Biogeosciences Discuss.,
doi:10.5194/bg-2016-231-AC1, 2016

Biogeosciences Discussions

Interactive comment on "The interaction between nitrogen and phosphorous is a strong predictor of intra-plant variation in nitrogen isotope composition in a desert species" *by* J. Zhang et al.

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Received and published: 18 August 2016

General comments: In this manuscript, Zhang et al. report new measurements of intra-plant variation in 15N, and discuss mechanisms that might be responsible for the observed patterns. Overall, the measurements provide a useful characterization of a previously little-studied species and ecosystem type, and the discussion provides a nice overview of some possible mechanisms. Response: Thanks for a careful review and constructive comments.

Comments: However, the attribution of particular mechanisms to the observed patterns is entirely speculative, and does not convincingly advance current understanding of controls on variation in 15N. Several suggestions to strengthen the manuscript are

made below. Response: It would be fantastic if a single paper can not only report a previously un-reported natural phenomenon but also provide a convincing explanation of the mechanism responsible for the observed phenomenon. Unfortunately most of the time science does not advance this way. Instead, scientists have to crawl along the path of research – discovery of phenomenon – proposal of hypothesis – research to test hypothesis – more research – more research . . .. . . finally convincing explanation. There is no short cut. In the revision, we have made sure we present our attribution of potential mechanisms as testable hypotheses for future research to tackle.

Specific comments: [1] Background. It would be helpful for the introduction and discussion to include more information that is specific to the nitrogen cycle in the study systems. In particular, key questions to address are: What are the sources of nitrogen for the study species? How does _15N vary among those N sources? Critically, is Nitraria tangutorum a nitrogen-fixer? The clustering of the _15N values in this study close to 0 per mille, and the fairly high tissue nitrogen concentrations, both suggest that Nitraria either supports nitrogen-fixing symbionts itself, or is obtaining nitrogen from another nearby fixer that occurs in the community. This would have important implications for interpreting intra-plant variation in _15N. Response: Nitrogen cycling in this remote desert region of China has rarely been studied. But it appears N. tangutorum is not a nitrogen fixer as we carefully examined the structures and morphology of excavated fine roots and did not observe any obvious nitrogen fixing features. There was a single conference report on the observation of the presence of endogenous nitrogen-fixing bacteria in N. schoberi. We have revised the site description to include these background information.

Comment: [2] Statistical methods. The authors have used an approach based on fixed effect models which indicates that the interaction between tissue N and P concentrations is the strongest predictor of variation in _15N. However, it is not clear that the fixed effect approach is appropriate here, so this statistical result may not be reliable. Since the samples were collected in a hierarchical sampling design (i.e., different organs

nested within the same nebka; different nebkas nested within the same site; multiple sites, etc.), it seems like analysis with mixed effect models would be a more appropriate way of testing for the best predictors of variation in _15N. Suggest either repeating the analysis with mixed effect models, or clearly justifying why fixed effect models were applied. Response: Thanks for this interesting comment. We believe most regressional models in natural science should be fixed effects models because natural scientists are generally interested in causal effects. We believe the fixed effects model is the correct model to be used here. This is because we are only interested in the detection of the existence or absence of any potential correlation between the specific effect (nitrogen isotope composition) and independent variables (N and P contents) across plant organs and we are not interested in how any peculiarities of nebkas and locations might or might not affect the specific effect. The only purpose of including multiple nebkas and locations is to increase the power of statistical analyses (i.e., to avoid using data from a single nebka at a single location and getting a spurious correlation). Furthermore, we are not interested in making inferences outside the observed dataset. For all these reasons, the use of a fixed effects model with stepwise regression is the correct option. We have added this explanation in the revision.

Comment: [3] Interpretation of statistical results. In lines 355-356, the authors state, "To our knowledge, no previous studies have systematically evaluated relationships between intraplant variations in _15N and organ N or P contents." In fact, some previous work has addressed these relationships. One analysis that is particularly relevant is Kalcsits, Lee A., Hannah A. Buschhaus, and Robert D. Guy. 2014. "Nitrogen isotope discrimination as an integrated measure of nitrogen fluxes, assimilation and allocation in plants." Physiologia Plantarum 151: 293-304. Since this is a fairly recent paper and was not cited by the authors, they may not be aware of it. However, the theory developed by Kalcits et al. has the potential to be quite helpful as a foundation for interpreting the Nitraria results reported here. Suggest reviewing this reference, and incorporating it into the discussion. Response: Thanks for pointing out to us the paper by Dr. Lee Kalcits and his collaborators. Indeed this paper and some other papers by

Dr. Kalcits are very relevant to our study. We have revised our discussion in view of findings by Dr. Kalcits.

Technical comments: Comment: [a] In the methods, suggest including details of digestions used to prepare samples for ICP-OES analysis. Response: Suggestion adopted.

Comment: [b] Both in the methods and in the figure legends, suggest specifying whether these are molar or mass ratios (i.e., C/N, N/P, C/P). Response: Suggestion adopted.

Comment: [c] Figure 2, Difficult to focus on plotted data because ANOVA codes are so large. Suggest shrinking size of font used for ANOVA codes to improve readability. Response: Suggestion adopted.

Comment: [d] Figure 5, Seems redundant. Perhaps the information here could be somehow combined with Fig. 3. Response: If Figure 5 is combined with Fig. 3, the resulted figure would be very complicated. It is probably better to keep them separated.

Interactive comment on Biogeosciences Discuss., doi:10.5194/bg-2016-231, 2016.