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Biogeosciences Discussions

Interactive comment on "Phosphatase activity and organic phosphorus turnover on a high Arctic glacier" *by* M. Stibal et al.

Anonymous Referee #1

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Stibal and colleagues present the results of MUP-based alkaline phosphatase assays of debris from within cryoconite holes atop an Arctic glacier. They also report on experiments to determine substrate, product, light and temperature effects on the enzymes. The results are generally consistent with those from other low-temperature aquatic environments, and provide some evidence of phosphorus deficiency within the microbial communities associated with the debris in the cryoconites. The authors also use ELF staining to try to identify the sites of AP activity within the debris. Finally, they attempt to estimate the potential for organic P turnover within the cryoconites and the possible contribution of debris to the P budget.

The paper is generally well written and easy to follow, and the figures and tables convey the results effectively. The AP data are important mainly because there are so few

from this type of extreme environment. The MUP and ELF methods appear sound, and the authors recognize some of the limitations of their approach. However, I have doubts about some of the wet chemistry (see below). Overall, the study results are not particularly novel, except for the evidence of a light effect on AP activity and the apparent lack of association of AP hotspots with microbial cells. I think these two findings are intriguing and warrant more discussion. The weakest part of the paper is the attempt to estimate OP and DOP turnover, which I do not believe can be done with these types of data alone.

Major issues

1) On p.2701 lines 17-20 you describe your SRP assay, and state that you used 1 cm cuvettes. Then on lines 20-21 you claim a detection limit of 0.015 uM. With a 10 cm cell the detection limit for the Murphy-Riley method is about 0.030 uM; on a good day 0.015 uM might be possible but I wouldn't trust the quantitation at these levels. With a 1 cm cell you are looking at 0.3 uM as a typical detection limit. This discrepancy is important because you later (p.2709 line 2) state that the SRP in your cryoconites is 0.75 ug/l (= 0.024 uM), a value an order of magnitude lower than the detection limit of your assay. Even if you actually used a 10 cm cell, you could not have measured such a low concentration with much confidence. In your experimental treatments, you say the SRP was 0.31 ± 0.05 uM (p.2704 line 27); even this is right at the detection limit with a 1 cm cell. Any conclusions regarding ratios (such as SRP:DOP) will therefore be highly suspect.

2) It would be very helpful to normalize your AP measurements to some measure of biomass. Even dry weight would be better than wet weight. ATP, chlorophyll, organic phosphorus – did you measure any of these on the debris? It looks like you have made measurements of organic P from the discussion on p.2711 line 28 to p.2712 line 1. With the normalization to wet weight you have no way of comparing your results to published studies of P-deficiency (e.g., Healy and Hendzel 1979, Freshwater Biol 9:429). The problem here is that cells may make AP even when not particularly P-

stressed, but the amount they make relative to their own biomass goes way up under P-stress. Without a measure of biomass, it is hard to directly compare your rates with those in other environments to evaluate the degree of P-deficiency.

3) I don't follow the argument on p.2710 lines 12-13 that higher AP activity in the dark than in the light implies that most of the activity is associated with heterotrophs. I can imagine a situation where autotrophic AP production might be stimulated by light, but why would heterotrophic AP production be stimulated by darkness? The result appears more consistent with some sort of photoinhibition of AP activity, which I have never heard of previously. This result deserves more discussion.

4) On p.2709 lines 18-22 you suggest that the AP is inducible (actually, repressible, in the case of these DIP additions) rather than constitutive. This is consistent with what is known about AP in other environments. However, later (p.2710 lines 20-22) you discuss your ELF results and suggest that 80% of the AP activity is not associated with microbial cells. How do you reconcile these points; in other words, cells may make less AP when exposed to phosphate, but how do you explain why AP activity in a sample is inhibited by phosphate if the enzyme is free?

5) The estimates of DOP and OP turnover rates on p.2711-2712 are just not believable. For one thing, you have no idea what the actual concentrations of substrate (phosphate monoesters) or product (orthophosphate) are in your system. Second, you don't know how much of the OP in the debris is even physically capable of interacting with AP under in situ conditions. Third, the conditions in your experimental treatments are so far from in situ conditions that they are unlikely to yield rates anywhere near in situ rates. For example, you heavily dilute the debris, then you incubate at a pH that is way different from that of the natural environment. Using the AP activities as a measure of P stress is reasonable, but trying to get at in situ turnover rates from these data is too much of a stretch.

Minor issues/questions

1) p. 2698 line 6 and elsewhere: What exactly is being "limited" by P? – Microbial biomass? Primary production? Heterotrophic production? Other? You need to be specific.

2) p. 2698 line 9 and elsewhere: It would be good to offer a more detailed characterization of the composition of this debris and where it comes from.

3) p. 2698 line 9: What constitutes "significant" activity?

4) p. 2698 line 10: I would say "inhibited" rather than "controlled" here.

5) p. 2698 line 12: Delete "rates" – redundant.

6) p. 2698 lines 14-15: Do you have any evidence that microbial biomass P actually increases in these systems over the ablation season?

7) p. 2698 lines 25-26: Do you have a citation for the assertion that the debris is the principal source of "the majority of nutrients"? Atmospheric deposition could be important, especially for nitrate. Nitrogen fixation could likewise be an important source of ammonium.

8) p. 2698 line 26: Define "nutrient poor".

9) P. 2699 lines 2-4: Could it be that inorganic nutrients just have no appreciable source, as opposed to them being "rapidly sequestered"?

10) P.2699 lines 6-8: Regarding the figure of 0.1 uM, you are talking about TP, yes? DIP is probably much lower than this in such environments. After all, DIP is in the picomolar range in many oligotrophic lakes (e.g., see Hudson et al. 2000, Nature 406:54).

11) p.2699 lines 8-11: What exactly is meant by "bound to the debris" here? Do you mean that the P is part of the chemical composition of the debris itself, or that it is adsorbed onto debris, or something else?

12) p.2699 lines 16-17: How do you ascertain that the P within the debris is "potentially

bioavailable"?

13) p.2699 lines 21-23: AP cleaves the phosphate group from phosphate monoesters only. Diesters, phosphonates, etc. are not rendered "bioavailable" by AP.

14) p.2700 lines 1-3: You should take a look at Lisle & Priscu 2004, Microb Ecol 47:427. With ELF stain they found AP activity was associated with bacteria in aggregates within Antarctic lakes.

15) p.2700 lines 9-12: I wouldn't consider organic P utilization by cryoconite microbes to be of "great significance for the whole Arctic ecosystem". I believe the Arctic is mostly ocean, for one thing. Perhaps reword this to refer to ice-covered terrestrial Arctic ecosystems, or something like that.

16) p.2700 lines 24-26: How do you quantify the amount of "readily available P" within the debris?

17) p.2701 lines 17-20: You should not try to equate SRP with DIP. SRP includes acid-labile organic P compounds as well.

18) p.2702 lines 22-24: Please explain your quenching correction.

19) p.2702 lines 27-29: Do you know anything about the mechanism of MU adsorption to the debris? Or are you implying that the MU was physically shaded by the debris particles? Something else?

20) p.2703 line 19: Remove space from "p hosphatase".

21) p.2704 line 27 and elsewhere: Please define these errors (e.g., sd, se, 95% conf.).

22) p.2705 line 1: I suspect that this %error is too low – did you propagate the errors of the TDP and SRP measurements used to determine DOP/TDP = (TDP-SRP)/TDP?

23) p.2705 lines 1-5: Do you have an estimate of how many of these cyanobacterial cells end up in solution as a result of your experimental procedures?

24) p.2705 lines 1-5: Are these cyanobacteria diazotrophic?

25) p.2707 lines 10-12: Please define TDC. If it includes inorganic carbon then TDC:TDP ratios tell you little, given that CO2 exchanges with the atmosphere.

26) p.2707 lines 18-23: This is an odd result. Perhaps the lack of inhibition has something to do with the AP being mostly unattached to cells? Also, I'm not following your logic regarding the unshown plot of AP activity versus DOP/TDP – what does the plot look like and what do you believe it is telling you?

27) p.2709 lines 4-5: Antarctic lake water does have "debris" of sorts, both suspended microbial aggregates and wind-blown sediment and desiccated cyanobacterial mat material that migrates over time through the ice. See Lisle and Priscu 2004 (cited above) and Priscu et al. 1998, Science 280:2095.

28) p.2709 lines 14-16: I wonder if the reduction in AP activity is a result of occupation of active sites by substrate that were initially all open.

29) p.2709 lines 23-26: Is there any direct evidence for a release of SRP into solution from debris as the cryoconites melt?

30) p.2710 lines 23-27: Wouldn't release of free AP into the medium offer a competitive advantage to bacteria, since they have a higher affinity for phosphate uptake at low concentration than do algae?

31) p.2711 lines 1-4: I believe intracellular phosphatases are typically acid phosphatases, and you describe your system as having an in situ pH of 5.4; hence, AP would be more likely to be inactivated in the medium than intracellular phosphatases, yes?

32) Are there birds in this part of the world, and if so, do they contribute to the P budget of the glacial surface?

Interactive comment on Biogeosciences Discuss., 6, 2697, 2009.