

1 **Microbial nutrient limitation in arctic lakes in a permafrost**
2 **landscape of southwest Greenland**

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4 **Benjamin Burpee¹, Jasmine E. Saros¹, Robert M. Northington¹, and Kevin S.**
5 **Simon²**

6
7 [1]{Climate Change Institute, and School of Biology & Ecology, University of Maine, Maine,
8 USA}

9 [2]{School of Environment, University of Auckland, Auckland, New Zealand}

10 Correspondence to: Benjamin Burpee (benjaminburpee@gmail.com)

11
12 **Abstract**

13 Permafrost is degrading across regions of the Arctic, which can lead to increases in nutrient
14 concentrations in surface freshwaters. The oligotrophic state of many arctic lakes suggests that
15 enhanced nutrient inputs may have important effects on these systems, but little is known about
16 microbial nutrient limitation patterns in these lakes. We investigated microbial extracellular
17 enzyme activities (EEAs) to infer seasonal nutrient dynamics and limitation across 24 lakes in
18 southwest Greenland during summer (June and July). From early to late summer, enzyme
19 activities that indicate microbial carbon (C), nitrogen (N), and phosphorus (P) demand increased
20 in both the epilimnia and hypolimnia by 74% on average. Microbial investment in P acquisition

1 was generally higher than that for N. Interactions among EEAs indicated that microbes were
2 primarily P limited. Dissolved organic matter (DOM, measured as dissolved organic carbon) was
3 strongly and positively correlated with microbial P demand ($R^2 = 0.84$ in July), while there were
4 no relationships between DOM and microbial N demand. Microbial P limitation in June
5 epilimnia ($R^2 = 0.67$) and July hypolimnia ($R^2 = 0.57$) increased with DOM concentration. The
6 consistency of microbial P limitation from June to July was related to the amount of DOM
7 present, with some low DOM lakes becoming N-limited in July. Our results suggest that future
8 changes in P or DOM inputs to these lakes are likely to alter microbial nutrient limitation
9 patterns.

10

11 **1 Introduction**

12 Permafrost degradation is one of the most prominent responses of arctic landscapes to
13 accelerated warming. Many factors can influence the thaw rate of permafrost (Zhang et al. 2005;
14 Jorgenson et al. 2010), but permafrost thaw is very sensitive to even small changes in air and
15 ground temperatures (Hinkel and Nelson 2003; Zhang et al. 2005; Romanovsky et al. 2007;
16 White et al. 2007). Permafrost is expected to continue to degrade in response to climate warming
17 (Jorgensen et al. 2001; Lawrence and Slater 2005; Jorgensen et al. 2006; Frey et al. 2009), with
18 some models predicting that in areas of continuous permafrost, near-surface permafrost extent
19 will decrease by 26-90% (Lawrence and Slater 2005; Anisimov and Reneva 2006) and soil active
20 layer depth will deepen by 30-100% (Stendel and Christensen 2002; Zhang et al. 2008a) over the
21 next century. Such changes are likely to alter biogeochemical cycling in soils and the aquatic
22 systems that receive material from soils.

1 The soil active layer controls much of the tundra landscape's hydrologic and
2 biogeochemical activity (Hinzman et al. 1991; Waelbroeck et al. 1997; Zhang et al. 2005; Schuur
3 et al. 2008; Frey et al. 2009), which in turn affects groundwater and nutrient inputs to arctic
4 aquatic ecosystems (Hobbie et al. 1999; Zhang et al. 2005; White et al. 2007). Degradation of
5 permafrost typically increases phosphorus (P) export to surface waters (Hobbie et al. 1999, Frey
6 and McClelland 2009) while changes in inorganic nitrogen (N) and dissolved organic carbon
7 (DOC) are less consistent. For example, with permafrost thaw, watershed DOC export in the
8 Yukon, Alaska, and Central Siberia decreased (Carey 2003; Kawahigashi et al. 2004; Striegl et
9 al. 2005; McClelland et al. 2007) whereas it increased in West Siberia (Frey and Smith 2005).

10 These water chemistry changes are important for the ecology of arctic lakes because they
11 alter nutrient and energy availability to phytoplankton and heterotrophic microbes. Hobbie et al.
12 (1999) demonstrated that permafrost thaw in Northern Alaska contributed 30% of inflowing
13 phosphate and nitrate into Toolik Lake. Long-term experimental manipulation of another lake at
14 that study site demonstrated that sustained P inputs increased primary production and increased
15 phytoplankton biomass (Hobbie et al. 1999). It was therefore speculated that P inputs from
16 permafrost degradation would increase lake eutrophication. However, another experimental
17 study on Alaskan arctic lakes indicated that N subsidies may be more important than P in
18 stimulating phytoplankton productivity (Levine and Whalen 2001). Despite these few studies,
19 the nature and magnitude of permafrost degradation effects on arctic lakes remain largely
20 uncharacterized and largely focused on phytoplankton production.

21 Heterotrophic bacteria are key to aquatic biogeochemical reactions and transformations
22 and they should be susceptible to changes in DOC and nutrient input to lakes in arctic systems
23 (Cotner and Biddanda 2002, Villar-Argaiz et al. 2002, Crump et al. 2003). Due to microbial

1 mineralization of C, arctic lakes can release significant amounts of greenhouse gases, such as
2 CO₂ and CH₄ (Kling et al. 1992). In arctic lakes, the source of DOM can shift seasonally from
3 the landscape (allochthonous DOM) to in-lake production (autochthonous) (Whalen and
4 Cornwell 1985). This can shift microbial community structure and production rates (Crump et al.
5 2003). In aquatic systems receiving nutrient subsidies, nutrient limitation of bacteria should
6 relax. This would increase the rate by which heterotrophic bacteria consume organic matter for
7 growth and respiration, resulting in increased CO₂ production in oxygenic environments, or CH₄
8 in anoxic ones (del Giorgio and Cole 1998; Smith and Prairie 2004). This has important
9 implications for arctic lakes that may receive nutrient subsidies through permafrost degradation.
10 For instance, microbial growth increased in lake and pond waters of the high Canadian Arctic
11 that received experimental P subsidies, indicating microbial P limitation (Graneli et al. 2004).
12 Alternatively, in lakes that receive fewer hydrological inputs of nutrients and DOM due to
13 increased soil active layer depth and catchment microbial activity, the size of DOM and nutrient
14 pools would decrease. Such a decrease would initiate a simplification of lake microbial food web
15 structure (Hobbie et al. 2000). Further investigating heterotrophic microbial activity in arctic
16 lakes at present is important to understanding future thaw-driven changes in nutrient inputs.

17 One way to assess the nutrient demands of microbial communities is to measure activities
18 of extracellular enzymes (EEA), used by microbes to cleave complex organic molecules into
19 smaller compounds that can be assimilated. Relative activities of enzymes associated with C, N
20 and P acquisition can be used to infer nutrient limitation following resource allocation models
21 (Sinsabaugh et al. 2008). Enzymes of interest in EEA studies commonly include β -1,4-
22 glucosidase (BG) that degrades cellulose and β -1,4-glucans to glucose for C acquisition
23 (Ljungdahl and Eriksson 1985; Sinsabaugh et al. 2008), β -N-acetylglucosaminidase (NAG) and

1 leucine aminopeptidase (LAP) which acquire N from chitin and polypeptides, respectively,
2 (Sinsabaugh and Foreman 2001; Sinsabaugh et al. 2008), and phosphatase (AP) which degrades
3 phosphomonoesters to obtain P (Turner et al. 2002; Sinsabaugh et al. 2008). These enzymes are
4 catalysts for terminal reactions in which organic matter is converted to monomer nutrients
5 (Sinsabaugh et al. 2008). As such, their activity reflects total microbial demand for C (via BG),
6 N (via NAG+LAP), and P (via AP). Recent work has established the use of EEAs as a method to
7 infer microbial nutrient limitation (Sinsabaugh et al. 2008; Moorhead et al. 2013, Hill et al.
8 2014), making EEA assays an extremely valuable tool for evaluating changing nutrient
9 concentrations in aquatic ecosystems.

10 We examined microbial nutrient limitation patterns, via EEA analysis, and water
11 chemistry in lakes spanning a range of nutrient availability in the continuous permafrost
12 landscape of southwest Greenland. While the Alaskan, Siberian, and Canadian Arctic have
13 shown consistent increases in air temperatures and active layer thickening since the mid-1970's
14 (Blunden and Arndt 2014), recent and abrupt ($>10^{\circ}\text{C}$) warming in western Greenland (Hanna et
15 al. 2012) coincides with deepening permafrost active layer only since the mid 1990's
16 (Christiansen et al. 2010). As a result, these relatively recent changes in Greenland provide a
17 unique situation in which we could examine patterns in microbe-nutrient relationships in a
18 landscape with relatively low permafrost loss, providing a baseline from which to gauge future
19 change. We measured EEA during the summer of 2013 in 24 lakes situated around
20 Kangerlussuaq, southwest Greenland. We hypothesized that most lakes would be P limited based
21 on previous findings with phytoplankton experiments in this area (Brutemark et al. 2006), but
22 that patterns in microbial enzyme allocation toward C, N and P would track variation in
23 lakewater DOC, dissolved inorganic N (DIN), and total P (TP) availability.

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2 Methods

2.1 Study site

The region around Kangerlussuaq, Greenland (67°00' N, 50°41' W, Figure 1) contains more than 20,000 lakes (Anderson et al. 2001) and is underlain by continuous permafrost estimated to be 300 m thick (Nielson 2010; Harper et al. 2011). The climate of this region is low Arctic continental with a mean summer temperature of 10.2° C. In Western Greenland, annual air temperature has increased by 3°C and annual melting degree days by 100% when comparing 2007-2012 to 1979-2000 (Mayewski et al. 2014). The region is semi-arid, receiving approximately 150 mm of precipitation per year, and even less at the ice sheet margin. Lakes in this study ranged in surface area from 0.02 to 0.8 km², and in maximum depth from 9 to 36 m. Most of these lakes are oligotrophic, with low nutrient concentrations characteristic of lakes in this region (Anderson et al. 2001, Perren et al. 2009). Lakes were first sampled in June shortly following ice-off. At that time, about half of the study lakes were weakly stratified. For those that were not, the “hypolimnion” sample depth was the estimated limit of the euphotic zone determined as twice the measured Secchi depth. Lakes were sampled again in July, during the period of stable thermal stratification for all lakes.

2.2 Environmental parameters

Physical and chemical variables of the lakes were measured to determine their relationship to microbial EEAs within the epilimnia of the study lakes. Temperature and pH were measured at the point of greatest lake depth using a submersible HydroLab Datasonde 5a. Epilimnetic and hypolimnetic water samples were collected with a van Dorn bottle. Water samples for measurement of dissolved nutrients and DOC were filtered through Whatman GF/F

1 filters that were pre-rinsed with DI water. Samples for total nutrients were unfiltered. All
2 samples were collected into acid-washed bottles and kept refrigerated until analysis. Dissolved
3 inorganic (NH_4^+ , NO_3^- , and PO_4^{3-}) and total nutrient (TN and TP) concentrations were analyzed
4 on a Lachat QuickChem 8500 flow injection analyzer. Nitrate was measured with the cadmium
5 reduction method, NH_4^+ with the phenate method, and PO_4^{3-} with the ascorbic acid method
6 (APHA 2000). TN and TP were determined by measurement of NO_3^- and PO_4^{3-} on unfiltered
7 water samples following digestion with persulfate (APHA 2000). Quantification limits on all
8 nutrients were $2 \mu\text{g L}^{-1}$ except for TN, which was $10 \mu\text{g L}^{-1}$. For statistical analyses, nutrient
9 values below the $2 \mu\text{g L}^{-1}$ quantification limit were replaced with 1. Dissolved inorganic
10 nitrogen: TP (DIN:TP) ratios were calculated, with DIN determined by the addition of NH_4^+ and
11 NO_3^- . The DIN:TP ratio is a useful metric for inferring nutrient limitation, moving from N to P
12 limitation with an increase from 1.5 to 3.4 (Bergstrom 2010). DOC was analyzed with an OI
13 Analytical Aurora 1030D TOC analyzer using wet chemical oxidation.

14 **2.3 EEA analysis**

15 Water samples for EEA analysis were collected in the same way as total nutrient samples
16 (i.e., not filtered). Due to the remote location of the lakes samples from June were stored frozen
17 (-20°C) for 60 days and samples from July were refrigerated for 30 days and then frozen for 30
18 days before analysis. Though the analysis of fresh samples is considered preferable due to the
19 uncertainty of whether freezing introduces bias into results, it is common for freshwater EEA
20 studies to freeze samples owing to logistical constraints (e.g. Simon et al. 2009; Clinton et al.
21 2010; Freimann et al. 2013; Parr et al. 2015). We are assuming that if freezing had any effect it
22 was similar across systems. EEA samples were thawed, processed and analyzed with a Thermo
23 Electron Corporation Fluoroskan Ascent FL fluorescence spectrophotometer using fluorescent-

1 labeled substrates following published methods (Sinsabaugh and Foreman 2001; Findlay et al.
2 2003). Fluorescent substrates were used to measure activity of BG (4-MUB- β -D-glucoside),
3 NAG (4-MUB-N-acetyl- β -D-glucosaminide), LAP (L-Leucine-7-AMC) and AP (4-MUB-
4 phosphate). Briefly, 200 μ L sub-samples from each lake sample were added in triplicate to 96-
5 well assay plates and combined with 50 μ L of substrate for a final saturated substrate
6 concentration of 200 μ M and assayed at 25°C. Controls for substrate and sample fluorescence
7 and quenching were included. Pilot assays were used to ensure substrate concentrations saturated
8 enzyme kinetics such that kinetic rates were equal to V_{max} , and readings were made during linear
9 increases in fluorescence. Throughout the analysis, steps were taken to standardize and optimize
10 the procedure following the suggestions of German et al. (2011).

11 **2.4 Data analysis**

12 Microbial nutrient limitation was inferred from activity of individual enzymes and from
13 ratios of multiple enzymes. Across ecosystems, nutrient acquisition effort as measured by BG,
14 NAG+LAP, and AP is typically close to 1:1:1 based on global empirical evidence and following
15 stoichiometric and metabolic theories (Sinsabaugh et al. 2008, 2009). Departures from these
16 values are indicative of differential microbial nutrient demand as microbes invest resources in
17 enzymes to acquire limiting nutrients. Degree of C limitation can be inferred from ratios of C to
18 nutrient acquiring enzymes (BG:NAG+LAP and BG:AP; Sinsabaugh et al. 2008, 2009). Further,
19 the stoichiometric ratios BG:NAG+LAP and BG:AP can be considered in concert to gauge
20 degree of microbial N or P limitation (Moorhead et al. 2013; Hill et al. 2014). This can be done
21 by plotting BG:NAG+LAP vs. BG:AP and measuring deviation from the 1:1 line which
22 indicates equal nutrient acquisition effort (Sinsabaugh et al. 2008; Sinsabaugh et al. 2009;
23 Moorhead et al. 2013; Hill et al. 2014). On these plots the distance from the origin to a data point

1 forms a vector. Deviation of the vector angle from the 1:1 (45°) line indicates increasing P
2 (higher angles) or N (lower angles) limitation (Moorhead et al. 2013; Hill et al. 2014). Figure 2
3 displays hypothetical data from a lake in June and July plotted onto a vector plot with the 1:1 line
4 drawn in dashes. The vectors from which angles are calculated are shown as arrows from the
5 origin to the individual data points. In June, the vector angle is positive with respect to the 1:1
6 line ($> 45^\circ$) indicating P limitation in this lake. However, in July nutrient limitation shifts from P
7 to N, as indicated by the negative angle with respect to the 1:1 line ($< 45^\circ$). We quantified vector
8 angles for our samples and display the data as degrees of deviation from the 1:1 line such that
9 positive values indicate P limitation and negative ones indicate N limitation (Figure 5). Lastly,
10 the distance of data points from the origin indicates microbial investment in C acquisition
11 relative to that of N and P, such that C demand is indicated by larger vector lengths (Moorhead et
12 al. 2013, Hill et al. 2014).

13 To test whether water quality parameters, nutrient concentrations, or enzyme-related
14 activities differed between months (June vs. July) or lake strata (epilimnia vs. hypolimnia), two-
15 tailed, paired t-tests were used. To determine whether certain factors, such as nutrient
16 concentrations or ratios, were related to enzyme activities, simple least squares linear regression
17 was used with a level of significance of $p = 0.05$. All statistical analyses were completed using R
18 (version 3.1.2).

19

20 **3 Results**

21 **3.1 Water quality parameters**

22 Several physical and chemical parameters varied from June to July in lake epilimnia
23 (Table 1; Supplementary Table 1). Surface water temperatures increased between June and July,

1 on average from 7.8° to 13.5°C ($p < 0.01$). pH was relatively consistent across lakes (5.8 – 7.7)
2 but on average increased from 6.6 in June to 7.0 in July ($p < 0.01$). DOC ranged tenfold from 4
3 to 40 mg L⁻¹ across study lakes, and slightly increased, on average, over the summer (12.1 to
4 13.4 mg L⁻¹, $p < 0.01$). DIN ranged from 2 to 25 µg L⁻¹, and increased from June to July (6 to 12
5 µg L⁻¹, $p < 0.01$), as did TN (467 to 554 µg L⁻¹, $p < 0.01$, range from 178 to 1132 µg L⁻¹). TP
6 ranged from <2 to 12 µg L⁻¹ and did not appreciably increase from June to July (4 to 5 µg L⁻¹, p
7 = 0.68). PO₄³⁻ was below quantification limits at all times. DIN:TP ranged from 0.17 to 22 and
8 increased from June to July (2.1 to 5.4, $p < 0.01$). TN was tightly related to DOC in both months
9 (June; $R^2 = 0.83$, $p < 0.01$; July $R^2 = 0.77$ $p < 0.01$). There were no relationships between TP and
10 DOC, or DIN:TP and DOC.

11 **3.2 Seasonal and spatial patterns in enzyme activities**

12 Activity of all enzymes increased from June to July in both the epilimnia (p values \leq
13 0.03) and hypolimnia (p values ≤ 0.02 ; Figure 3). Averaged between lake strata, BG activity
14 increased 73%, NAG+LAP 79%, and AP 70% from June to July. Averaged across lakes,
15 absolute activities of single enzymes differed between epilimnetic and hypolimnetic samples. For
16 BG, epilimnetic activities were 1.7x higher than those of hypolimnia in June ($p = 0.01$) and 1.3x
17 higher in July ($p = 0.42$). For NAG+LAP, epilimnetic activities were 1.1x and 1.3x higher than
18 those of hypolimnia in June and July ($p < 0.01$ and $p = 0.01$, respectively). There were no
19 differences in AP activities between strata in either month (p values > 0.05).

20 Ratios of C to nutrient-acquiring enzyme activity varied by lake strata and time (Figure
21 4). Hypolimnetic BG:AP was consistently lower than epilimnetic BG:AP, though the difference
22 was not significant in July (June $p = 0.01$, July $p = 0.09$). In June, epilimnetic BG:NAG+LAP
23 was greater than that of the hypolimnia ($p = 0.03$), but in July there was no difference ($p = 0.72$).

1 There were no seasonal differences in BG:AP or BG:NAG+LAP within the same stratum (p
2 values < 0.05). BG:NAG+LAP was greater than BG:AP in epilimnia and hypolimnia in June (p
3 values < 0.01) but not in July (p values > 0.05). These data suggest microbes were investing
4 more in P and less in N acquisition across lakes in June but not July.

5 Vector angles were mostly positive indicating consistent microbial P limitation across all
6 lakes in both time periods (Figure 5). In the epilimnia, the angle magnitude was about 3 times
7 lower in July than in June suggesting relaxed P limitation later in the year (p < 0.01, Table 2).
8 This was less obvious in the hypolimnia where June and July data were much more similar (p =
9 0.04). Angles in the hypolimnia were 1.3x greater than those of the epilimnia in June and 3.6x
10 greater in July (p values < 0.01). On the vector plots, distance of data points from the origin did
11 not change between June and July within the same strata (Table 2). This indicates that microbial
12 investment in C acquisition did not appreciably vary with respect to N- and P-acquiring
13 enzymes. In June, the investment in microbial C acquisition was greater in the epilimnia
14 compared to the hypolimnia (mean vector length 8.9 vs. 4.7, p = 0.02). In July, however, this
15 trend was no longer significant (p = 0.54), indicating distributed C acquisition of similar
16 magnitude across lake strata in late summer.

17 **3.3 Relationships between water chemistry and enzyme activities in lake** 18 **epilimnia**

19 Due to sampling constraints, DOC was measured in epilimnetic water only. There was a
20 strong, linear increase in epilimnetic AP activity with increasing DOC concentration in June and
21 July ($R^2 = 0.73$ and 0.84 , respectively, p values < 0.01, Figure 6). BG activity was not related to
22 DOC in June or July. Likewise, the activities of the N-acquiring enzymes, NAG+LAP, were
23 unrelated to DOC in both seasons. None of the absolute EEAs were related to epilimnetic

1 DIN:TP. When considered as enzyme ratios, epilimnetic investment in C:P acquisition (BG:AP)
2 decreased with DOC concentration in June ($R^2 = 0.24$, $p = 0.01$) and was unrelated to DOC in
3 July. C:N acquisition (BG:NAG+LAP) was unrelated to DOC in both months.

4 The magnitude of epilimnetic microbial P limitation, described by vector angles,
5 increased with rising DOC concentration in both months (June and July $R^2 = 0.67$ and 0.57 ,
6 respectively, p values < 0.01 , Fig. 6). There were no relationships between vector angles and
7 DIN:TP (data not shown). Likewise, there were no statistically significant relationships between
8 vector length (i.e. C-limitation) and water chemistry (data not shown).

9

10 **4 Discussion**

11 Our results reveal a pattern of microbial P limitation across these southwest Greenland
12 arctic lakes. Vector analysis indicated more severe P limitation in June compared to July, despite
13 DIN:TP increasing in July. The overall pattern of P limitation of bacterioplankton and
14 phytoplankton is consistent with previous research of plankton alkaline phosphatase activity in
15 two lakes in the same study region, which also suggested P limitation of plankton communities
16 (Brutemark et al. 2006). P limitation can be a factor that controls lake algal and microbial
17 productivity and trophic status. In the High Canadian Arctic, for instance, P subsidies to lake and
18 pond water caused increased microbial growth, indicating P as the primary limiting nutrient
19 (Graneli et al. 2004). This was supported by another study completed across 20 lakes in Quebec,
20 Canada, which demonstrated that TP, and not DOC, controlled microbial growth rates,
21 respiration rates, and growth efficiency (Smith and Prairie 2004). Further, P availability
22 controlled the fate of DOC, such that in oligotrophic, low-P concentration environments, DOC
23 was mostly used for respiration (converted to CO_2), rather than being incorporated into biomass.

1 Various measures of enzyme activity indicated a positive relationship between P
2 limitation and DOC, contrary to our expectation that DIN:TP might be a stronger predictor.
3 Here, we quantitatively measured and reported it as DOC, but for discussion, DOM is a more
4 appropriate term as it includes organic N and P as well as carbon. DOM is a broad group of
5 organic compounds with varying lability depending on the source, chemical structure, and N and
6 P content (Mineau et al. 2013, Parr et al. 2015). In these lakes, DOM positively co-varied with
7 TN but had no relationship with TP. Collectively, these enzyme and water chemistry data
8 suggest that the DOM in these lakes may provide a readily available source of N, while higher
9 DOM concentrations are associated with enzyme-mediated microbial P acquisition.

10 DOM can contain distinct nutrient pools available for microbial consumption when
11 conditions become stoichiometrically favorable. For instance, in a study of N-limited humic
12 lakes in Northern Sweden, DOM-associated P was used by bacterioplankton and phytoplankton
13 when N was added into the experimental systems (Jansson et al. 2001). Furthermore, in the same
14 study the authors showed that bacterioplankton production was strongly controlled by DOC,
15 such that bacterioplanktonic production in water containing 15-20 mg L⁻¹ of DOC could not
16 stimulated by further nutrient addition. However, DOM is not consistently a source of P in all
17 lakes. Phosphorus amendments, in addition to simulated sunlight, were important in stimulating
18 microbial degradation of DOC in an experiment using water from a southern Sweden humic
19 lake, suggesting P limitation (Kragh et al. 2008). Another study of humic lakes located in
20 southern Sweden demonstrated that P alone was not sufficient for stimulating microbial
21 respiration and production; a source of labile C (glucose) was also required (Vidal et al. 2011).
22 Together, these studies demonstrate that the interactions between bacteria and DOM are

1 complex—DOM can either behave as a source of bioavailable material, providing bacteria with
2 energy and certain nutrients, or remain a recalcitrant, unavailable pool of organic compounds.

3 DOM remained an important factor in determining microbial P demand and limitation
4 from June to July, suggesting DOM is the dominant source of nutrients available for microbial
5 degradation and use. Lakes with low DOM appeared to have more seasonality of nutrient
6 limitation than those with higher concentrations—as can be inferred from vector angles in Figure
7 7A, only low DOM lakes switched from P to N limitation in July, while higher DOM lakes
8 remained consistently P limited. If the DOM pool is representative of N availability, it follows
9 that N limitation would be more likely in low than in high DOM lakes. A seasonal shift in the
10 type of DOM pool in low DOM lakes could also be contributing to seasonal differences in
11 DOM-related nutrient dynamics. Crump et al. (2003) investigated bacterioplankton community
12 dynamics in relation to DOM in Toolik Lake, Alaska. In spring DOM was flushed from the
13 landscape into an inlet stream, and was labile due to extended soil and plant leaching, freeze-
14 thaw processing, and microbial cell lysis. Moreover, this DOC was transported across the surface
15 of the frozen tundra rather than the subsurface soils. The quality of this DOC then decreased as
16 leaching of organic material decreased and the active layer deepened, allowing microbial
17 degradation of DOM during transport.

18 In some areas of the Arctic, discharge-normalized DOC export to arctic surface waters
19 has decreased in recent decades (Striegl et al. 2005), with permafrost thaw and soil active layer
20 deepening contributing to this trend (Carey 2003; Kawahigashi et al. 2004; Striegl et al. 2005;
21 Striegl et al. 2007). If lakewater DOM concentrations and quality are also declining, it is likely
22 that nutrient subsidy and limitation patterns will also change. Our data suggest that lakes
23 receiving less DOC may become less P-limited and move towards N limitation, since DOM is

1 being suggested as an N source. Control of microbial production of enzymes and nutrient
2 limitation may shift from organic matter to inorganic nutrients that are primarily flushed into the
3 lakes during snowmelt and ice-off. Conversely, if DOC input into lakes were to increase due to
4 increased terrestrial production within catchments, lakes would be predicted to become
5 universally P-limited. Changes in nutrients sources and concentrations will affect microbial and
6 autotrophic productivity (Smith and Prairie 2004; Elser et al. 2007). It is likely that as the amount
7 of lake DOM changes, DOM composition and lability will shift as well. Bacterial community
8 structure has been shown to change in correspondence with DOM quality in arctic lakes, as some
9 bacteria prefer more labile compounds while other species are adapted to utilizing recalcitrant
10 forms (Crump et al. 2003). In this study, the seasonal source and quality of the DOM pool might
11 have been inferred by the inclusion of oxidative enzymes, such as phenol oxidase or peroxidase,
12 which are responsible for degrading terrestrially-derived compounds such as phenols and
13 aromatics, respectively (Sinsabaugh et al. 2008). Though BG is assumed to broadly represent C
14 acquisition activity, oxidative enzyme activity may be an important metric in future studies.

15 Bacteria are of primary importance to freshwater ecosystems, as they transfer energy and
16 nutrients often contained in organic matter to higher trophic levels (Azam et al. 1983), yet
17 relatively little research on microbial ecology has been conducted in arctic lakes. It is therefore
18 important to consider microbial responses to factors that will be changing in the near future (such
19 as active layer depth and DOM concentrations) in order to understand ecological effects and
20 directions of future change. We found that microbes in southwest Greenland lakes are generally
21 P-limited, and that the strength of microbial P limitation decreased by mid-summer. Further,
22 DOM was very important in determining microbial nutrient demands and limitation due to its
23 potential as an N source. Lakes within the permafrost landscape of this study region are likely to

1 experience shifts in nutrient limitation patterns as aquatic-terrestrial linkages potentially weaken
2 (due to active layer increase) and DOM inputs decline. This study establishes current microbial
3 nutrient limitation patterns that will allow us to assess response to future changes in this region.
4

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11

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Table 1. Epilimnetic physical and chemical data across the 24 lakes in June and July 2013. Significant changes ($p \leq 0.05$) between June and July means indicated by asterisk (*). Depth was not measured in July. S.E. = standard error.

	June			July		
	Mean	S.E.	Range	Mean	S.E.	Range
Depth (m)	19.7	1.67	8-36	-	-	-
pH	6.6	0.08	5.8-7.2	7.0*	0.07	6.4-7.7
Temperature (°C)	7.8	0.40	5.3-11.6	13.5*	0.35	9.1-17.4
DIN ($\mu\text{g L}^{-1}$)	6	1.2	2-25	12*	1.1	4-22
TN ($\mu\text{g L}^{-1}$)	467	47	178-1042	554*	61	197-1132
TP ($\mu\text{g L}^{-1}$)	4	0.7	<2-12	5	0.7	<2-11
DOC (mg L^{-1})	12	1.9	4-35	13*	2.2	4-40
DIN:TP	2.1	0.37	0.17-6.0	5.4*	1.4	0.55-22

Table 2. Nutrient Limitation and C acquisition as indicated by mean vector angle and length for both months. Significant changes ($p \leq 0.05$) between June and July means indicated by asterisk (*). S.E. = standard error.

		June			July		
		Mean	S.E.	Range	Mean	S.E.	Range
Epilimnia	Vector Angle (°)	16	2.82	-8 - 41	4*	5.52	-32 - 41
	Vector Length	8.9	1.75	1.6 - 40.2	9.9	2.98	0.2 - 58.4
Hypolimnia	Vector Angle (°)	20	3.08	-6 - 45	15*	4.93	-30 - 43
	Vector Length	4.7	1.10	0.2 - 24.8	7.3	2.69	0.2 - 62.8

1 Figure 1. Map of study site, with the 24 study lakes surrounding Kangerlussuaq, Greenland
2 indicated by dots.

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

10 Figure 3. Absolute enzyme activities for: A) β -1,4-glucosidase (BG), B) β -N-
11 acetylglucosaminidase and leucine aminopeptidase (NAG+LAP), and C) alkaline phosphatase
12 (AP) across epilimnia and hypolimnia of study lakes, from June to July. Error bars are standard
13 deviation. Units of activity are $\mu\text{mol mL}^{-1} \text{hr}^{-1}$.

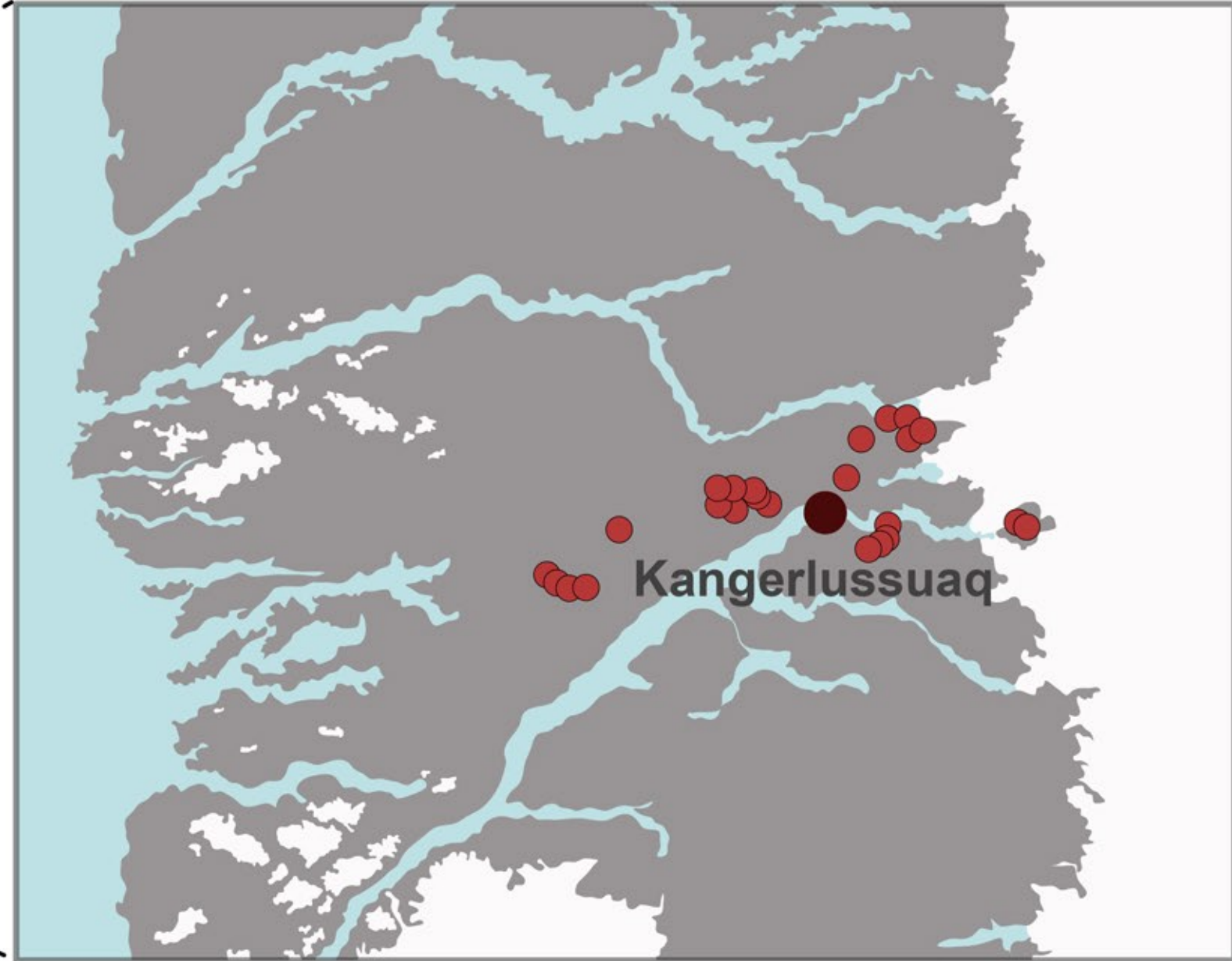
14 Figure 4. EEA ratios of BG relative to those of A) NAG+LAP and B) AP across lake strata for
15 June and July. Error bars are standard deviation.

16 Figure 5. Scatterplot of microbial enzyme ratios (BG : NAG + LAP vs. BG : AP) about the 1:1
17 line. Included is C:P and C:N acquisition data of lake epilimnia (circles) and hypolimnia
18 (triangles) from June (gray) to July (black). Dotted line indicates 1:1 (45 °) line. Vector angles
19 (indicative of nutrient limitation) are calculated from these plotted data points, as deviation from
20 the 1:1 line.

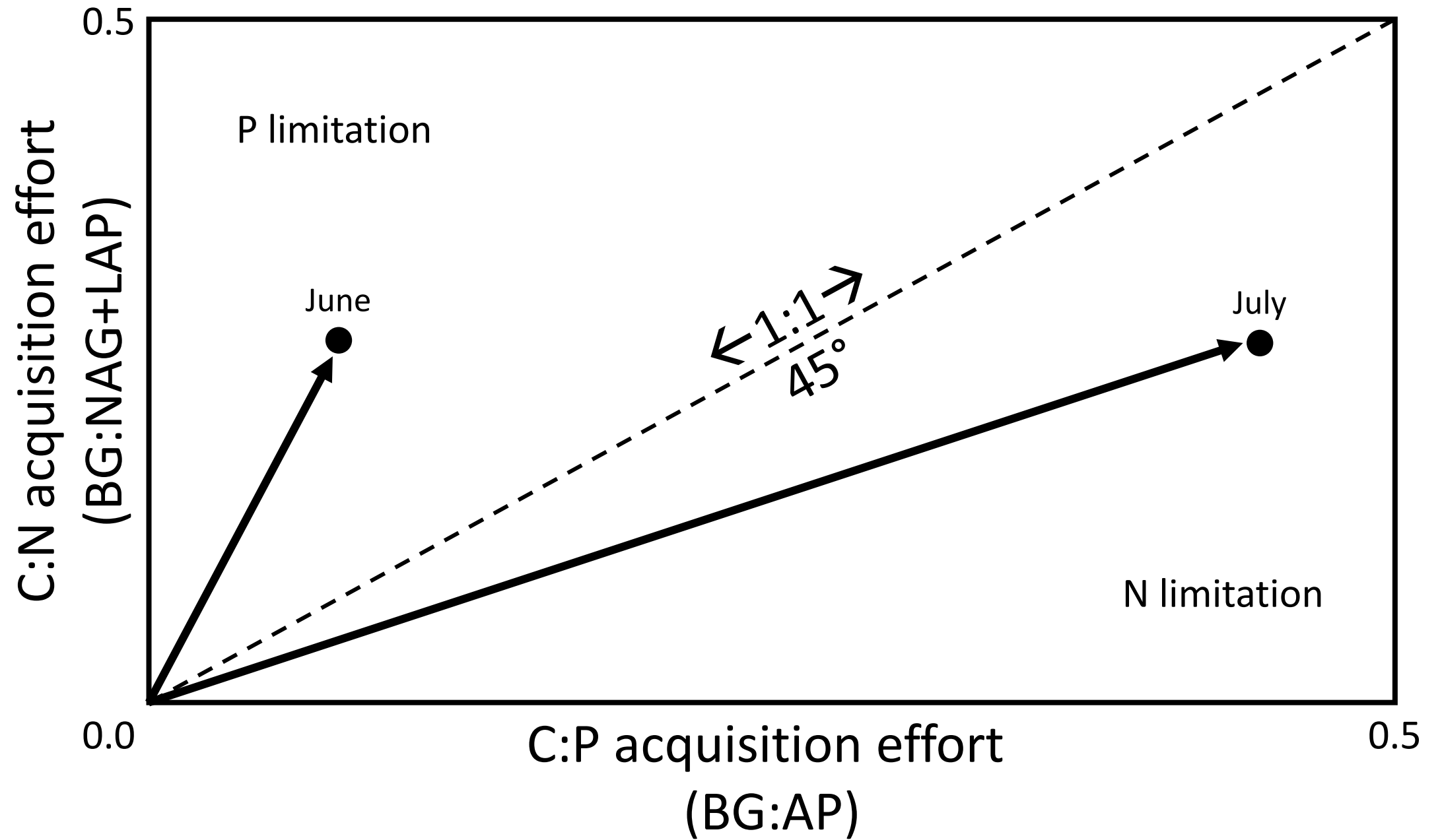
1 Figure 6. Response of A) AP, B) BG, and C) BG:AP to DOC in lake epilimnia. Data from June
2 are indicated in grey triangles, data from July are indicated in black circles. Only significant
3 relationships are displayed.

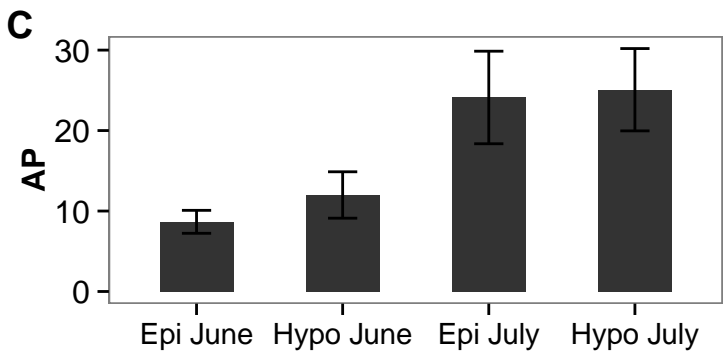
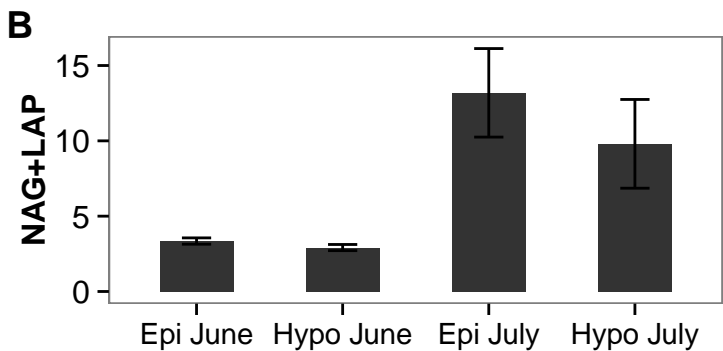
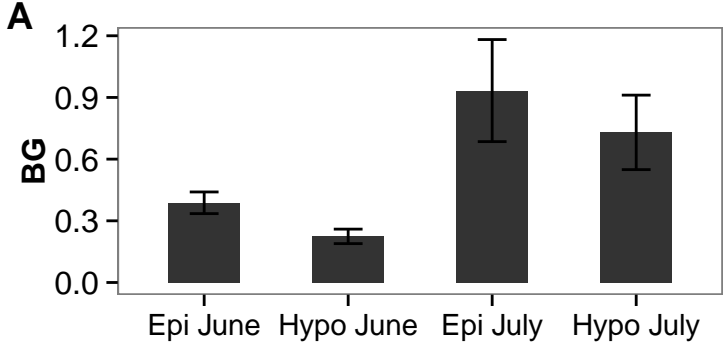
4 Figure 7. Vector angles in response to A) DOC, and B) epilimnetic DIN:TP. Data from June are
5 indicated in grey triangles, data from July are indicated in black circles. Dotted line indicates the
6 boundary between P limitation (positive values) and N limitation (negative values). Only
7 significant relationships are displayed.

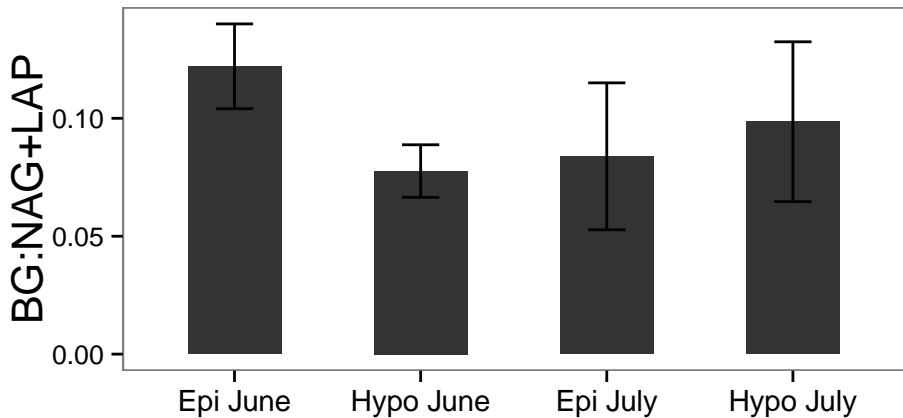
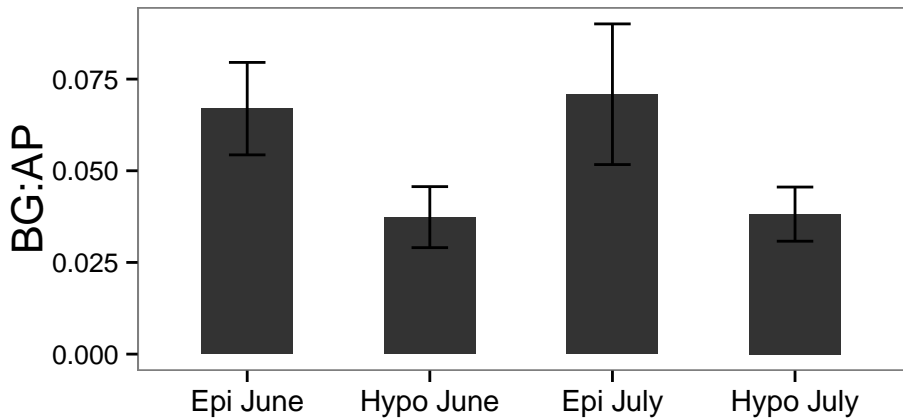
GREENLAND

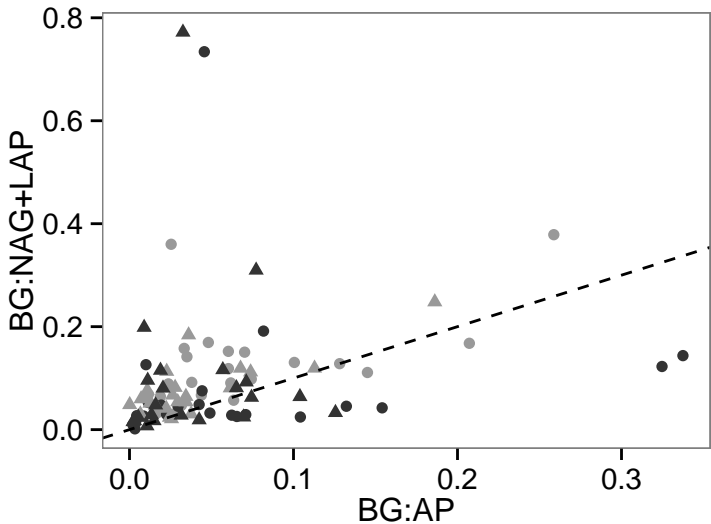


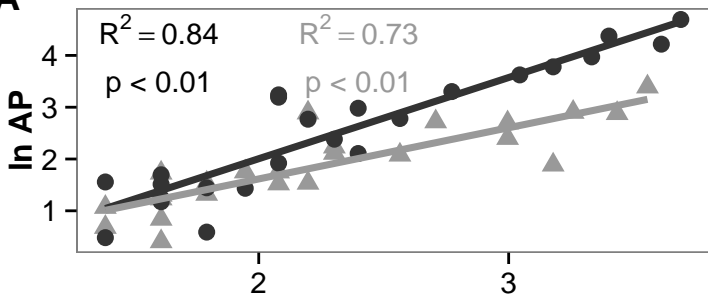
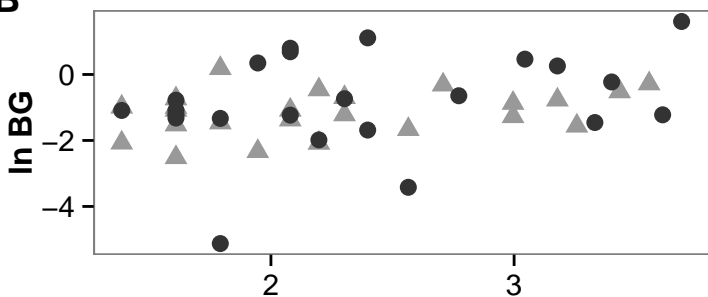
Kangerlussuaq









A**B****C**