

## Letter from Editor with Reviews of Initial Submission

Dear Prof McGlynn,

Your manuscript has now been peer reviewed and the comments are accessible in PDF format from the links below. Do let us know if you have any problems opening the files.

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We would be grateful if you could address the comments in a revised manuscript and provide a cover letter giving a point-by-point response to the concerns.

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We look forward to receiving your revised manuscript by 23 October 2009. If you imagine that it will take longer to prepare please give us some estimate of when we can expect it.

Please don't hesitate to contact me if you have any problems or questions regarding your manuscript.

With best wishes,

xxxxxxxxxxxxxxxxxxxxx  
Scientific Editor

## Reviewer One, Initial Submission

**Date:** 22 September 2009

**Reviewer number:** 1

### **Reviewer's report:**

The authors aim to describe landscape-scale variation in stable isotopes and trophic position of gypsy ants (*Aphaenogaster araneoides*). They performed a sampling of seven areas distributed across a landscape of 500 ha and sampled colonies of ants and leaf litter (baseline) for carbon and nitrogen stable isotope analysis. They provide two different results: A correlation between  $\delta^{15}\text{N}$  of ants and leaf litter and total phosphorus in the soil, and a multiple regression model relating the ant's 'trophic position' and different predictor variables describing the soil and ant colonies. The authors seem to have results that are certainly interesting and merit publication in a scientific journal. However, from my point of view, the paper in its present state lacks acceptable description of the results and needs major changes before it can be accepted for publication.

- Major Compulsory Revisions

1. The first result aims to provide a description of the main factors affecting d15N variability in the soil, but the authors explore only a single parameter, total phosphorus (TP). Although TP might be certainly the main reason for d15N variability, they should show (at least!) a correlation matrix with other soil parameters, such as other elements and their ratios, decomposition rates, etc. Then if TP is the only one with such good correlation they can claim it is the driving of the d15N variability. They should also do the same for d13C. In a parallel study (McGlynn et al., 2009), the same authors describe these different parameters sampled from the soil. Therefore, it is surprising and intriguing why they do not use them at all. In addition, what is the hypothesis for the relationship between TP and C and N stable isotopes? This is a bit confusing at the moment, especially at the discussion.

2. The second result is related with the so called 'trophic position' of the ants. Provided that ants are being described as generalists, they sampled leaf litter as a trophic baseline and calculate the difference between ants and the baseline (termed 'trophic position' at the moment). Trophic position is usually calculated in multiple-species food web studies, in order to account for changing feeding habits of predators under the hypothesis that if a predator changes its prey from, for example, a first order consumer to a second order consumer, its d15N will increase due to the increase of d15N in each trophic step. This is only valid if one assumes that the discrimination factor (increase of d15N from diet to consumer's tissue) is constant, or most likely its changes are smaller than the shift due to diet change. In their case, they expect a discrimination of 3.4 and find a discrimination factor ranging from 2.99 to 3.51. This would indicate that their ants potentially feed on leaf litter as nitrogen source, and that the variability in d15N is low and likely related with physiological changes in d15N fractionation. The factors found to explain the variability in d15N and d13C discrimination also point to this hypothesis. But in any case they report any convincing evidence that the changes in discrimination factor are due to a change in the trophic position (e.g. feeding habits) of their ants. Therefore I suggest they change the term trophic position by the more conservative 'discrimination factor'. The authors are recommended to revise recent review publications on the subject (e.g. Martínez del Rio et al., 2009). This would imply changing the title and part of the introduction. A second requirement is related with the variability in d13C discrimination. They do not provide any value of it, other than d13C ranges from -27.73 to -27.09!!! But what is the discrimination factor for d13C? 0.01, 0.1, 1, 10???

- Minor Essential Revisions

3. Introduction. Change to factors affecting discrimination factors. 4. Results. First line should be part of the discussion.

5. Page6. First line. 'mean trophic position' and the rest of the text, replace by d15N discrimination factor. Include results of d13C discrimination factor, as well.

6. Discussion. It needs rewriting and clarification of concepts in general. For example, the 3rd paragraph, page 7. It is very speculative. They should contrast their results with the relatively vast literature on factors affecting  $\delta^{13}\text{C}$  discrimination (fractionation on some papers).

7. Methods. Absolute trophic position is not used in the paper, remove its description from methods.

- Discretionary Revisions

8. The spatial variability might not be fully described with only seven points. Perhaps they could consider not mentioning in the title as one of the main results of the paper.

#### References

Martínez Del Rio, C.M., Wolf, N., Carleton, S.A. & Gannes, L.Z. (2009) Isotopic ecology ten years after a call for more laboratory experiments. *Biological Reviews*, 84, 91-111.

Mcglynn, T.P., Fawcett, R.M. & Clark, D.A. (2009) Litter Biomass and Nutrient Determinants of Ant Density, Nest Size, and Growth in a Costa Rican Tropical Wet Forest. *Biotropica*, 41, 234-240.

**Level of interest:** An article of limited interest

**Quality of written English:** Acceptable

**Statistical review:** Yes, and I have assessed the statistics in my report.

#### Reviewer Two, Initial Submission

**Date:** 30 September 2009

**Reviewer number:** 2

#### **Reviewer's report:**

In this study the authors quantify sources of isotopic variation in a tropical ant. The manuscript focuses heavily on stable isotopes as a tool for understanding trophic position, but the authors provide a nice background on the method and on the study system. Moreover, the manuscript reports some interesting results, particularly those relating spatial heterogeneity in soil nutrients to variation in ant trophic position as measured by stable isotopes. This result seems an important addition to the list of other caveats for applying stable isotopes to food webs.

#### Major Compulsory Revisions

The main limitation of the current study is that, although there are some pretty clear effects on ant isotopic signatures, the causal mechanisms driving such variation are not abundantly clear. For example is the gradient in soil P also a gradient in leaf litter and detritivore/decomposer community composition? Or does the change in ant  $^{15}\text{N}$  stem from innate differences in consumer metabolism over a range of P concentrations? The discussion only briefly

touches on this issue. Ideally some measures of leaf litter, decomposer, and detritivore composition could be presented. At least a more detailed discussion is needed describing what is known about how the community changes and the significance of colony size and growth on ant foraging.

I was also struck by the lack of discussion of some of the results. In particular it is interesting that the authors found no effect of other ants on the isotopic signatures of *A. araneoides*. Does this result make sense to the in light of what is known about species interactions in this system? Or do they attribute this result to stable isotopes being too crude to pick up diet shifts?

Finally, Tables 1 and 2 present several variables related to ant C and N isotopic ratios, yet values for these variables are not presented anywhere. Ideally some of this information could be shown, at least the significant variables, to help the reader interpret more easily the results. Is this data being analyzed and included in another manuscript? Perhaps it could be included as an appendix.

Minor Essential Revisions -pp. 5 and 6: units (‰) are needed for C and N delta values. -p. 6, lines 4-5: These values are for the ants or leaf litter? -p. 8, lines 5-7: I'm not sure you can say this without explicit isotopic measures on a wide range of potential food sources.

-p. 9: some ecologists may consider the 3 isotopic measures at the colony scale pseudoreplicated

-p. 10, last sentences: There seem to be two conflicting metrics of trophic position. Is the second presented anywhere?

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

## Cover letter accompanying first revision

Date: 5 October 2009

Dr. XXXXXXXXXXXX:

As requested, I have uploaded a manuscript now entitled, "Spurious and functional correlates of the isotopic composition of a generalist across a tropical rainforest landscape" to be evaluated for BMC Ecology.

I have been able to accommodate the concerns of both reviewers, resulting in an improved manuscript.

The first major concern of Reviewer 1 was the focus on phosphorus to the

exclusion of other potential limiting nutrients. As requested, I provided a correlation table between the isotope ratios of the ants and all nutrients for which we have values, indicating that the association between phosphorus and nitrogen isotope discrimination is absent for all other nutrients. This table provides validation for the model selection procedure. Because the emphasis on phosphorus was designed a priori given the known role of phosphorus, I have made the rationale for this emphasis more overt in the introduction. Note that it is not statistically valid to conduct multiple regressions using all available nutrients given the number of nutrients and localities in the study.

The second major concern of Reviewer 1 was use of N stable isotopes to infer relative trophic position. I agree with the cautions against overinterpretation, and have complied with the request to discuss differences in N and C stable isotope enrichment among sites merely as  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  discrimination factors. As a consequence, I have altered text along these lines, including changes in interpretation and an alteration in the title.

I have made all the minor revisions prescribed by Reviewer 1.

Reviewer 2 requested more discussion of the relationship of soil phosphorus with other forest properties. I have included more specific information in the introduction about the relationship between soil P and the density of arthropods in leaf litter. Further discussion about potential mechanisms that may explain whether phosphorus limitation changes metabolic processes that alter the rate of N fractionation is too speculative in my opinion, though the present paper indicates further work in this direction.

The changes that I made in the discussion in response to Reviewer 1 should also satisfy the concerns of Reviewer 2, about comparing competing hypotheses about isotopic discrimination factors and dietary choices of ants. Reviewer 2 indicated that the isotopic values for the ants be provided in the manuscript. I have presented the means and ranges in the text and tables, and the individual values are found within the figures. If the individual variates for each site are this important, I could prepare a supplementary data file, though it would be atypical for a dataset and manuscript of this scope.

Last, Reviewer 2 indicated that sampling three colonies per site might be construed as pseudoreplication. I agree that using multiple colonies per site, and only one nutrient value per site, would be pseudoreplication and I would not use such an approach. As indicated at the end of this paragraph in the methods, all analyses only include the mean values of colonies at each site, with only a single value per site for each variable, indicating that the unit of measurement and statistical comparison is equivalent to the unit of independence and that the degrees of freedom in the analyses are appropriate.

I have made all the minor changes to the manuscript recommended by Reviewer 2.

If any additional alterations to the manuscript are required, please let me know.

Sincerely,  
[submitted electronically]  
Terry McGlynn

## Reviews of First Resubmission

### Response from Editor from First Revision

Dear Prof McGlynn,

Your manuscript has now been peer reviewed and the comments are accessible in PDF format from the links below. Do let us know if you have any problems opening the files. [redacted]

We would be grateful if you could address the comments in a revised manuscript and provide a cover letter giving a point-by-point response to the concerns.

Please also ensure that your revised manuscript conforms to the journal style ([http://www.biomedcentral.com/info/fora/biology\\_journals](http://www.biomedcentral.com/info/fora/biology_journals) ). It is important that your files are correctly formatted.

We look forward to receiving your revised manuscript by 23 October 2009. If you imagine that it will take longer to prepare please give us some estimate of when we can expect it.

You should upload your cover letter and revised manuscript through xxxxxxxxx. You will find more detailed instructions at the base of this email.

Please don't hesitate to contact me if you have any problems or questions regarding your manuscript.

With best wishes,  
xxxxxxxxxxxxxxxxxxxxxx  
Scientific Editor  
BioMed Central

## Reviewer One, First Resubmission

**Date:** 28 October 2009

**Reviewer number:** 1

### **Reviewer's report:**

The authors have responded satisfactorily all the concerns found in the previous version of the manuscript. Therefore I recommend this manuscript for publication in BMC Ecology

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

## Reviewer Two, First Resubmission

**Date:** 3 November 2009

**Reviewer number:** 2

### **Reviewer's report:**

The authors convincingly show two main results relating to the isotopic signature of a generalist tropical ant, namely 1) ant N isotopic ratios depend largely on leaf litter N isotopic ratios that vary over relatively small spatial scales, and 2) C and N isotope fractionation rates are tied to a select few biotic and abiotic conditions. These results are notable, particularly the first, which provides an important caveat to the ad hoc application of stable isotope analysis to terrestrial food webs. The revised manuscript improves upon the original by including additional detail on soil nutrients in the introduction and table 1, and the paper now appropriately includes some hesitation regarding inferring trophic position from uncalibrated N isotopes.

Based on the methods it appears that the authors conducted multiple regressions with 7 total replicates. Such a low level of replication would likely impair their ability to distinguish among the effects of different variables. If nothing else, the authors should include a caveat regarding the interpretation of results given the low replication.

The other issue with the current manuscript is the remaining lack of biological detail underlying the observed effects. Specifically, it would be surprising if the plant and decomposer communities are consistent among sites that vary so greatly in soil P. The lack of such community composition data need not preclude publication of the manuscript, but they would improve the mechanistic insights provided by the manuscript and hence appeal to a wider audience.

Minor comment:

Figure 1: Although the points represent the mean of three subsamples it is probably not appropriate to include error bars, unless a nested design was used

in the regression model.

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

## Cover letter for 2nd revision

Dr. xxxxxxxxxxxxxxxxxxxx:

As requested, I have uploaded the second revision manuscript now entitled, “Spurious and functional correlates of the isotopic composition of a generalist across a tropical rainforest landscape.” We address the comments of reviewer 2, including our revisions in a point-by-point manner:

... Such a low level of replication would likely impair their ability to distinguish among the effects of different variables. If nothing else, the authors should include a caveat regarding the interpretation of results given the low replication.

We agree that caution is required for interpretation using multiple regression models featuring seven localities. We have inserted a caveat in the methods section as suggested. Our original experimental design was developed to mitigate the difficulties associated with a low number of replicate sites. For each parameter we measured, we made multiple measurements and utilized the mean, and in the analysis we used AIC to identify a single result which was the most parsimonious; the results maintain a high level of significance under a sequential Bonferroni correction.

... it would be surprising if the plant and decomposer communities are consistent among sites that vary so greatly in soil P. The lack of such community composition data need not preclude publication of the manuscript, but they would improve the mechanistic insights provided by the manuscript and hence appeal to a wider audience.

We agree that information about the composition of plant and decomposer communities would add to the value of this manuscript, and would enable us to make stronger inferences about mechanisms that can account for the pattern that we describe. The inclusion of these data, if they were available in adequate detail, would change our hypothesis at hand. We have made a point to cite a relevant paper on how soil P alters the density of detritivorous arthropods; these results should hopefully inspire other research groups to address the broader questions that we have identified as we continue to pursue them as well.

Figure 1: Although the points represent the mean of three subsamples it is probably not appropriate to include error bars, unless a nested design was used in the regression model.

As suggested, we have modified Figure 1 to remove the error bars.

Sincerely,

[submitted electronically]

Terry McGlynn

## Letter of Acceptance

Authors: Terrence P McGlynn, Hee K Choi, Stefanie T Mattingly, Angela Upshaw, Evan K Poirson and Justin Betzelberger

Title : Spurious and functional correlates of the isotopic composition of a generalist across a tropical rainforest landscape

Journal: BMC Ecology

MS : 6922267529400233

Dear Prof McGlynn,

We are delighted, in principle, to accept the above manuscript for publication in BMC Ecology.

However before acceptance, our editorial production team needs to check the format of your manuscript, to ensure that it conforms to the standards of the journal. They will get in touch with you shortly to request any necessary changes or to confirm that none are needed.

If you have any problems or questions regarding your manuscript, please do get in touch.

Best wishes,

xxxxxx

xxxxxxxxxxxxxxxxxxxx

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