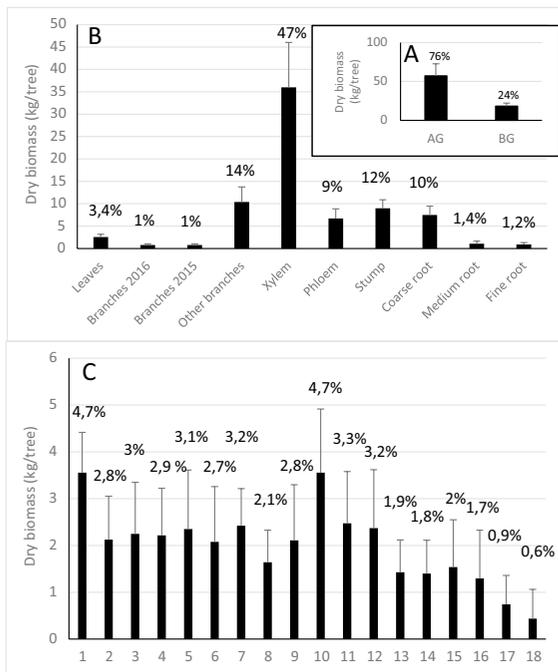
## Results

### Biomass partitioning

Here we present data on biomass partitioning in five 20-year-old oak trees. The average height of the trees was 10.22 ± 0.8 m and the average diameter at breast height was 13.44 ± 1.5 cm (Table 1). The trees had a total biomass of 75 kg ± 18 kg, ranging from 57.5 kg to 101.6 kg. The distribution of the total biomass was in favor of the aboveground part, which represented 76% of the total biomass (Fig. 1A). The aboveground compartments considered were leaves, branches of the current and previous years, other branches,

**Table 1** Morphological characteristics of the five trees

Tree	Height of tree (m)	Diameter at Breast Height (cm)
1	10.6	15.7
2	10.5	12
3	8.96	13
4	11.05	14
5	10	12.5
Mean	10.22 ± 0.8	13.44 ± 1.5

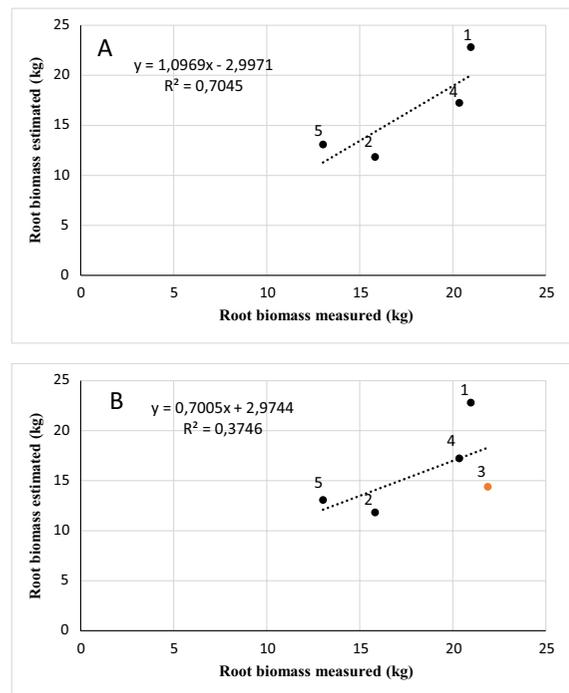


**Fig. 1** Quantity of dry biomass expressed in kg/tree in above-ground (AB) and belowground (BG) compartments (A), sub-compartments (B) and in the 18 xylem rings (C). Rings 1 and 18 correspond to years 2016 and 1999 respectively. Values are mean and standard deviation of 5 field replicates from five trees. The number above the bars are the percentage of the dry biomass in the considered compartment relative to the whole tree

phloem and xylem, the latter containing nearly 50% of the tree’s total biomass and 63% of the above-ground biomass (Fig. 1B). The biomass of the two successive branch compartments (branches of 2015 and branches of 2016) were quite similar representing  $0.76 \pm 0.3$  kg and  $0.78 \pm 0.2$  kg respectively, i.e.

1% of the total biomass of the trees. The trunk biomass associated to individual rings ranged from  $0.4 \pm 0.6$  kg to  $3.6 \pm 0.9$  kg depending on the year. The limits of the sapwood to heartwood varies among the trees (ring 11 for tree 1, ring 11 for tree 2, ring 10 for tree 3, ring 11 for tree 4 and ring 8 for tree 5). The largest rings were those formed in 2016 (ring 1) and 2007 (ring 10) (Fig. 1C). From ring width measurement, we observed a decrease in annual growth from ring 2 to ring 10 (2015 to 2007), a sharp spike in ring 10, and then subsequent decrease from ring 11 to ring 18 (2008 to 2001), with small fluctuations.

The belowground biomass which represents 24% of the total biomass was made of coarse roots and the stump at 90% (Fig. 1B). Fine and medium roots (< 1 cm) put together represented 2.6% of the total biomass of the tree, similar to the order of magnitude of the leaves which represented 3.4% of the total biomass of the tree.

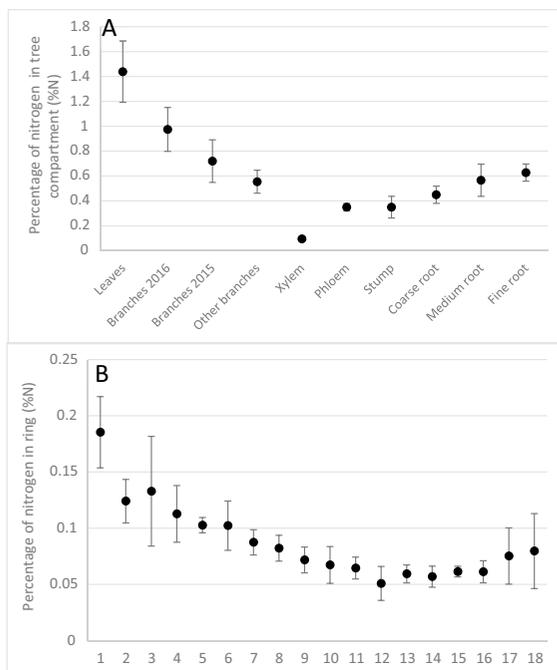


**Fig. 2** Correlation between the root biomass measured by summing the individual biomass of fine, medium and large roots with the root biomass estimated using DBH and the Eq. 2 from (Drexhage and Colin 2001), with all the trees except the tree 3 (A) or with all the five tree (B). The number under the point refers to the number of the tree (see Table 1)

The result of the root biomass estimated using allometric equation is shown in Fig. 2. The estimated biomass was close to actual (measured) biomass ( $R^2=0.7045$ ) when tree 3 was not included ( $r^2=0.3746$ , when tree 3 was included).

### Nitrogen distribution

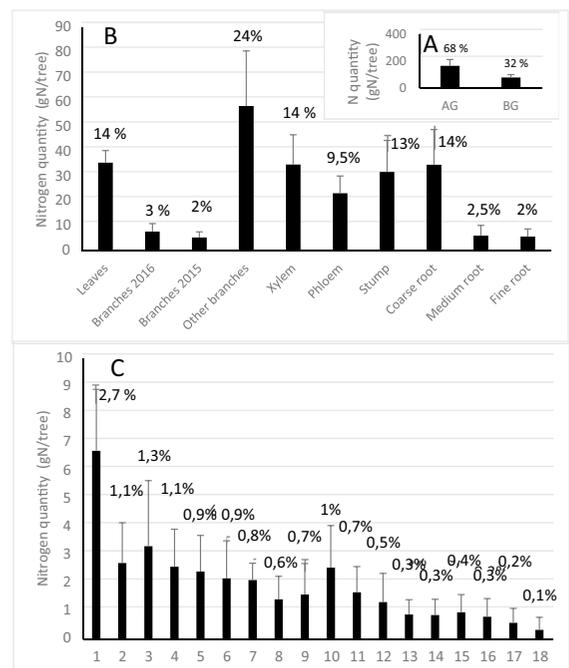
Figure 3 presents the percentage of nitrogen in the tree compartments. Among all of the sub-compartments, the nitrogen percentage was significantly higher in leaves (Fig. 3A), than that in the xylem, phloem and stump. Other than leaves, the compartments with the highest nitrogen were the branches:  $0.97 \pm 0.17\%N$  in the current year's branches and  $0.72 \pm 0.17\%N$  in the previous year's branches. Fine roots contained  $0.63 \pm 0.07\%N$ . The percentage of nitrogen ranged from  $0.09 \pm 0.01$  to  $0.56 \pm 0.01\%N$  for the other compartments (xylem, phloem, stump, coarse root, medium root and other branches). On average, the xylem had the lowest concentration of nitrogen.



**Fig. 3** Percentage of nitrogen in sub-compartments (A) and in the the 18 xylem rings (B). Rings 1 and 18 correspond to years 2016 and 1999 respectively. Values are mean and standard deviation of 5 field replicates from five trees

In the xylem, the concentration of nitrogen depended on the age of the ring ( $p < 0.05$ ): the percentage of nitrogen in the first ring (younger ring) was higher than in the older rings (and significantly higher than in rings 11 to 16) (Fig. 3B). The lowest value of the percentage of nitrogen in the ring was 0.05% (in ring 12). Nitrogen decreases sharply from ring 1 to ring 2 (student T-test  $p=0.06$ ) and then decreased steadily up to ring 12 (which was the maximum inner limit of the sapwood), reaching a minimum after which the N concentration increased up to ring 18 (i.e. throughout the heartwood), reaching 0.75% of the dry mass, i.e. half the average concentration of rings 1–2.

The total quantity of nitrogen in a tree represented  $243 \pm 56$  g N / tree, 68% of which was aboveground and 32% belowground (Fig. 4A). The majority of the tree nitrogen was found in the branches and the roots, these two compartments alone accounted for 57.5% of the total tree (Fig. 4B). The trunk (xylem



**Fig. 4** Quantity of nitrogen expressed in g N /tree in above and below ground compartments (A), sub-compartments (B) and in the 18 xylem rings (C). Rings 1 and 18 correspond to years 2016 and 1999 respectively. Values are mean and standard deviation of 5 field replicates from five trees. The number above the bars are the percentage of the nitrogen quantity in the considered compartment relative to the whole tree

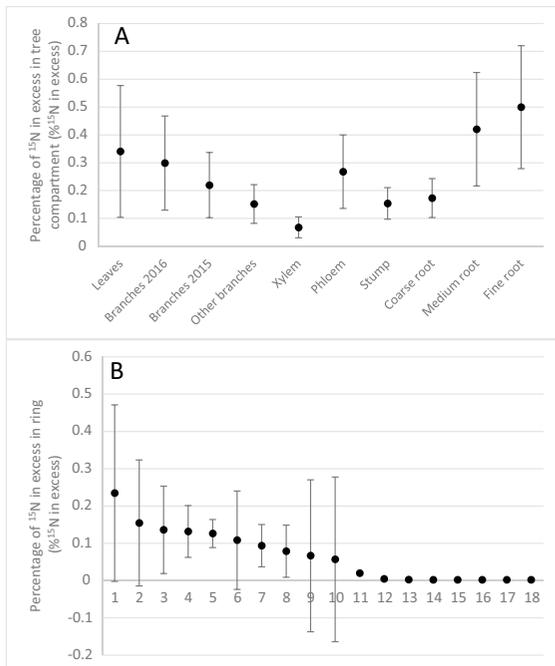
plus phloem) contained around the same amount of nitrogen as the other branches (23.5% versus 24%). Similar amounts of nitrogen (around 7 g N/tree) were recovered in the 2016 branches and in the latest ring of the xylem (ring 1, also formed in 2016), (Fig. 2B and C). The nitrogen content of this latest ring represents 2.7% of the nitrogen content of the tree and around 20% of the xylem nitrogen. Both the branches of the previous year (2015) and the fine roots contained around 5 g N/tree. Medium roots contained around  $6 \pm 4$  g N/tree.

<sup>15</sup>N distribution

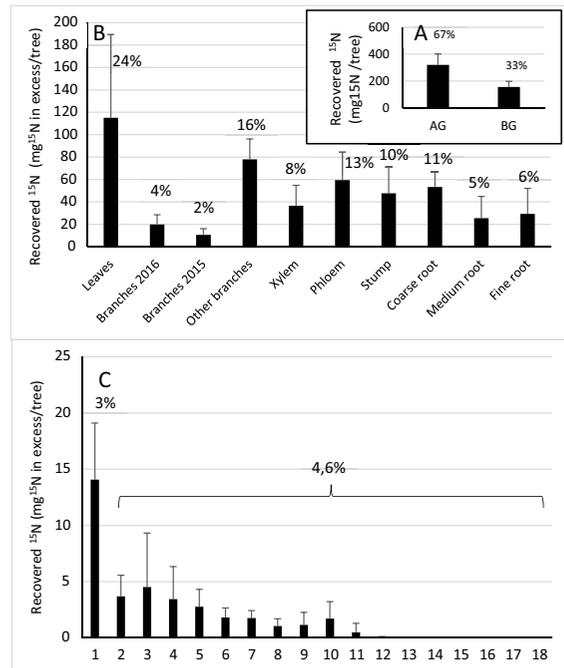
Figure 5 presents the percentage of <sup>15</sup>N in excess in the tree’s compartments. These values indicate the proportion of nitrogen coming from the soil labelling in comparison with the total nitrogen present in the compartment. The percentage of <sup>15</sup>N in excess was higher in the fine and medium roots (more than 0.4% of <sup>15</sup>N in excess) (Fig. 5A). The leaves and the newer branches (2016) are the second <sup>15</sup>N rich

compartment with values around  $0.3 \pm 0.16\%$  <sup>15</sup>N in excess (Fig. 5A). Phloem was also rich in <sup>15</sup>N with a value of  $0.27 \pm 0.13\%$ . The other compartments had lower values. In the xylem, <sup>15</sup>N in excess was found in all the sapwood (from ring 1 to ring 10) and especially in the new ring of 2016, but not in the heartwood (from ring 12 to 18). The ring 11 formed in 2006 was in the sapwood or the heartwood (dependent on the tree and location in the tree) and showed intermediate value (Fig. 5B).

The total tree <sup>15</sup>N recovered was  $475 \pm 60$  mg <sup>15</sup>N in excess which represents only 26% of the 1.82 g of <sup>15</sup>N initially applied to the soil in spring (Fig. 6A). The recovered <sup>15</sup>N was mainly found in aboveground part (67%) (Fig. 6A) especially in the leaves (24%), then in the stump and coarse root when summed (21%) followed by the trunk (20% when the phloem and xylem were summed) (Fig. 6B). The <sup>15</sup>N in excess contained in the first



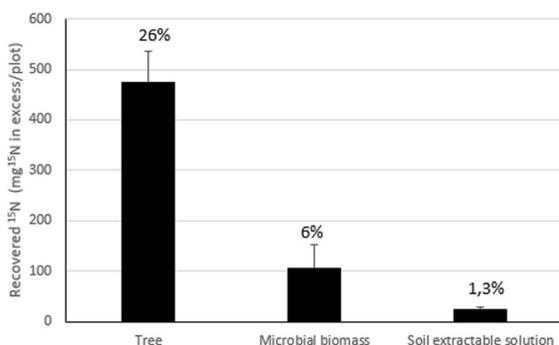
**Fig. 5** Percentage of <sup>15</sup>N in excess in sub-compartments (A) and in the 18 xylem rings (B) in %<sup>15</sup>N in excess. Rings 1 and 18 correspond to years 2016 and 1999 respectively. Values are mean and standard deviation of 5 field replicates from five trees



**Fig. 6** Quantity of recovered <sup>15</sup>N expressed in mg <sup>15</sup>N in excess/tree in aboveground (AG) and belowground (BG) compartments (A), sub-compartments (B) and in the 18 xylem rings (C). Rings 1 and 18 correspond to years 2016 and 1999 respectively. Values are mean and standard deviation of 5 field replicates from five trees. The number above the bars are the percentage of the <sup>15</sup>N in excess in the considered compartment relative to the whole tree

ring of the xylem (Fig. 6C) (i.e.  $14 \pm 5$  mg  $^{15}$  N in excess) is in the same order of magnitude as that in the branches of the current or previous years and represented around 3% of the  $^{15}$  N recovered in the tree. As shown in Fig. 6C, the first ring of the xylem accounted for 3% of the  $^{15}$  N recovery while the other rings collectively accounted for 4.6%. In relation to the tree trunk (the xylem), the first four rings of the xylem contain 70% of the  $^{15}$  N with the first ring taking 38% of it. The other rings of the sapwood (from ring 2 to ring 10) contained  $22 \pm 4$  mg  $^{15}$  N in excess that is much lesser than what we found in the stump ( $48 \pm 23$  mg  $^{15}$  N in excess), fine roots ( $29 \pm 22$  mg  $^{15}$  N in excess), medium roots ( $25 \pm 19$  mg  $^{15}$  N in excess) and coarse root ( $53 \pm 13$  mg  $^{15}$  N in excess). The less studied compartments (coarse root, stump, xylem and other branches) together recovered 45% of the  $^{15}$  N.

Out of the 1.82 g of  $^{15}$  N that was applied to the trees at the beginning of the experiment, only 0.604 g was recovered in the EP (tree, microbial biomass and the extractable soil solution) in Autumn which represented 33% of the original applied  $^{15}$  N (Fig. 7). At this point, most of the recovered  $^{15}$  N was found within the tree (79%), while only 17% and 4% were recovered in the microbial biomass and extractable soil solution, respectively.



**Fig. 7** Quantity of recovered  $^{15}$  N expressed in mg  $^{15}$  N in excess/plot in Tree, microbial biomass and soil extractable solution. Values are mean and standard deviation of 5 field replicates. The number above the bars are the percentage of the  $^{15}$  N in excess in the considered compartment relative to the  $^{15}$  N applied in spring

## Discussion

The objective of the paper was to report an exhaustive assessment of total dry matter (biomass pools), total nitrogen (N), and total  $^{15}$  N and distribution in all compartments of the trees as N flux.

### Biomass repartition

The aboveground compartment was found to contain more biomass than the belowground compartment. Similar results have previously been obtained in both deciduous species such as oak (Barbaroux et al. 2003) and evergreen species such as Scots pine (Xiao et al. 2003). While we found that 76% of biomass in the aboveground and 24% belowground, Barbaroux et al. (2003) found these values to be 87% (stem, 51%; branches, 8% and leaves, 2%), and 13% belowground. The proportion of belowground compartment biomass might have been lower in Barbaroux et al. 2003 (13%) compared to our finding (24%), because they did not consider stump (only fine roots, medium roots and coarse roots). Difference in age (20 years in our case and 45 years in Barbaroux et al. 2003) might also explain the difference observed as age-dependent preferential biomass allocation is proven to be higher in the aboveground with increasing age of the tree (Helmisaari et al. 2002; Yang et al. 2019).

The result of more biomass in the aboveground compartment than in the belowground compartment may be due to high NPP allocation to the aboveground compartment (Barbaroux et al. 2003). Biomass variation among rings was likely due to physiological processes and effects of weather and environmental conditions (Verheyden et al. 2004; Delpierre et al. 2016).

Recent advancement in biomass estimation involved the use of allometric equations that employ biomass prediction models to estimate both aboveground (Crow 1978; Cunia 1987; Brown et al. 1989; Houghton et al. 2001; Chave et al. 2001, 2014) and belowground biomass (Zianis et al. 2005) using DBH only (Ter-Mikaelian and Korzukhin 1997; Návár 2009) or DBH-h (DBH and height) equations (Wang 2006). In European species, most of the biomass equations concentrated on aboveground parts, particularly for branches and foliage with very few equations targeting belowground components (Zianis et al. 2005). Currently, for *Quercus petraea*, there is only

one allometric equation (DBH only) that can be used to estimate root biomass (Drexhage and Colin 2001). The root biomass we estimated using this (Drexhage and Colin 2001) allometric equation was close to actual (measured) biomass ( $p=0.70$ ) when tree 3 was not included ( $r^2=0.37$ , when tree 3 was included). The reason why tree 3 reduced the  $r^2$  was not well understood but we hypothesized that to be due to the lowest height attributed to tree 3 (height=8.96 m) compared to all other trees (mean height=10.22 m). We therefore suggest that for more robust allometric estimation of biomass, DBH-h equations that take into account the height of the tree should be employed.

### Nitrogen distribution

We found 68% of nitrogen in the aboveground compartment and 32% in the belowground compartment. Studies comparing aboveground and belowground nitrogen distribution in deciduous species are insufficient, but studies on evergreen species such as citrus are available and shows similar trend to our deciduous study. Tadashi et al. 1982 found 61% of nitrogen in the aboveground and 39% in the belowground, in citrus trees (*Citrus unshiu* Marc.) that were 4 and 6 years old. This similarity might be due to the similarities in the diversity of the aboveground compartments measured: Tadashi et al. (1982) studied fruits, current year leaves, current year twigs, 1-year-old leaf, 1-year-old twig, 2-year-old twigs, 3–4-year-old branches, 5–10-year-old branches, while we focused on leaves, branches of the year, branches of the previous year, other branches, phloem and xylem rings in our work. Additionally, similar belowground compartments were studied between both studies, such as the belowground stump, coarse roots, medium roots and fine roots.

Nitrogen quantities and concentration were not stable in the xylem rings but relatively decreased with the aging of the rings. More nitrogen was found in the sapwood (0.107 gN/100gDM) (from ring 1 to ring 10) than in the heartwood (0.064 gN/100gDM) (from ring 12 to 18). This is similar to the finding of Meerts (2002), who found lower nitrogen concentrations in heartwood (0.117 gN/100gDM) than in the sapwood (0.174 gN/100gDM) in 47 angiosperm species. Similarly, Härdtle et al. (2014) and McLauchlan and Craine (2011), respectively found in the heartwood

of *Quercus petraea* and *Quercus alba* around 1 mgN gDM<sup>-1</sup> and 1.2 mgN gDM<sup>-1</sup> while in the sapwood 1 to 2.2 mgN gDM<sup>-1</sup> and 2.22 mgN gDM<sup>-1</sup>. Fluctuations of nitrogen among rings were observed due to high radial mobility of xylem N within trees (Elhani et al. 2003). There was also a sharp decrease from ring 1 to ring 2 in terms of biomass and N quantity, which might be due to the accumulation of N originating from leaf resorption into ring 1, probably in the form of N reserve (Bazot et al. 2013). Our results show that most of the nitrogen was stored in perennial tissues (trunk, branches, stump and coarse root), which was likely due to the timing of the sampling. In autumn, leaf senescence occurs and nitrogen in the leaves is translocated to perennial storage components (Millard 1995; Aerts 1996; Babst and Coleman 2018). But we still found a large proportion of nitrogen in the leaves (14%) at this date. That could be explained by the delay between the onset of protein degradation and the efficiency of the nitrogen export (Sample and Babst 2019). Nitrogen compounds resulting from the leaf protein degradation could be stored in the vacuole (Sample and Babst 2019) before being withdrawn to vegetative storage components for use in the spring and summer. We estimated that at the sampling date (DOY 287, October 13), the trees were at the mid-period of leaf senescence as the leaf nitrogen percentage was 1.58% at this date, 2.21% at LAI max (data not shown), and the %N in the soil litter around 1% during November (Nicolas Delpierre, personal communication). Following Du et al. (2021) and Vergutz et al. (2012) we calculated the nitrogen resorption efficiency at the sampling date to be 44%. The maximum resorption efficiency was 64% when calculated with the % of N in the soil litter. A similar value was obtained on *Quercus serrata* by Migita et al. (2007).

### <sup>15</sup>N distribution

After the soil labelling at budburst, the <sup>15</sup>N was analyzed in the different tree compartments at the end of the vegetation season in autumn. We found that 67% of the recovered <sup>15</sup>N within the tree was found in the aboveground compartment, while 33% was found belowground. Zeller et al. (2001) traced a 3 year <sup>15</sup>N labelled litter decomposition in *Fagus sylvatica* and found 61.1% of recovered <sup>15</sup>N in the aerial biomass, with 38.8% in the root biomass.

According to El-Zein et al. (2011a) from April 10 (DOY 100), i.e. before bud burst, to May 7 (DOY 166), when bud burst was completed, leaves attracted most of the applied  $^{15}\text{N}$  (up to 68% in oak), which was lower than the 14% we found the difference might be due to short sampling-labelling time frame such that if sampling would be repeated after relatively longer period, higher values might be recorded. Difference in age of the trees (45 years in El-Zein et al. (2011a, b) and 20 years in our study) might also be a reason for the variation with our finding because translocation of N, P and K were shown to increase with tree age in relation to enlarged stem wood biomass (Colin-Belgrand et al. 1996). Another explanation could be the high turnover of leaf nitrogen over time to maintain the integrity of some enzymes like Rubisco and thus the probable dilution of the  $^{15}\text{N}$  mobile pool of leaves with unlabeled N. 12% of  $^{15}\text{N}$  was present in phloem and 8% in the xylem which may be an indication of concomitant resorption (through phloem) and soil nitrogen uptake (through xylem). This is supported by Amponsah et al. (2004); Jordan et al. (2012) and Bazot et al. (2016) which shows the occurrence of autumnal N uptake in Pine, Peach and Oak trees respectively.

We found  $^{15}\text{N}$  in all the sapwood rings, with increased concentrations in the newest ring, but not in the heartwood ring, probably due to phloem-xylem transfer via the symplast of horizontal ray parenchyma cells (Pfausch et al. 2015). Bidirectional nitrogen exchange between phloem and xylem has been experimentally proven in Norway spruce trees (*Picea abies* [L.] Karst; Geßler et al. 2003). That also could be explained by the fact that the latest ring was built during the year 2016, with the  $^{15}\text{N}$  coming from the labeled soil through sap flow that was particularly high in the ring of the current year (Granier et al. 1994).

The origin of the  $^{15}\text{N}$  in other rings is undetermined. They may come from the sap flow via the non-embolised tiny vessels of the sapwood (Čermák et al. 1992), through all the most recent and most ancient rings, or from inter-rings transfer during the growing season or during the resorption process. Additionally, it cannot be excluded that the evaporation of the sap-flow during the freeze-drying process has left a residual  $^{15}\text{N}$  in the sap tissues. Hart and Classen (2003), studying  $^{15}\text{N}$  trends in rings 14 years after labeling, found that the  $^{15}\text{N}$  value of rings was a maximum

one year after the labeling and also that the  $^{15}\text{N}$  value decreases in the most recent rings. While suggesting nitrogen transfer from older to newer rings, their study reported no  $^{15}\text{N}$  transfer to older rings. Our result also shows that no nitrogen from N absorption has been transported to the heartwood.

The global  $^{15}\text{N}$  budget repartition between the tree and soil microbial biomass showed that still 18% of the  $^{15}\text{N}$  retrieved (6% of the initial labeling) was located in the microbial biomass at the end of the growing season, confirming the role of the microbial biomass as nitrogen sink in the forest (Rennenberg and Dannenmann 2015; Bazot et al. 2016; Maxwell et al. 2020). The small percentage of  $^{15}\text{N}$  retrieved (33%) indicates that other fluxes (such as leaching, denitrification and incorporation into the understorey vegetation) should be measured to obtain a more exhaustive budget. Volatilization was also possible since the soil was not covered after  $^{15}\text{N}$  labelling as in El-Zein et al. (2011a) for example.

However, in addition to all the physiological and biochemical processes occurring within the tree, possible isotopic exchange between the N pools would be a possible limitation of this study.

## Conclusion

This study has enriched the literature by providing detailed information on the contribution of oak tree compartments to biomass and nitrogen content. The study confirmed the importance of less commonly studied compartments as nitrogen sinks.

While we found  $^{15}\text{N}$  in all the sapwood and at variable proportions in the xylem rings, the transport mechanism of the  $^{15}\text{N}$  cannot be fully explained, i.e. to distinguish the source of the  $^{15}\text{N}$ : whether from the soil via uptake or resorption from leaves to trunk and/or mobility between rings. Further research is needed to better understand this processes especially because N isotopic fractionation associated with uptake and translocation has been shown to affect  $^{15}\text{N}$  variations in tree rings (Poulson et al. 1995).

Out of the 1.82 g of  $^{15}\text{N}$  that was applied to the trees at the beginning of the experiment, only 0.604 g (33% of original quantity applied) was recovered in the trees; there is therefore a need for further research

to identify the lost  $^{15}\text{N}$  through leaching, denitrification and/or volatilization.

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#### Declarations

**Conflict of interest** The authors declare that they have no conflict of interest.

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