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Interactive comment on "Contribution of previous year's leaf N and soil N uptake to current year's leaf growth in sessile oak" *by* Stephane Bazot et al.

Anonymous Referee #1

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General comments

This paper presents a study examining N sources for seasonal growth in *Quercus petrea*. Experimental measurements of N cycling phenology is limited and the manuscript represents a valuable addition to the literature. The author use a clever application of ^{15}N 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

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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 ^{15}N recovery is calculated using a pre-experiment baseline without any consideration as to whether background ^{15}N content may change over time. 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. 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. 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. 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 ^{15}N recovery seem similar there are no error bars representing measurement uncertainty nor clear indication of how many points are on the lines. Given that there are only 15 points per series (Table 1), could these be shown on the graph to indicate periods where ^{15}N content is inferred by a fitted line rather than a measurement?

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? Were samples taken randomly and from all trees at all dates? How were the phloem and xylem sampled? How were twigs and leaves selected? Were multiple replicates taken at each time,

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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?

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 ^{15}N abundance' (l118, 0.3M). It is not clear to me if this former is a 'control' unfumigated treatment and the latter is the ^{15}N-fumigation treatment, or if a control (unfumigated) ^{15}N 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, ^{15}N extracts from low [N]/[^{15}N] 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, were ^{15}N 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.

Specific comments

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

L74 – nitrogen deposition, if known, might be useful to include here as this study con-

cerns N additions. High soil N availability may affect the origin of N for growth.

L77 – how big were the trenched areas? What was the spacing of the trees?

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.

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?

L92 – how long was the plastic tarpaulin in place? Was this long enough to prevent losses from leaf leaching (l237) from reaching the soil?

L110 – were these ground by hand, or in a mill? Were the samples dried, e.g. in an oven, before this?

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

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.

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?

L241 – Maybe this needs a little more elaboration. Allocation of ^{15}N 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 ^{15}N is going.

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

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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 root ^{15}N 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.

L304-305 – With no indication of uncertainty, it is rather speculative to interpret differences this small as real changes!

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.

L344 – a reference for cessation of glutamine synthetase activity would be useful.

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.

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 ^{15}N sprayed", and "Budburst" rows necessary, given that it is the same in all treatments? Budburst could instead be indicated by an entry in the

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table.

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.

Technical corrections

L27 – the sentence 'the literature describes. . .' is unnecessary.

L75 – include authority with species name

L89 – 'on' April 20, or 'by' April 20?

L106 – the 'leaf mass area' (LMA) should be 'leaf mass per area'.

L124 – is this the same six trees as measured?

L129 – (PRN) would be easier to interpret if it immediately follows 'proportion of recovered 15Nitrogen'

L144 – The sentence 'That why results was expressed as the mean of both trees' is poor English and should read 'That is why results were. . .'

L 147 – 'leafy season' - > 'growing season'?

L232 – Substantial fraction is ambiguous

L238 – the lack of contamination could be supported by referring to figure 1d.

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.

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L309 – this sentence is very long – could it be split up?

References – numerous cases where super- or subscript is not used in reference list (e.g. line 412 ‘15N’)

Figure legends: remove ‘the’ from ‘the tree 1’ and ‘the tree 2’.

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?

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