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Discussions

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Interactive comment on "Exploring the distance between nitrogen and phosphorus limitation in mesotrophic surface waters using a sensitive bioassay" *by* Enis Hrustic et al.

Anonymous Referee #2

Received and published: 27 September 2016

The authors present a data set and analysis method aimed at diagnosing and quantifying the excess bioavailabilities of N and P within aquatic systems. The new data presented appears to be a rich resource and the proposed analysis technique appears to have merit. Having said this, I found the manuscript difficult to assess on first reading and I would encourage the authors to consider expanding both the text and presentation of the data to make accessibility clearer to the general reader. Below I make some general comments and suggestions which I would like to see the authors address in any revised version of the manuscript.

Specific comments:

Both conceptually and in terms of practical calculation the estimates of 'N*' and 'P*'

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[Discussion paper]

derived from the experimental protocol described differ from the typical usage, which has in the past been based on a strict definition (e.g. N* = Nitrate – 16 x Phosphate). In order to avoid confusion within the literature I would prefer the use of different terminology (e.g. maybe 'apparent excess N or P') or, at the very least, a strict definition of the terms used. In particular, at present I remain unsure how and whether the resulting values of derived 'N*' and 'P*' might depend on system characteristics, e.g. including the biological characteristics of the system. Indeed, given the time dependence of the results, it seems likely that derived values are a complex and variable system property. Additionally, I would like to see the authors' thoughts on whether the variable sizes and turnover times of bioavailable dissolved organic N and P pools might influence their results? Additionally, I was unclear how the presence and turnover of natural DOP pools, both AP-hydrolysable and otherwise might be influencing the results?

Related to the above, it would be useful to have some additional information on the systems studied, e.g. including inorganic and organic N and P concentrations and potentially some characteristics of the planktonic community?

Given the focus of the paper, I would like to see some more details on the APA measurements. In particular what substrate was used (MUFF-P?) and at what concentration. Also how long were the APA incubations and were multiple timepoints measured within the incubation? Concentrations of substrate are potentially particularly important and I would be interested in the authors' opinion on whether it would matter if the substrate concentration was saturating or not for the derived response surface?

Some of the measured APA values are negative, what does this represent? I assume some form of blank correction within the fluorometric measurements? Please be more specific on methods.

Additional comments on specific sections within the text:

Abstract:

Line 19: I suggest '...primary limiting nutrient...'

Section 1:

Line 16: suggest '...fixers potentially having a competitive...' i.e. the evidence in the cited study is largely on the basis of hypothetical modelling

Line 22: define N* and P* (e.g. N* = Nitrate – 16 x Phosphate) on first use (also note above, I would prefer use of different terminology for derived apparent excesses of N and P)

Line 44: It was not immediately clear to me that the assays described can be used to quantitatively asses the excess of overall bioavailable N or P in a system and, despite spending some time with the authors' code and data (which are very usefully provided within the supplement), I still remain to be entirely convinced. Firstly, there is clearly an assumption that the organisms in the community react to P limitation through increasing APA (see abstract of Thingstad and Mantoura 2005). While this may be the case, it remains an assumption and should probably be explicitly stated as such. I think there is also an underlying assumption that the community level APA response is directly proportional to the overall availability of all forms of bioavailable N and P within the system. The authors can correct me if this is wrong, however, I think that the equation on line 2, page 4 suggests there is this assumption of a linearity of response? Overall however I will admit that I struggled to follow some of the authors' arguments, particularly on the first read through. I would therefore suggest that the authors could provide a fuller treatment and explanation for their analysis method for the data presented.

Related, it would be good to fully separate out the equations, number them and explicitly refer to them as/when required. Some additional simple graphical schematic plots and further graphical presentation of the extensive data set might also aid accessibility (see below).

Section 2:

Line 4: Rephrase. Please provide some background to the experiment. At this stage the experimental mesocosms have not been described/introduced.

Line 30 (section 2.3): please provide information on both the incubation time for APA, substrate used, concentration etc. (see above).

Page 6, lines 1-12: given that the value of r has a direct impact on the calculations of any excess bioavailable N or P within the system, I was left wondering how the lack of a consistent explanation for the variability in this derived parameter between the different sets of experiments could potentially influence the authors' conclusions. Overall I might argue that caution should be applied to quantitative conclusions based on the technique described until a more complete understanding of the responses is available. Related, does 'r' really represent a consumption ratio? I think it likely represents the equivalence ratio between the influence of added N and P on APA rates within each individual system studied, but it isn't immediately obvious to me that this would be the same as the overall nutrient consumption ratio, in particular due to the potential for variable turnover rates of different pools.

Figure 1: this was useful in helping to understand the technique, however a similar schematic of the opposite case (i.e. where No is negative) would be useful.

Figure 2: I found this figure difficult to interpret on first sight. After using the authors' code to analyse their data I eventually got the hang of what was being presented, however I wonder whether there might be a more simple graphical way to display the data. e.g. could a similar plot to figure 1 be produced with the responses to individual values within experiments contoured/coloured rather than presented as a surface? This may aid a reader in picking out the N = No + rP line and associated intercepts etc. Maybe such a presentation could be used in addition to the format in Figure 2, as the latter admittedly does have the benefit of displaying the magnitude of residuals. Overall I would encourage the authors to consider a wider presentation of the data, as the manuscript is currently short and hence there is ample space available. Related, a key strength of

the data appears to be the repeatability of results, so I would suggest presentation of some more individual experiments would be useful to the reader.

Finally, running the authors MATLAB codes on the data provided I found some discrepancies with the information provided in table 1. I haven't checked all the experiments, but, for example, for the 'M7' experiment for Tvärminne I get:

General model: myfit(x,y) = A/(1+exp(s*(1/sqrt(1+r^2))*(r*x+No-y)))-B Coefficients (with 95% confidence bounds): A = 8.027 (7.681, 8.373) s = 20.67 (19.18, 22.15) r = 1.616 (1.555, 1.677) No = 0.6882 (0.676, 0.7004) B = 0.1094 (0.06078, 0.158)

Thus the estimate for No appears different (albeit only minor) to that stated in the table, as does the confidence interval for s?

Similarly, for 'Fjord' I get:

General model: myfit(x,y) = A/(1+exp(s*(1/sqrt(1+r^2))*(r*x+No-y)))-B Coefficients (with 95% confidence bounds): A = 7.41 (7.156, 7.664) s = 37.87 (34.98, 40.76) r = 2.482 (2.39, 2.573) No = 0.6771 (0.6676, 0.6867) B = 0.2197 (0.1826, 0.2569)

Which again seems different to info in Table 1?