Referee # 1:

General comments

This paper presents a study examining N sources for seasonal growth in Quercus petrea. 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 that15N 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).

L143-147 : 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.

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:

L255-260: "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. "

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 L262-285.

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.

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. Leaves / twigs were pooled, ground in fine powder and analyzed (15N and %N). At few dates several aliquots were analyzed to check the repeatability of the analyzes. The results show a good repeatability: for example, this table shows the values obtained for some repeated analysis. For these samples an average is made between the two replicates.

	Tree	DAL	%N	$d^{15}N$
Leaves	L1	1	2,14	6547,43
Leaves	L1	1	1,81	6429,00
Leaves	L2	1	1,99	5400,31
Leaves	L2	1	1,85	5477,90
Leaves	L1	126	1,43	1770,64
Leaves	L1	126	1,28	1425,89
Roots	L1	126	0,88	1325,73
Roots	L1	126	0,66	1153,65
Roots	L2	126	0,79	1256,25
Roots	L2	126	0,81	1393,10
Twigs	L1	318	1,34	2215,44
Twigs	L1	318	1,24	2361,02
Leaves	L1	337	3,94	1500,32
Leaves	L1	337	3,89	1625,35

This type of replicate could not be done at all dates due to the excessive number of sample that would have generated.

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.

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 of sampling after labelling for each compartment and each labelled tree.
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.

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: L110-118: "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 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."

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, we have, at few dates, checked the repeatability of the analysis by analyzing two aliquots of a compartment. See table above.

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 : L130-139: "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".

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.

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.

L77 – how big were the trenched areas?

Response: It was mentioned L80: 5m² in average

What was the spacing of the trees?

Response: At least 20m, see L81.

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.

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.

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: L94-98: "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".

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: L121-129 : "All plant tissues and soil samples were brought to the laboratory in a cooler, frozen, lyophilized and ground to a fine powder with a ball mill before analyses. For analyses, all sampled of each compartments were pooled. An aliquot of each powder (1 mg) was transferred into tin capsules (Elemental

Microanalysis, UK, 6 x 4 mm, ref. D1006, BN/139877). Total N concentration of plant and soil samples, was analysed by dry combustion using an N auto-analyser (Flash EA 1112 series, Thermofinnigan). ^{15}N abundance was quantified in the same plant and soil fine powder aliquots with a mass spectrometer (PDZ Europa, University of Davis, Isotopes Facility, California)".

L121 – I feel that something is needed here to justify this approach rather than having a concurrent control unlabeled set of trees.
Response: L143-147: "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".

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. L165-168.

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).

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 L273-275.

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.

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).

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%), which confirms the limitation of N soil uptake during winter. L338-343: "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".

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.

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.

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.

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.

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.