Response to Anonymous Referee #2

Comment: This manuscript reports patterns in isotopic discrimination between leaf and heterotrophic bulk tissue after one growing season in two desert sites in China. The authors found interesting relationships between the discrimination and nitrogen concentration (N) of the heterotrophic tissues, thus hypothesizing that resource allocation is partly driving discrimination for this species. The sampling design is sufficient and the methods and analysis are relatively straightforward. The article is clear and well written and the topic is relevant to readers of Biogeosciences.
Response: *Many thanks for this reviewer's support of our study and insightful suggestions. We have revised the manuscript substantially by taking all comments made by this reviewer into consideration. As a result, four new paragraphs and a new figure have been added.*

Comment: Main comments: What I am primarily concerned about is the presentation and interpretation of the results. In its present form, I find the article is short enough so that a further discussion of site differences could be explored more and the review and justification of N as a driver of isotope enrichment/fractionation could be better explained.
Response: *In response to this comment, we have added a discussion of site differences in vegetation and soil nutrients and their implications on the different degrees of heterotrophic enrichment of 13C at the two study sites. See lines 490-507.*

Comment: One of the main findings not discussed in depth is the difference in shrub biomass between the two sites. Plants within the Dengkou site were significantly larger than the Minqin site and thus the growth rate could also be greater. This is important because of the relationship between growth rate and N:P ratio, albeit the generality of this phenomena is still an active area of research (Niklas et al., 2005 Ecology Letters; Elser et al., 2010 New Phytologist). This highlights a further weakness in the analysis. The relationship depicted between N and N:P in Figure 5 (middle row) is an important basis for the paper, I would argue. One can see that the patterns are largely driven by nitrogen, as mentioned by the authors, but I think it is clear that spread in the data are more or less differences in site, rather than differences in organ N concentration. This is also reflected in the root length differences, the smaller plants from the Minqin site also have longer roots, perhaps because they are mining for nutrients and/or water. To their credit, the authors mention the correlative nature of their findings; however, I find the relationship between N and 13C enrichment rather speculative and needs further bolstering. Perhaps there are data available on site nutrient availabilities? These data might add more to the discussion.
Response: *Thanks for the sharp reading and insightful suggestions. In response to these suggestions, three paragraphs and a new figure have been added in revision (lines 490-524 and Fig.S2). Literature review has been also strengthened to support the interpretation of our observational results and the conclusion drawn. We added information on soil nitrogen (N) contents at the two sites (from previously published sources) in the discussion. Soil phosphorus (P) contents have not been measured at either site. Consistent with the reviewer's suggestion, available soil N data do show that the Dengkou site was more fertile than the Minqin site. This is also consistent with the difference in biomass (particularly fine roots) N contents between the two sites. Although we don't have information on soil P contents at the two sites, we suspect that soils at the Dengkou site contained higher P than the Minqin site did as higher P contents were found in the roots at the former than at the latter site. Of course, these differences in site*

fertility likely interacted with the difference in water availability in controlling the growth of vegetation at the two sites (Minqin was drier than Dengkou). So it would be very difficult to tease out the impacts of nutrients vs. water on differential vegetation growth.

We are really intrigued by the reviewer's suggestion that the theoretical relationship between the relative growth rate of plants and the N/P stoichiometry advocated by Niklas et al. (2005) and Elser et al. (2010) *might have implications for heterotrophic 13C enrichment compared with leaves. The Minqin site had lower N/P ratios in plant organs and also lower vegetation stature than the Dengkou site did, which seems not in agreement with predictions by the model of Niklas et al. (2005) (see their Figure 4). It may be that the difference in levels of water limitation at the two sites confounded the effect of N/P ratio on plant growth. Regardless how N/P ratio is related to plant growth, it would be difficult to speculate how differences in N/P ratio affect the heterotrophic 13C enrichment via its effect on (relative) growth rate of plants.*

We agree with the reviewer that the between-site differences are at least as large as the within-organ variations at the same site in terms of heterotrophic 13C enrichment and organ nutrient contents and stoichiometric ratios. But even within the same site, the effect of heterotrophic organ N on this organ's 13C enrichment over the corresponding leaves can be clearly seen. The fact that the patterns of the two sites line up nicely when they are put together further gives us confidence in our conclusion that N is a key factor in heterotrophic 13C enrichment. Of course, all we have shown is just a correlation. It would be nice to conduct controlled nutrient and water experiments to derive definitive cause-effect relationships.

Comment: If the authors can reconcile the issues mentioned above, then I think the development of N as a driver of 13C enrichment of heterotrophic tissues needs to be further developed. The review of the literature on post-photosynthetic discrimination was fine, but I think a hypothesis based on plant physiology or allocation is needed to move this issue and research forward – for example, explain why an increase in N will lead to an increase in respiratory CO_2 refixation. A small paragraph in the introduction on the role of N in 13C enrichment of heterotrophic tissues or even in leaf isotopic discrimination (e.g., Cernusack et al., 2007) might be helpful in preparing readers for the discussion later.

Response: *Many thanks for these suggestions. There have been a number of studies on the relationship between leaf nitrogen concentration and leaf carbon isotope discrimination. Therefore we have followed the reviewer's suggestion and added a small paragraph in the introduction to discuss these studies. We have not found any previous study that focused on how N affects 13 enrichment of heterotrophic tissues. In addition to adding a paragraph in the introduction section, we have also revised the discussion section so that previous studies on the relationship between organ N and respiratory CO_2 refixation are discussed more clearly. See lines 110 – 125, lines 490 – 524 and Fig. S2.*

Response to Anonymous Referee #1

Comment: The paper describes a careful study where the authors related the differences in the carbon isotope composition between leaves and heterotrophic tissues to nitrogen, phosphorus and carbon concentrations (and ratios between these elements). The aim of the work was to explore if the generally well-known 13C enrichment of heterotrophic vs. autotrophic tissues is driven by nutrients. The authors found a clear relationship between the 13C enrichment on the one hand and C/N and N/P ratios as well as N contents of heterotrophic tissues on the other. When the tissue N content was normalized against leaf N the relationship did not improve (but was more or less comparable). From these findings the author conclude that the relationship between tissue N content and 13C enrichment is due to processes in heterotrophic tissues rather than in the leaves or related to leaf export. As a main candidate for such a process the authors propose PEPC activity, which has been shown to be related to N content. The manuscript is very well written and the structure and the story line are very clear and the finding is novel and interesting for the isotope community. The methodologies applied are adequate and the paper fits well into the scope of Biogeosciences. There are a few minors things I feel the authors should address before the manuscript can be published in BG:
Response: *We appreciate this reviewer's support and encouragement of our study. We have adopted all the suggestions made by this reviewer in our revision*.

Comment: The PEPC hypothesis: The authors follow a clear line of argumentation and they give a reference (Berveiller et al. 2010) to support their assumption that PEPC activity increases with N. However, it is still speculation - which I like - and it is not based on measurements of PEPC activity. This speculative nature of the conclusions gets fully clear in the discussion but I feel the authors should be more carefully in their wording in the abstract - "probably" sounds to strong without having the background from the discussion.
Response: *Thanks for pointing this out. We totally agree with this reviewer's assessment. In revision, we used 'hypothesized to be' to replace 'probably' in the abstract and also relevant texts in the main body.*

Comment: Fractionation during phloem loading: It is generally assumed that phloem loading itself is not causing fractionation but that rather the unreacted sugars loaded into the phloem are 13C enriched compared to the primary assimilates (because the non-exported (structural) compounds in the leaves are 13C depleted) (cf. Hobbie and Werner 2004). The same might happen associated with phloem transport – Continuous unloading of sucrose from the phloem, metabolic conversion of part of the sucrose and reloading of the rest. Lignin and other substances produced become 13C depleted (kinetic and equilibrium isotope effects) and the retrieved sugars thus 13C enriched (e.g. Gessler et al. 2014); this point might need clarification in the text.
Response: *We are grateful to this reviewer for this detailed and clear explanation of the relationship between 13C enrichment/depletion and phloem loading/unloading. We have revised relevant texts to reflect the correct understanding of this relationship*.