

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

### **Anonymous Referee #3**

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The manuscript deals with microbial phosphorus cycling in Arctic supraglacial environments, i.e. in cryoconite holes on a glacier. The authors present phosphatase activity of the microbial community in cryoconite debris. The bulk activity was assayed with MUP and phosphatase sites were localised using the ELF technique. In several incubation experiments, the authors suggested overall P limitation of the microbial community associated with cryoconite debris and calculated turnover rates of organic P on the glacier. The manuscript is well written, however, a thorough revision of the text is necessary. The authors have got a good data set, worth publishing in Biogeosciences, but some methods are currently unclear, oversimplified, or misinterpreted. Therefore, based on this methodological criticism (see General comments), some results are clearly overinterpreted, hence, some conclusions are likely unjust and should be carefully reconsidered.

## General comments:

1) Soluble reactive P (SRP) must not be interpreted as dissolved inorganic P (p. 2701, line 17, and elsewhere). SRP is preferred to DRP in current limnology, highlighting that the term soluble has distinct meaning than dissolved. Furthermore, the phosphorus reacting with molybdate blue may not be entirely inorganic P, not even orthophosphate, either. SRP rather may be taken as a proxy of readily bioavailable P. I too doubt your detection limit of SRP unless it is clearly defined. Consequently, total dissolved P as well as dissolved organic P may not be correctly estimated (p. 2701, lines 21–24) and, in particular, properly interpreted (e.g., p. 2707, lines 20–23).

2) Phosphatase assay. From the Methods, one may believe that Michaelis-Menten kinetics was the standard assay; however, Table 2 suggests that it was not that case. You should clarify, whether a single point assay (which concentrations) or saturation kinetics was used. If the former was the case, how many times the kinetics was measured? Why did not you use some commercial software for direct plotting data by non-linear regression instead of the Eadie-Hofstee transformation (p. 2703, lines 12–15) that, in many cases, may reveal imperfect estimates of kinetic parameters?

3) Phosphatase kinetics. A mixture of enzymes recently has been found in many aquatic or soil samples, which does not follow the simple Michaelis-Menten model as you assumed (p. 2703). If (groups of) enzymes differ by their affinity (p. 2708, bottom), they could be distinguished kinetically. In lake plankton indeed, bacterial phosphatases used to have an order-of-magnitude higher affinity (lower  $K_m$ ) compared to algal ones. Therefore, unless you estimate saturation kinetics over a wide range of substrate concentrations, you can hardly assume that several-times higher activity at 100  $\mu\text{M}$  than at 0.5  $\mu\text{M}$  confirms that the former concentration is saturating (p. 2705, lines 24 and 28, p. 2707, line 18). If two kinds of enzymes with the distinct affinity are present in your sample, your data (Table 2) may be interpreted alternatively that way that high-affinity enzymes are saturated at 100  $\mu\text{M}$ , whereas low-affinity ones are not. Consequently, the low-affinity enzymes (say with  $K_m$  of tens of  $\mu\text{M}$ ) would not be saturated at all (the

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saturating concentration is considered to be as high as nine-fold  $K_m$ ). Also inhibition effects of P addition (e.g., on p. 2707, lines 18–20) would be more properly quantified if sets of saturation kinetics (with different P additions) were used to calculate an inhibition constant.

4) Higher phosphatase activity in the dark (p.2710, lines 12–13) cannot distinguish heterotrophic vs. autotrophic phosphatase activity, during a 30-min incubation either. Indeed the proportion of phosphatase sites on autotrophic (Chl+) cells was almost identical in the dark and light (4.3% and 4.5%, respectively, Table 2)!

Specific comment:

p. 2698, line 12, and elsewhere (pp. 2705–2711, Table 2, Fig. 3): Phosphatase activity rates is a terminological duplicity. The activity is, by definition, the rate of enzymatic hydrolysis.

p. 2703, lines 1–2: What do you mean with MU sequestration by the microbial community? Neither MU nor MUP is sequestered by microbes, is it? MU may be absorbed to the debris, MUP is cleaved by the (microbial) phosphatases. What is fluorescence quenching of MU? Absorption behaviour of MU and MUP may not be the same either.

p. 2703, line19: correct “p hosphatase”

p. 2706, lines 2–4: How were filamentous bacteria quantified? Were single cells in the filaments well visible? The enumeration method (p. 2704) should be described accordingly.

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