Ref. #1: Recent transcriptomic work has shown that each of these different states can be quite complex with somewhere on the order of 200-600 proteins being up or down-regulated based on the nutritional state of the microbes. It seems that only looking at a couple of those proteins is likely to lead to idiosyncratic conclusions. I am not suggesting that the authors should necessarily be using a transcriptomic approach, but given this information, I think it is trivial to draw a line at a 45 degree angle and suggest that anything above the line is P-limited and anything below the line is N-limited. This kind of information just has not been substantiated enough to say whether that line should be 45 degrees, 48 degrees or 10 degrees.

***We understand the concerns of the reviewer and the advantages offered by transcriptomic approaches. However, enzyme-based modeling of microbial nutrient limitation is a well-recognized approach that has its foundations in a large body of primary literature which has investigated microbial enzyme activities in relation to nutrient dynamics and environmental factors since the early 1970s.

It is of course correct that there are many classes of enzymes involved in the microbial conversion of OM into nutrients (Ljungdahl and Eriksson 1985; Kirk and Farrell 1987; Sinsabaugh 2005; Sinsabaugh et al. 2010), and further, that the expression of the entire suite of enzymes is dependent on nutritional state. However, while many enzymes are active in a general lake ecosystem, only a few will have relatively high activities (Sinsabaugh and Foreman 2001). The four enzymes selected for this study have been repeatedly utilized in terrestrial and aquatic studies alike, slightly varying in combination according to the nature of the research being conducted and the systems of interest (e.g. Sinsabaugh et al. 2010; Mineau et al. 2013; Moorhead et al. 2013; Hill et al. 2014; Parr et al. 2015); this is due to the fact that these enzymes represent the catalysts for *terminal* reactions in which organic matter (OM) is converted into monomer nutrients, as stated in this study (Sinsabaugh et al. 2008; 2010). Important to the extracellular enzyme activity (EEA) assay method is understanding that the bulk of enzymatic pathways devoted to OM degradation converge into those of hydrolytic, terminal catalysts (Allison et al. 2007; Moorhead et al. 2013). BG, NAG, LAP, and AP-mediated breakdown generate low-molecular mass compounds that are readily bioavailable. Together, the activities of these indicator enzymes represent the final steps of OM degradation and are therefore proxies of the total amount of microbial enzyme activity devoted to C, N, or P acquisition (Moorhead et al. 2013).

Significant research has been conducted that shows the inverse correspondence of nutrient availability to specific enzyme activities, for example AP (Wetzel 1981; Chrost & Overbeck 1987; Chrost 1991; Olander & Vitousek 2000; Sinsabaugh et al. 2008; Hill et al. 2010a; b). N-acquiring enzyme activities in relation to N availability is more complicated, but evidence suggests that inorganic N depresses hydrolytic N-acquiring enzyme production (Olander & Vitousek 2000; Stursova et al. 2006) and organic N subsidization induces their production (Sinsabaugh et al. 1997; Weintraub and Schimel 2005; Allison 2007). The development of enzyme studies over the past few decades have established links between environmental nutrient availability and relative enzyme activities, linking ecological stoichiometric and metabolic theories (Sinsabaugh et al. 2009; Sinsabaugh & Shah 2012; Moorhead et al. 2013), leading to models that allow for the prediction of microbial nutrient acquisition efforts and limitation patterns. In support of these models, we note that Referee 2 indicated that we “employed a proper and well-established method for the measurement of enzyme and data interpretation”. In short, there is robust evidence that precedes this study to suggest that enzymes are accurate and sensitive indicators of

microbial nutrient demand, acquisition efforts, and limitation, and we have cited these studies throughout the manuscript in support of this.

> Ref. #1: I also have concerns about the execution of the study in particular, although I know it is problematic doing research in remote places, the authors froze the samples for transport from the sites back to the USA or NZ where analyses were conducted. No mention is made of any controls or quality control to determine if freezing had any effect on enzyme activity. This would be particularly problematic if it affected some enzymes differently than others which would certainly affect the conclusions of the study. It would also be useful to know that each of the enzymes was measured at the Vmax, providing a solid quantitative measure of enzyme activity.
>
> Ref. #2: I can see that collection and storage of samples were highly difficult due to the location of study sites, but freeze-and-thawing affects and often substantially interfere with enzyme activities. The authors should explain possible problems or a source of error due to the sample treatment.

***Storage of enzyme samples was indeed dictated by the remote location of our study sites and the transport options available to us. There is no current consensus on the effects of freezing on measures of enzyme activity. For example, in a review of enzyme methodology German et al. (2011) found no consistent effects of freezing. Some studies have found no significant difference of enzyme activities from soil samples stored refrigerated or frozen (Lee et al. 2007; DeForest 2009). Wallenius et al. (2010) propose that freezing has only minor effects on enzyme activity samples, especially within uniform sample types. In streams, the effects of freezing can be variable (Smucker et al. 2009) although explicit tests are restricted to a few locations. We note that there is precedence in other investigations that have successfully used frozen water samples for EEA analysis (e.g. Simon et al. 2009; Clinton et al. 2010; Freimann et al. 2013; Parr et al. 2015). Since there was no way to analyze fresh samples from Greenland, control samples were not available. To address this issue, we will add the following caveat into the methods section:

"Due to the remote location of the lakes samples from June were stored frozen (−20 ◦C) for 60 days and samples from July were refrigerated for 30 days and then frozen for 30 days before analysis. *Though the analysis of fresh samples is considered preferable due to the uncertainty of whether freezing introduces bias into results, it is common for freshwater EEA studies to freeze samples owing to logistical constraints (e.g. Simon et al. 2009; Clinton et al. 2010; Freimann et al. 2013; Parr et al. 2015). We are assuming that if freezing had any effect it was similar across systems.* EEA samples were thawed, processed and analyzed…"

It is important that each of the enzymes is measured at V_{max}, which was why we experimentally determined the saturating concentration of substrates prior to the study. To make this clearer, we will add the following amendment to the methods section:

"Pilot assays were used to ensure substrate concentrations saturated enzyme kinetics*, such that kinetic rates were equal to* V_{max}, and readings were made during linear increases in fluorescence. Throughout the analysis…"

> Ref. #1: Another concern here is that the authors refer to the organisms producing the exoenzymes as 'bacteria', but I doubt they looked to see if there were also archaea and/or eukaryotes in their samples. If so, they should mention it.

***This is an excellent point. Though we did not determine archaeal, algal, or even bacterial abundance, we are cognizant of the fact that extracellular enzymes in aquatic environments are produced by phyto- and bacterioplankton alike. This is why both bacteria and phytoplankton studies were considered throughout the paper. Though we were careful with our wording, we inadvertently specified 'bacteria' in a few places. We refer now exclusively to "microbes" to encompass all possible taxonomic groups that could be involved in enzyme production.

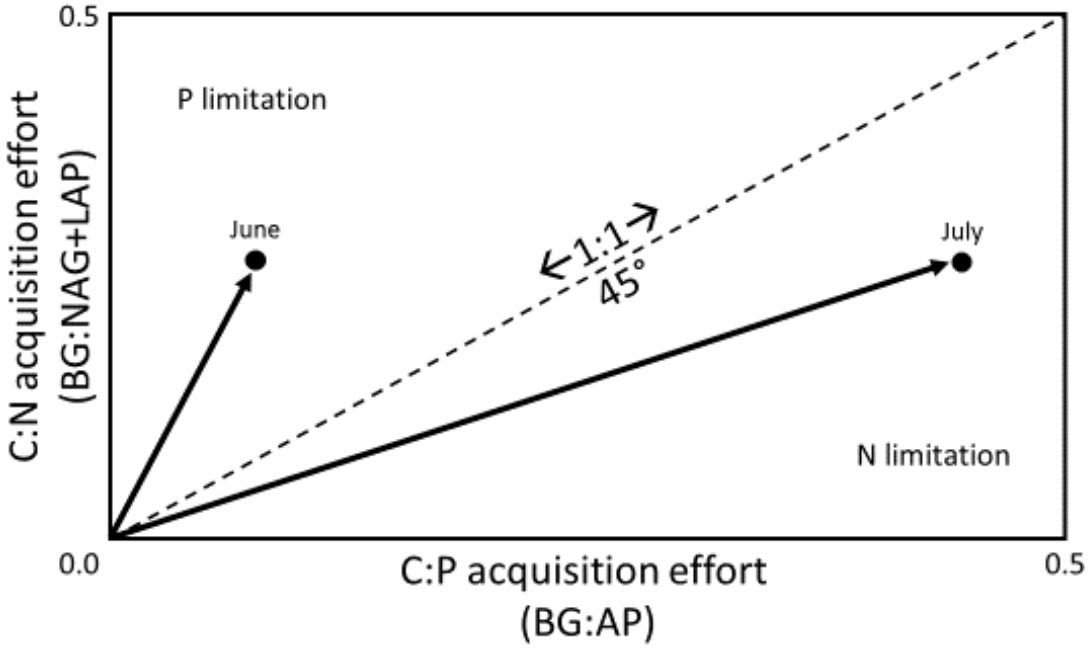
> Ref. #1: Presentation quality (rating: 2) The authors use an approach adopted from Moorhead et al. 2013 to determine nutrient limitation from enzyme data whereby a vector length and angle are calculated. I had to look at several papers before I found a decent description with the mathematics of this approach (Hill et al. 2014). The description should also be included in the present manuscript because most readers will not be very familiar with it.

***We cite Hill et al. 2014 when explaining this approach, and will insert the following into line 18 of p. 11870 to make the method more intuitive to readers:

"Figure 2 displays hypothetical data from a lake in June and July plotted onto a vector plot with the 1:1 line drawn in dashes. The vectors from which angles are calculated are shown as arrows from the origin to the individual data points. In June, the vector angle is positive with respect to the 1:1 line ($> 45^{o}$) indicating P limitation in this lake. However, in July nutrient limitation shifts from P to N, as indicated by the negative angle with respect to the 1:1 line ($< 45^{o}$)."

And we will add the following figure and caption:

Figure 2. An example of vector plot analysis for a hypothetical lake sampled in June and July. The 1:1 line is drawn in dashes and separates zones of P imitation (above) from N limitation (below). Vectors for each data point are drawn in arrows. Their angles indicate microbial nutrient limitation, such that the positive angle value with respect to the 1:1 line in June indicates P limitation, while the negative one in July indicates a shift to N limitation. The lengths of the vectors are also indicative of microbial C acquisition efforts, which in this example is greater in July than in June.



Figures will be renumbered in the final submission to make this addition consistent.

> Ref. #1: It is also complicated by the fact that several of the figure axes and captions in the paper seem to be mis-labeled or not labeled at al. Units in Fig. 3 are not given and the ratios in that figure for BG:NAG+LAP are on the order of 10-60. But then in Fig. 4, the axis for BG:NAG+LAP is in the range of 0-0.8. The caption says that what is plotted are the vector angles, i.e., not the activity, but in Table 2 the vector angle ranges are around -10 to +45. So it is really not clear what is being plotted in Fig. 4. Figure 4 also seems like a more convoluted plot than it needs to be. If BG is in the numerator for each axis, it cancels itself out and essentially they are plotting NAG+LAP against AP and therefore should be labeled that way. Figs. 5 and 6 also need units to be labeled.

***Units in Figure 3 are not given due to the fact that the quantities being shown are ratios, and therefore without units. You may likely be referring to Figure 2, where the units are not given on the axis labels, but are instead reported in the figure caption in order to save space. This has been the

convention in other enzyme papers, and we use it here. For Figures 5 and 6, the variables are ratios or natural log (ln)-transformed quantities are therefore without units. You picked up on a discrepancy in our data that was due to a calculation error—the BG:NAG+LAP ratios reported in Figure 4 are accurate, whereas those in Figure 3 are erroneous. This mistake will be fixed for the final submission, but importantly, it does not change our significant findings and overall conclusions of the paper.

Figure 4 is indeed complex, and we heed your suggestions. We address this by changing the caption:

"Figure 4. Scatterplot of microbial enzyme ratios (BG : NAG + LAP vs. BG : AP) about the 1:1 line. Included is C:P and C:N acquisition data of lake epilimnia (circles) and hypolimnia (triangles) from June (gray) to July (black). Dotted line indicates 1:1 (45 °) line. Vector angles (indicative of nutrient limitation) are calculated from these plotted data points, as deviation from the 1:1 line."

We disagree that BG should be cancelled out, though it is the numerator in both variables. If we were seeking regression between the two variables, then BG would cause covariation. However, this is not our intention. We are instead interested in the angles generated by the data points plotted using the two ratios *and* we were interested in determining microbial C acquisition efforts by vector lengths, despite the fact that C acquisition did not show important trends in this study. Regardless, obtaining and analyzing C-acquisition data would not have been possible if BG had been factored out.

> Ref. #1: More specific comments: p. 11873: Why did they use DIN: TP as an index of nutrient limitation? A more appropriate comparison would be DIN:DIP or TN:TP. p. 11874 line 15: I don't think you can necessarily infer that the DOM supply was poor in P from this relationship. There can be (and likely are) other sources of N and P other than DOM. Also, it is the supply relative to the requirements of the organisms that would determine this relationship.
>
> Ref. #2: High correlation between DOM and TN and absence of such relationship between DOM and TP do not necessarily indicate the difference in availability between N and P. Rather, different chemical properties of organic N (which is mostly directly bonded to C) and organic P (mostly tied as an ester bond) could be the reason for that.

***We believe that DIN:TP is a more accurate account of nutrient limitation than DIN:DIP or TN:TP based on the work of Bergstrom (2010) in which DIN:TP serves as a better indicator of phytoplankton nutrient limitation than TN:TP in oligotrophic lakes. Though our enzyme samples include activities from both algal and bacterial organisms, presumably with different stoichiometric requirements, the DIN:TP ratio seemed most appropriate, especially since DIP (PO_4^{3-}) was frequently below detection limits. Further, DIP is typically not used because P can cycle extremely rapidly and so the total pool of P is considered a better index of P availability. This argument does not hold for N, as it does not cycle as quickly. Also, as stated in the manuscript, TN and DOC positively covaried, making it difficult to separate their effects, and importantly, the size of the TN pool in high DOM lakes does not necessarily correlate to bioavailability of N.

To avoid suggesting that DOM is a poor P supply on p.11874, we will change the sentence to the following: "Collectively, these enzyme and water chemistry data suggest that the DOM in these lakes

may provide a readily available source of N, while higher DOM concentrations are associated with enzyme-mediated microbial P acquisition."

> Ref. #2: One reservation for the paper is about C- mineralizing enzyme. Most of DOM delivered to lakes could be composed of highly recalcitrant carbon for which beta-glucosidase may not be a representative enzyme. Decomposition of phenolic or humic materials is known to be harnessed by oxidase activity (e.g., phenol oxidase or laccase), which in turn may limit the activities of other hydrolases (see, Freeman et al., 2001). Enzymes involved in mineralization of recalcitrant carbon should be discussed somewhere in the manuscript.

The authors note the importance of oxidative enzymes in the degradation of recalcitrant forms of OM. BG is a more commonly utilized enzyme in aquatic literature, as it is assumed to broadly represent C acquisition activity. However, an enzyme such as phenol oxidase could have provided insight into degradation rate of terrestrially-derived C. We will address this in the manuscript discussion on page 11876:

"Bacterial community structure has been shown to change in correspondence with DOM quality in arctic lakes, as some bacteria prefer more labile compounds while other species are adapted to utilizing recalcitrant forms (Crump et al., 2003). *In this study, the seasonal source and quality of the DOM pool might have been inferred by the inclusion of oxidative enzymes, such as phenol oxidase or peroxidase, which are responsible for degrading terrestrially-derived compounds such as phenols and aromatics, respectively (Sinsabaugh et al. 2008). Though BG is assumed to broadly represent C acquisition activity, oxidative enzyme activity may be an important metric in future studies*."

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