Referee's Report

Critical loads of nitrogen deposition and critical levels of atmospheric ammonia for mediterranean evergreen woodlands

by Pinho, Theobald, Dias, Tang, Cruz, Martins-Loução, Máguas, Sutton, and Branquinho

Summary
This research uses indicator groups of epiphytic lichens on cork oak to establish lichen community responses to a gradient in ammonia from a local source (pig barn) in a rural part of Portugal. Threshold responses are calibrated against passive sampler estimates of total inorganic nitrogen deposition and ammonia to estimate critical loads and levels.

General comments

This paper contributes to the literature on critical loads in Mediterranean climates with regard to nutrient nitrogen. There are few reports, especially for Europe, yet background levels of nutrient nitrogen appear to be elevated nearly universally in Europe compared to historic background levels. Therefore more discussion and science is needed to debate and establish critical loads. This paper contributes to that discussion. It is a culmination of a progression of studies by the primary authors using well-established methodology to measure lichen response to nutrient nitrogen deposition.

One criticism I have of this paper is that the critical loads and levels (CLL) selected are quite high compared to studies in North America and to the CL for Ammonia first published by Cape et al. 2009 that has become the standard for Europe. The authors propose higher CLLs for rural agricultural areas ('semi-natural areas') compared to background areas. But background areas used in the study already have enhanced N deposition from nitrate and nitric acid. Therefore it appears that these new CLLs are built for an already anthropogenically influenced community that has a higher response threshold than pre-industrial, pre-agricultural (i.e. natural background conditions). This point needs to be clarified in the paper, i.e. that the response threshold used to select the CL is designed for a modern background level of N that already exceeds 10 kg ha yr, when historic natural background could be assumed (based on N deposition in remote areas in other parts of the planet) to be less than 1 kg ha-1 yr. Therefore these semi-natural area CLLs by default accept a certain level of degradation and are not the same as CLLs for natural background conditions.

A related point is that there is increasing evidence that lichens respond not only to ammonia but to other forms of deposition including nitrates, nitric acid, and ammonium. That is why the background site total N deposition is a concern. A new paper should be appearing soon in Ecological Applications based on a study in Southern California using passive samplers for multiple pollutants, provides good evidence that lichens are not only responding to ammonia but to other forms of N deposition in this Mediterranean ecosystem:

1. Sarah Jovan, Jennifer Riddell, Pamela Padgett, and Thomas H. Nash, III. 2012. Eutrophic lichens respond to multiple forms of N: implications for critical levels and critical loads research. Ecological Applications (In Press).

A final point needing clarification is the selection of the CLs from the data. It was not clear to me that the response thresholds selected (LDVoligo and LDVnitro values) were indeed the point at which the community began to suffer adverse effects, rather it seemed that the percentage of nitrophytes and oligotrophs were changing continuously with increased deposition along the entire study area and therefore it appeared to me that the cleanest site already exceeds response thresholds. So I would like to see a better justification for the response thresholds selected or lowering of the CLs.

Some new sources from North America are now available that suggest much lower CLs for lichens of Mediterranean ecosystems. I would like to see the authors discuss these results as part of the justification for higher CLLs selected in Portugal

1. Pardo et al. 2012. Assessment of Nitrogen Deposition Effects and Empirical Critical Loads of Nitrogen for Ecoregions of the US. USDA Forest Service Northern Research Station General Technical Report NRS-80. (This document is the N American equivalent of Bobbink et al. for Europe)
2. Linda Geiser, Sarah Jovan, Doug Glavich, Matt Porter. 2010. Lichen based critical loads for atmospheric nitrogen deposition in Western Oregon and Washington Forests,USA. Environmental Pollution 158: 2412-2421
3. Jennifer Riddell, Thomas H. Nash III, Pamela Padgett. 2008. The effect of HNO3 gas on the lichen Ramalina menziesii. Flora 203: 47–54
4. M.E. Fenn, E.B. Allen, S.B. Weiss, S. Jovan, L.H. Geiser, G.S. Tonnesen, R.F. Johnson,
5. L.E. Rao, B.S. Gimeno, F. Yuani, T. Meixner, A. Bytnerowicz. 2010. Nitrogen critical loads and management alternatives for N-impacted ecosystems in California. Journal of Environmental Management 91: 2404-2423.

Specific Comments

Title

The title clearly and concisely describes the research project and reflects the content of the manuscript. One comment is that the lichen community measurements were made exclusively on cork oak yet the title suggests that the same results would be encountered in evergreen woodlands in general. Perhaps some statement in the discussion or introduction should explain the extrapolation.

Abstract

The abstract briefly summarizes the purpose of the work, what was done, what was found, and the significance and includes no extraneous information. However this particular section of the paper contains many more grammatical errors than the rest of the paper which need to be corrected for clarity.

Introduction

- A discussion of the North American literature should be included either here or in the discussion or both.
- Otherwise this section is well researched and written and includes not only a good literature review but a brief overview of the objectives, and methods. I would suggest adding the scientific name of the study organism (cork oak) here.
- Personally, I feel that the traditional statement of hypotheses is a very useful way to structure a paper and is a core part of scientific thinking and methodology. So I wish that more authors these days would state an actual hypothesis in the introduction. In

this case, it is not so critical, but it could be interesting to see how the paper would change if a hypothesis was stated here rather than an objective.

Materials and Methods

- The materials and methods generally presents appropriate materials and methods in sufficient detail to allow the results to be repeated and is written in the past tense. If not included in the introduction, the scientific name including the taxonomic authority of cork oak should be included here.
- On page 11146 I wanted to see some mention of the current total N deposition in the study area relative to prehistoric and preindustrial levels to give me some context for the exposure levels in this study. Later I saw some mention of this in the discussion. Also I wanted to know what data exist to suggest that the species composition and diversity is not also harmed/altered at the control site compared to historical natural background conditions. Finally, what were the statistical methods used to test that the difference between the control site LDV and pigbarn sites LDVs were significant?

Tables and Figures

- All the table and figures appeared to me justified and necessary. Figures are sharp and lettering is proportionate to the size of the figure. Tables are presented in the most simplified and condensed manner.
- Table 1. Why were the dates included with the taxonomic authorities? Has there been some new rule change that requires inclusion of dates? Note misspelling of maximum.
- Figure 1. Consider including the location of the wind direction meter on the figure.
- Figure 2. It is amazing that deposition of N reaches over 400 kg ha yr only 130 m from the cleanest site in the study area. Even though the annual deposition drops rapidly from the barn, it seems to me that there could be episodic levels at 130 m much exceeding annual average levels that could be affecting these communities. Similarly ammonia is 35 ug m3 at the barn, an extremely high level and drops to an average of 2-3 at the cleanest site, but perhaps there are episodes of much higher levels. I think this warrants discussion in the paper, i.e., how do you know that any of these sites are not affected by episodic bursts of atmospheric N as atmospheric conditions and daily concentrations fluctuate. This could explain why the proposed CLLs are so high.
- Figure 3. It seems that if the regression line were extended (i.e. had there been sites in areas with lower deposition), that it would have resulted in selection of much lower CLs. Please justify the selection of sites used to establish the response threshold.
- Figure 4. Same comment as Figure 3. For example, if the line in figure B were extrapolated to the left, a CL similar to Cape et al. 2009 could have been selected. There is no sign that there was any leveling off of LDVoligo scores at the cleanest sites.

Results and Discussion

See 'General Comments' above.

Page 11150. See Geiser et al. 2010 (see general comments section), who found that climate, especially precipitation can influence lichen critical loads.

Because these data are based on one small area in Portugal, statistically speaking, the CLLs should really only apply to this area. What is the justification for extrapolating to Mediterranean ecosystems in general?

Citations

- The sources of all facts and ideas are cited throughout the paper.
- Authors present information from other sources in their own words.

References

The full citations are provided for all cited references.

Style

Subsections of the paper are logically organized. The appropriate information is included in the appropriate sections of the paper. The paper has minor grammatical errors throughout that could be easily fixed with attention from the authors.