

## ***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.***

**Anonymous Referee #1**

Received and published: 11 July 2016

General comments: In this manuscript, Zhang et al. report new measurements of intra-plant variation in  $\delta^{15}\text{N}$ , 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. 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  $\delta^{15}\text{N}$ . Several suggestions to strengthen the manuscript are made below.

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

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systems. In particular, key questions to address are: What are the sources of nitrogen for the study species? How does  $\delta^{15}\text{N}$  vary among those N sources? Critically, is *Nitraria tangutorum* a nitrogen-fixer? The clustering of the  $\delta^{15}\text{N}$  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  $\delta^{15}\text{N}$ .

[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  $\delta^{15}\text{N}$ . 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 nebkha; different nebkhas 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  $\delta^{15}\text{N}$ . Suggest either repeating the analysis with mixed effect models, or clearly justifying why fixed effect models were applied.

[3] Interpretation of statistical results. In lines 355-356, the authors state, "To our knowledge, no previous studies have systematically evaluated relationships between intra-plant variations in  $\delta^{15}\text{N}$  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 Kalcsits 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.

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Technical comments:

[a] In the methods, suggest including details of digestions used to prepare samples for ICP-OES analysis.

[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).

[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.

[d] Figure 5, Seems redundant. Perhaps the information here could be somehow combined with Fig. 3.

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