

Persistent nitrogen limitation of stream biofilm communities along climate gradients in the arctic

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28 Abstract

29 Climate change is rapidly reshaping arctic landscapes through shifts in vegetation cover and
30 productivity, soil resource mobilization, and hydrological regimes. The implications of these
31 changes for stream ecosystems and food webs is unclear and will depend largely on microbial
32 biofilm responses to concurrent shifts in temperature, light, and resource supply from land. To
33 study those responses, we used nutrient diffusing substrates to manipulate resource supply to
34 biofilm communities along regional gradients in stream temperature, riparian shading, and
35 dissolved organic carbon (DOC) loading in arctic Sweden. We found strong nitrogen (N)
36 limitation across this gradient for gross primary production, community respiration and
37 chlorophyll-a accumulation. For unamended biofilms, activity and biomass accrual were not
38 driven by any single physical or chemical driver across this region. However, the magnitude of
39 biofilm response to N addition did: in tundra streams, biofilm response was constrained by
40 thermal regimes, whereas variation in light availability regulated this response in birch and
41 coniferous forest streams. Furthermore, heterotrophic responses to experimental N addition
42 increased across the region with greater stream water concentrations of DOC relative to inorganic
43 N. Thus, future shifts in resource supply to these ecosystems are likely to interact with other
44 concurrent environmental changes to regulate stream productivity. Indeed, our results suggest
45 that in the absence of increased nutrient inputs, arctic streams will be less sensitive to future
46 changes in other habitat variables such as temperature and DOC loading.

48 Introduction

49 Global climate change is currently altering the ecological structure and functioning of arctic
50 landscapes. Warmer temperatures and modified precipitation patterns affect key biophysical
51 features of high latitude ecosystems, including the length of growing seasons and the magnitude
52 and timing of soil frost and snowmelt (ACIA 2004). In turn, these conditions have led to greater
53 terrestrial productivity (Mao et al. 2016), shifts in the spatial distribution of trees and shrubs (Xu
54 et al. 2013), altered seasonal hydrology (Déry et al. 2005), and changes in the mobilization and
55 export of soil resources (McClelland et al. 2007). While research on climate-sensitivity in the
56 arctic has overwhelmingly emphasized terrestrial ecosystems, the close connection between
57 running waters and the catchments they drain make it likely that these observed changes on land
58 also influence the productivity of streams and rivers (Davis et al. 2013). Yet predicting how

59 running waters will respond to climate change at high latitudes remains a major challenge and
60 requires that we understand how concurrent alterations to thermal, light, flow, and resource
61 regimes interact to shape patterns of biological activity. To this end, our study explores how these
62 interacting factors influence stream biofilm productivity along a climate gradient in arctic
63 Sweden.

64
65 The effects of catchment greening, expanding tree lines, increased runoff, and altered resource
66 supply on arctic streams will ultimately depend on the response of microbial biofilms to changing
67 habitat conditions. Stream biofilms constitute a complex aggregation of algae, bacteria, and fungi
68 embedded in a polysaccharide matrix attached to organic and inorganic surfaces in benthic and
69 hyporheic habitats (Lock et al. 1984, Battin et al. 2016). These assemblages are responsible for
70 much of the metabolic activity in streams and rivers (Battin et al. 2008), serve as vital energy
71 sources to higher trophic levels (McCutchan & Lewis 2002), and mediate key biogeochemical
72 processes, including greenhouse gas production (Rasilo et al. 2017) and nutrient uptake (Lupon et
73 al. 2016).

74
75 Stream biofilms support autotrophic and heterotrophic processes that are differentially sensitive
76 to changes in habitat condition. Photosynthesis in streams is obviously linked to incident light
77 (Hill et al. 1995), but algal growth may also be limited by inorganic nutrients (Reisinger et al.
78 2016), and constrained by physical disturbance (Biggs 1995), flow velocity (Peipoch et al. 2016),
79 and water temperature (Rasmussen et al. 2011). Heterotrophic processes share some of these
80 same physical and chemical drivers; in particular, nutrient supply (Burrows et al. 2015) and
81 thermal regimes (Jankowski et al. 2014) can strongly influence the activity of stream bacteria and
82 fungi. However, heterotrophs are additionally influenced by the chemical structure and quantity
83 of organic carbon available to fuel metabolism (e.g. del Giorgio & Cole 1998, Burrows et al.
84 2017), which derives from soils and detritus (Rasilo et al. 2017), and algae (Scott et al. 2008).

85
86 Climate change in the arctic will shift a number of these physical and chemical drivers, and stream
87 biofilm responses will ultimately depend on how these factors interact. For example, warmer
88 temperatures are very likely to elevate rates of biological activity in northern streams (Friberg et
89 al. 2009, Hood et al. 2017), yet these responses may be constrained or amplified by concurrent

90 changes to light and/or resource supply (Cross et al. 2015). Similarly, while arctic streams are
91 often well lit and can support comparatively high rates of gross primary production (GPP; Huryn
92 et al. 2014), the encroachment of riparian zones by trees and shrubs may constrain algal growth
93 through shading (cf. Warren et al. 2016), regardless of changes in temperature and/or resource
94 availability. Finally, observed increases (McClelland et al. 2007) or decreases (Lucas et al. 2016)
95 in the loading of dissolved organic carbon (DOC) and nutrients to arctic streams also have the
96 potential to alter biofilm processes. Indeed, these ecosystems are often oligotrophic (Peterson et
97 al. 1983, Gudmundsdottir et al. 2011), and fertilization experiments have shown clear ecosystem
98 responses to enrichment (Peterson et al. 1993). However, such experiments to date have
99 addressed the role of nutrient availability in isolation, and the significance of resource limitation
100 in the face of other factors that potentially exert stronger and/or interactive influences on biofilm
101 production (e.g., light, temperature, and disturbance) remains poorly understood (Kendrick &
102 Huryn 2015).

103
104 In this study, we ask: How does resource availability interact with different aspects of the
105 physical habitat template to influence biofilm accrual in arctic streams? To answer this, we used
106 nutrient diffusing substrates (NDS) to measure autotrophic and heterotrophic biofilm activity and
107 resource limitation in streams located along a regional climatic and vegetation gradient in arctic
108 Sweden. This gradient encompasses tundra-, birch-, and coniferous-dominated catchments that
109 reflect broad-scale changes in precipitation and air temperature. From the standpoint of stream
110 habitat, this gradient captures large changes in 1) seasonal water temperature linked to the timing
111 of snowmelt, 2) incident light arising from variable riparian canopy cover, and 3) DOC and
112 possibly nutrient loading from catchment soils. Our design thus provides an opportunity to assess
113 stream biofilm activity and biomass accrual and its potential resource limitation in response to
114 multiple changes in land-water connections that we might expect for a warmer arctic. Further, to
115 place our findings in a broader context, we also summarized published results from similar
116 bioassays carried out in streams and lakes across arctic ecosystems.

117 118 **Materials and methods**

119 **Study site**

120 We used 12 streams in northern Sweden to investigate how resource availability influences
121 biofilm accrual and activity. Four streams each drain sub-catchments dominated by one of three
122 arctic landscape units: heath-tundra, birch forest, or coniferous forest (“stream types”, Fig. 1).
123 The streams are located some 200 km above the Arctic Circle and are distributed along a regional
124 vegetation gradient ranging from 540 m.a.s.l in the tundra catchments to about 340 m.a.s.l in the
125 coniferous, with birch forest catchments in the middle of this gradient. Climate varies along this
126 gradient from continental in the coniferous to maritime in the tundra. Accordingly, precipitation
127 increases from ca. 490 mm yr⁻¹ in the coniferous catchments to 840 mm yr⁻¹ in the tundra
128 catchments, while annual temperature is relatively unchanged around -1.7 °C (climate data,
129 SMHI 2016). Total atmospheric N deposition (wet+dry) is <1 kg N ha⁻¹ yr⁻¹ (Bergström et al.
130 2013). Annual growing season length is about 100 days yr⁻¹ and it is considerably longer in the
131 coniferous catchments compared to tundra (Karlsen et al. 2008). Snow depth has increased in the
132 area over the last climatic period (Kohler et al. 2006) but the duration of ice cover in Lake
133 Torneträsk has decreased (Callaghan et al. 2010). Discontinuous permafrost exists in the
134 surroundings of our study area although highly connected to peat soils (Åkerman & Johansson
135 2008, Gislén et al. 2017) and thus unlikely to be present in our studied catchments. All studied
136 streams are headwaters with catchment areas less than 6 km²; stream depth varied between 10 to
137 50 cm during the study period and stream width was always less than 3m (S1). Spot
138 measurements of flow velocity (EM flow meter, Valeport, Devon, U.K.) were relatively stable
139 over time, with an average of 0.23 (±0.02 standard error, hereafter SE) m s⁻¹ across stream type
140 and season.

141 142 Resource limitation experiment

143 We conducted a resource limitation experiment using NDS surfaces to grow microbial biofilms
144 following Tank et al. (2006). We replicated the experiment three times (late August 2014, June
145 2015, and July 2015) to capture the range of physical and chemical conditions during the arctic
146 growing season. Briefly, we filled 30-mL plastic cups with 2% agar solution and capped them
147 with a 30-mm diameter top. Porous ceramic tops were used to mimic inorganic surfaces that
148 favor the accrual of autotrophic dominated biofilms. We constructed an additional set of NDS
149 cups capped with a 30-mm cellulose sponge top to mimic organic surfaces, which favor
150 heterotrophic organisms in the biofilm, but do not entirely exclude autotrophs (see Johnson et al.

151 2009). Accordingly, in this study, rates of respiration were, on average, thirteen times higher on
152 organic than inorganic surfaces. Both inorganic and organic NDS were enriched with 0.5 M
153 NaNO_3 (N treatment), 0.5 M KH_2PO_4 (P treatment), both (NP treatment), or unamended agar (A
154 treatment). For the organic NDS, we also added 0.5M $\text{C}_2\text{H}_3\text{NaO}_2$ (acetate, C treatment) and a
155 combination of all resources (CNP treatment). Four replicates of each treatment (16 inorganic
156 and 24 organic NDS in total per stream) were randomly attached with cable ties to a stainless
157 steel L-bar, placed underwater in main channels at a depth of approximately 20 cm. NDS were
158 deployed for 19 days, which is the recommended time period for maintaining elevated rates of
159 diffusion and enrichment (Tank et al. 2006, Bernhardt & Likens 2004, and Lang et al. 2004 for
160 additional information about diffusion rates). Upon removal, we placed surfaces individually in
161 50 mL Falcon centrifuge tubes filled with unfiltered stream water. At retrieval, the depth of the
162 NDS was more variable, from 5-40 cm. Samples were stored refrigerated (4°C) until analyses the
163 following day.

164
165 Biofilm primary production and respiration were measured using the modified dark bottle method
166 (Johnson et al. 2009). We replaced all water in the centrifuge tubes in the lab with unfiltered
167 stream water (oxygenated and close to room temperature), without headspace or noticeable
168 bubbles and of known dissolved oxygen (DO) concentration measured using a handheld DO
169 probe (YSI, Yellow Springs, U.S.A). Centrifuge tubes were then incubated in a Sayno MLR-351
170 growth chamber under light (at an average of $130 \mu\text{mol m}^{-2} \text{s}^{-1}$) for 3h and a final DO
171 measurement was taken at the end of incubation. After light incubations, water was replaced
172 again in each tube (with oxygenated water of known DO concentration as above) before the same
173 surfaces were incubated again for 3h in the dark. Note that organic NDS surfaces were only
174 incubated in dark for 3h. In addition, three centrifuge tubes from each stream were filled with
175 unfiltered stream water and incubated as controls in the light and dark, to correct for any
176 background DO changes during the incubations. Following incubations, organic surfaces were
177 pooled by stream and treatment and frozen at -80°C for later chlorophyll-a (Chl-a henceforth)
178 analysis.

179
180 Metabolic rates were calculated as the difference in DO between start and finish of incubations,
181 correcting for any DO change in the controls, and presented per surface area ($\mu\text{g O}_2 \text{cm}^{-2} \text{h}^{-1}$).

182 Gross primary production (GPP) was calculated on inorganic surfaces as mass of oxygen
183 produced during light incubation plus oxygen consumed during dark incubation. We calculated
184 community respiration (CR) as the mass of oxygen consumed during dark incubation on organic
185 surfaces. All presented CR data in the results thus represent organic surfaces, which were
186 intended to emphasize heterotrophic processes (Johnson et al. 2009). Additional analysis of algal
187 specific growth rate (i.e. GPP per unit of Chl-a) is available as supplementary material (S6).

188
189 Physical and chemical parameters
190 During all NDS deployment periods, we recorded light and temperature data every hour using
191 HOBO pendant loggers (Onset Computer Corporation, Borne, U.S.A.). We attached one or two
192 loggers to each set of NDS and converted lux to photosynthetically active radiation (PAR; using a
193 conversion factor of 0.0185 according to Thimijan & Heins 1983). Light data are presented as
194 daily photon flux ($\text{mol photons m}^{-2} \text{ day}^{-1}$) and percent time during deployment above a threshold
195 ($100 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$) that has been shown to be important to photosynthesis in streams (Hill
196 et al. 2009). We measured water velocity and concentrations of nitrate (NO_3^-), ammonium
197 (NH_4^+), soluble reactive phosphate (SRP) and DOC at beginning and end of each deployment
198 period. Dissolved inorganic nitrogen (DIN) was calculated by summing NO_3^- and NH_4^+ . Finally,
199 DOC:DIN ratio was calculated based on molar mass. Samples for water chemistry were filtered
200 in the field ($0.45 \mu\text{m}$ Millex HA filter, Millipore) and either frozen before analysis (for nutrients)
201 or analyzed unfrozen after acidification with 6 M hydrochloric acid (for DOC). $\text{NO}_3\text{-N}$ (ISO
202 13395:1996; Method G-384-08 Rev. 2), $\text{NH}_4\text{-N}$ (ISO 11732:2005; Method G-171-96 Rev. 12)
203 and SRP (ISO 6878:2004; Method G-297-03 Rev. 1) were analyzed colorimetrically using a
204 SEAL Analytical AutoAnalyzer 3 (SEAL Analytical, Mequon, WI, U.S.A.); DOC was analyzed
205 on a Shimadzu TOC-VcPH total organic carbon analyzer.

206
207 Chl-a analysis

208 We analyzed Chl-a accumulated on inorganic NDS with a BenthosTorch (bbe Moldaenke,
209 Germany) on the day of retrieval. The BenthosTorch is a hand held instrument that analyses Chl-a
210 pigments in-situ by fluorescence. This instrument has been shown to compare well with
211 conventional spectroscopic-based methods for Chl-a analysis (Kahlert & McKie 2014),
212 especially when the biofilm is thin (Echenique-Subiabre et al. 2016), as was the case in our study.

213 The BenthosTorch measures an area of 1.1 cm² and we systematically measured three separate
214 locations on each NDS surface to produce a representative mean Chl-a number for each surface.

215
216 To assess to which extent organic surfaces may have also served as substrate for benthic algae,
217 we measured Chl-a on all organic surfaces by spectrophotometric absorbance following Steinman
218 et al. (2007), including correction for pheophytins. Prior to extraction, organic surfaces were
219 stored frozen at -80 °C (to reduce Chl-a degradation, Graff & Rynearson 2011) for 1 to 2 years
220 depending on the time of deployment. Organic surfaces were thawed and put in centrifuge tubes
221 with 90 % acetone for 24 hours prior to analysis of the extract on a JASCO UV
222 spectrophotometer (model V-630/650/660/670, Japan).

223
224 Statistical analyses

225 Differences in stream physico-chemical characteristics (DOC, DIN, DOC:DIN ratio, SRP, light
226 and temperature) among deployment period and/or landscape units were tested using one-way
227 analysis of variance (ANOVA). We assessed spatial variation in resource limitation of stream
228 microbial biofilms with linear mixed-effects models (LMMs) using the 'lme4' R package. NDS
229 treatment, landscape type and deployment period were fixed factors in each LMM (this model
230 achieved a better parsimonious fit than having deployment period be a random factor) with
231 replicate sites as a random variable. Replicate samples (4 of each NDS treatment per deployment)
232 were averaged prior to analysis, i.e. not treated as true replicates. Nutrient and/or C limitation was
233 determined as a significant enhancement of Chl-a, GPP or CR values on amended relative to
234 unamended NDS. A significant increase in N+P or C+N+P over N, P or C alone is defined as
235 secondary limitation (or serial limitation, sensu Harpole et al. 2011). Multiple comparisons of
236 mean NDS treatment and landscape unit responses followed each LMM and was performed using
237 the 'multcomp' R package. The treatment response to N amendment (RR_N) was calculated by
238 dividing Chl-a, GPP and CR on N amended NDS by unamended NDS. We used step-wise
239 multiple regression based on Akaike information criterion (AIC) to predict variation in biofilm
240 responses explained by physical (water temperature, light) and chemical (DIN, DOC, DOC:DIN)
241 variables (hereafter r^2). This was done both with unamended response variables and the RR. All
242 statistical analyses were performed in R (packages used for the step-wise regression was Hmisc,
243 MASS, leaps and car) and the threshold for statistical significance was set at $\alpha = 0.05$.

244

245 Results

246 Stream physico-chemical characteristics

247 DIN concentrations were below $20 \mu\text{g L}^{-1}$ and SRP below $5 \mu\text{g L}^{-1}$ in all streams during the study
248 period and there was a consistent decrease in DIN concentration between June and August (One-
249 way ANOVA; $p=0.01$; Table 1). Also, DIN concentrations tended to be lower in tundra
250 compared to both coniferous and birch streams; however, this difference was not significant
251 (One-way ANOVA; $p=0.08$). DOC and DOC:DIN ratio was notably highest in coniferous
252 streams ($3.0\text{-}8.5 \text{ mg C L}^{-1}$, $420\text{-}780$ respectively) and lowest in tundra streams (0.7 to 3.4 mg C L^{-1} ,
253 $100\text{-}450$). Overall, water temperature varied between 1.5 and 11.4°C . For tundra streams,
254 average water temperature increased from 1.7°C in June to 8.7°C in August. By comparison,
255 coniferous and birch streams reached their highest temperatures (ca. 8.0°C) in July and averaged
256 ($\pm\text{SE}$) 6.3°C (± 0.9) and 4.0°C (± 0.3), respectively, in June.

257
258 Accumulated light varied between 2.9 and $33.6 \text{ mol photons m}^{-2} \text{ day}^{-1}$ and was considerably
259 higher in the tundra when compared to birch and coniferous streams ($p<0.05$; Fig. 2). While this
260 daily accumulated measure indicates high levels of incident light throughout the season, the light
261 threshold (% time of day that light was above $100 \mu\text{mol m}^{-2} \text{ s}^{-1}$) shows that the birch and
262 coniferous streams experienced high levels of light for only 2.5 and 4.8 hours per day in August,
263 respectively, while they had twice this amount of time in June. By comparison, the time above
264 this threshold in tundra streams was high throughout the summer (12.5 , 11 and 8.7 hours in June,
265 July, and August, respectively).

266

267 Nutrient limitation experiment

268 Biofilm activity and biomass accrual on unamended NDS

269 Chl-a accumulation (on inorganic and organic surface) and rates of GPP and CR on unamended
270 NDS were greatest in birch and coniferous streams during July and August, and lowest in tundra
271 streams in June (Fig. 3, and S3). More specifically, Chl-a on unamended NDS ranged from 0.10
272 to $1.88 \mu\text{g cm}^{-2}$ on inorganic surfaces and from 0.07 to $0.7 \mu\text{g cm}^{-2}$ on organic surfaces with
273 highest biomass in coniferous and birch streams during August and the lowest in tundra streams
274 in June and July (One-way ANOVA; all $p<0.05$). GPP on unamended NDS ranged from 0.88 to

275 8.24 $\mu\text{g O}_2 \text{ cm}^{-2} \text{ h}^{-1}$ and CR from 0.50 to 5.41 $\mu\text{g O}_2 \text{ cm}^{-2} \text{ h}^{-1}$. GPP and CR on these different
276 surfaces were closely correlated across sites and seasons ($r=0.76$, $p<0.01$, $n=34$) and were
277 uniformly highest in birch streams during July (Fig. S3).

278
279 Unamended biomass accrual and rates of GPP and CR were weakly only correlated to physico-
280 chemical variables. Based on the multiple regression models, Chl-a accrual on both inorganic and
281 organic surfaces declined with greater light availability ($r^2=0.43$, $p<0.05$, Fig. 5b) and on organic
282 surfaces was positively related with higher DOC concentration ($r^2=0.60$, $p<0.05$), which
283 corresponds to differences in stream types (i.e. lower light and higher DOC in coniferous
284 streams). Rates of GPP and CR both increased with water temperature and DIN concentration
285 and decreased with higher DOC concentration (GPP: $r=0.34$, $p=0.001$, CR: $r=0.33$, $p=0.002$).

286
287 Response to resource additions
288 Stream biomass accrual and rates of GPP and CR was primarily N limited in all streams types,
289 and on both surfaces (LMM, all $p<0.01$, Fig. 3, S3). Neither P nor C amendments alone had a
290 significant effect for any response variable except for birch streams, where GPP was marginally
291 inhibited by P addition (S5). While not significant, CR on C amended surfaces was on average
292 30% greater than unamended NDS. Chl-a accrual showed secondary P limitation in all stream
293 types on inorganic surfaces (as evidenced by significantly greater Chl-a accrual on NP compared
294 to N, $p<0.01$), and in coniferous streams on organic surfaces ($p<0.01$). Similarly, CR was
295 secondarily limited by P in all sites (i.e., NP treatment was higher than N treatment, all $p<0.01$)
296 and additionally by C in coniferous streams (CNP treatment was higher than NP treatment,
297 $p<0.01$). Biomass specific rates of GPP were slightly lower in NP treatment compared to other
298 treatments and unamended NDS, and it was highest in Tundra streams in July (LMM, all
299 $p<0.05$). All of which suggests that biomass specific GPP was highest in low nutrient NDS (S6).

300
301 We used the response ratio of N (RR_N) to assess secondary controls by light availability,
302 temperature, DIN, DOC and DOC:DIN ratio on biofilm activity and biomass. We focused only
303 on N because responses to N treatment were significant in all stream-types and response
304 variables, while there were diverse responses to CNP, NP and P. For Chl-a, multiple regression
305 models based on all sites indicated that RR_N was enhanced in warmer and lighter conditions and

306 depressed by higher DIN concentrations ($r^2=0.46$, $p<0.01$ for inorganic surfaces and $r^2=0.37$,
307 $p<0.01$ for organic surfaces). In these models, all variables were significant ($p<0.05$) except for
308 DIN in the model for Chl-a on organic surfaces (full models including AIC selection in S4).
309 Based on the multiple regression models on CR and GPP, the RR_N was greatest with higher DOC
310 and lower DIN concentrations ($r^2=0.46$, $p<0.01$ for CR and $r^2=0.25$, $p<0.01$ for GPP, all variables
311 significant). This means that the RR_N of both CR and GPP was positively related to the
312 DOC:DIN ratio of the stream water ($r^2=0.48$ $p<0.01$ for CR and $r^2=0.18$, $p<0.01$ for GPP, Fig. 4).
313 Chl-a on the other hand was not related to DOC:DIN ratio in the stream water. The average RR_N
314 (\pm SE) pooling all sites was 1.7 (\pm 0.09) for Chl-a on inorganic surfaces, 3.0 (\pm 0.18) for Chl-a on
315 organic surfaces, 1.6 (\pm 0.06) for GPP, and 2.2 (\pm 0.11) for CR.

316
317 The assessment of RR_N for each landscape unit and season separately highlighted the identity of
318 secondary controls over biofilm activity and temporal differences in the magnitude of nutrient
319 response. First, coniferous streams tended to have a stronger overall responses to N addition
320 (RR_N across all response variables = 2.74 ± 0.11) than birch ($RR_N = 2.41\pm 0.12$) and tundra ($RR_N =$
321 2.38 ± 0.13) streams. In addition, in tundra streams, the RR_N of all response variables increased
322 significantly with higher water temperature (all regressions, $p<0.05$; Fig. 5c). At the other end of
323 the landscape unit gradient, the RR_N for Chl-a and GPP in coniferous streams increased with
324 greater light availability (Fig. 5d). Birch streams show weak positive, but non-significant,
325 relationships between the RR_N and both temperature and light (data not shown). Furthermore, the
326 RR_N for all streams increased over the course of the summer from June to August (average RR_N
327 June= 2.20 ± 0.27 , July= 2.65 ± 0.24 and August= 2.74 ± 0.22 , ANOVA, $p=0.04$), meaning the streams
328 were more responsive towards the end of the season.

329

330 Discussion

331 We clearly demonstrate that biofilm activity and biomass accrual in our arctic study streams are
332 N limited throughout the summer, despite major differences in habitat properties along the
333 vegetation gradient. In fact, for unamended biofilms, neither activity nor biomass accrual was
334 strongly driven by stream temperature, incident light, or DOC loading across space and time.
335 However, these physical and chemical variables did influence how biofilms responded to N
336 enrichment, with effects that differed according to the landscape unit (coniferous to tundra).

337 Specifically, temporal changes in water temperature constrained biofilm responses to N addition
338 in tundra streams, while variability in incident light played this role for autotrophs in forested
339 catchments. Also, variation in DOC:DIN provided additional controls over heterotrophic
340 responses to experimental N supply in all stream types. Such interactions have important
341 implications for how arctic streams respond to ongoing climate change. In this context, our
342 results suggest that biofilm nutrient limitation can be sufficiently strong to dampen how these
343 ecosystems respond to other climate-related factors, including increased temperature, altered light
344 regimes, and shifts in DOC loading.

345

346 Nutrient limitation of autotrophs

347 Biofilm Chl-a and GPP were strictly N limited in all stream-types (tundra, birch and coniferous
348 catchments), with secondary limitation of P (i.e., where the NP treatment was significantly higher
349 than N) only for Chl-a in tundra and coniferous streams. These results contrast with studies of
350 nutrient limitation in the North American arctic, which have emphasized the importance of P at
351 short (Peterson et al. 1983) and long (Slavik et al. 2004) temporal scales. However, N limitation
352 has been observed for phytoplankton in sub-alpine lakes of arctic Sweden (Bergström et al.
353 2013), as well in streams (Burrows et al. 2015) and lakes (Bergström et al. 2008) of boreal
354 Sweden. The persistence of N limitation along our gradient, despite variable climatic and
355 catchment characteristics, is not surprising given low DIN concentrations observed across sites
356 and seasons (average $12 \mu\text{g DIN L}^{-1}$). By comparison, DIN concentrations in the P limited
357 Kuparuk River (AK, USA) were reported as high as $80 \mu\text{g N L}^{-1}$ during summer (Peterson et al.
358 1993, Slavik et al. 2004), with similar values observed for nearby tributaries (Benstead et al.
359 2005). However, consistent N limitation across our sites does not match predictions based on the
360 molar DIN:SRP ratios, which were routinely high enough to suggest P limitation (average ratio:
361 39 ± 5 SE). One potential reason for this discrepancy is that SRP only accounts for ~50% of total
362 P (TP, SLU monitoring data from Abiskoajokka, 2000-2013) in our study area, and indeed
363 DIN:TP has been shown to be the more predictive ratio for understanding phytoplankton nutrient
364 limitation (Morris & Lewis 1988, Ptacnik et al. 2010). Thus, we are perhaps missing some
365 portion of the bioavailable P pool associated with dissolved organic matter (DOM, e.g., Soares et
366 al. 2017). Regardless, the effects of N addition were clear, and predictions of aquatic nutrient

367 limitation from N:P ratios often do not match results from bioassays in more oligotrophic systems
368 (Levine & Whalen 2001, Tank & Dodds 2003, and Ogbebo et al. 2009).

369
370 To consider these results in a broader context, we summarized similar bioassay experiments
371 published for arctic freshwaters (103 lakes and 1 river, Fig. 6). Overall, this summary highlights
372 the widespread significance of nutrient limitation in these ecosystems but also a lack of nutrient
373 limitation assays in arctic running waters when compared to lakes. Specifically, 85% of bioassays
374 showed a significant response to resource addition and the average response ratios (for N, P, and
375 NP) tended to be higher than those reported for freshwaters globally (Elser et al. 2007, see Fig. 6
376 for comparison). In addition, the frequency of N versus P limitation across these systems is
377 roughly equal. Single N limitation was reported for 20% of the systems studied with a similar
378 amount (22%) reporting P limitation. As highlighted in global assessments of nutrient limitation
379 (Harpole et al. 2011), 43% of studied lakes and streams responded greatest to N and P added in
380 combination. However, we were not able to enumerate cases where the response to NP additions
381 were greater than single additions of N or P (i.e., true co-limitation). Regardless, when combined
382 with our results, this literature survey suggests that a previous emphasis on P limitation (Peterson
383 et al. 1993) does not necessarily apply across the arctic. Importantly, these patterns call for a need
384 to better understand how catchment processes interact with climatic factors and atmospheric
385 inputs to regulate variation in the supply and form of N versus P delivered to arctic freshwaters
386 (Bergström et al. 2015), particularly running waters, which are notably underrepresented in this
387 context.

388

389 Nutrient limitation of heterotrophs

390 Primary N limitation was also observed for CR in all stream-types and throughout the study
391 period. CR was secondarily limited by P in all stream types (i.e., NP treatment was significantly
392 higher than N treatment) and by C in coniferous streams. Interestingly, these results suggest that
393 nutrient limitation was strong enough to override the effects of adding labile carbon alone, even
394 in tundra streams where DOC concentrations were $<2 \text{ mg C L}^{-1}$. Similar heterotrophic nutrient
395 limitation and lack of response to C addition has been observed in Canadian arctic lakes with
396 extremely low DOC concentrations (Granéli et al. 2004), as well as in boreal streams (Franke et
397 al. 2013). By contrast, strong and persistent C limitation to heterotrophs has been reported

398 throughout the year in boreal streams with a large, but relatively recalcitrant DOC pool (Burrows
399 et al. 2017), as well as in North American streams with higher nutrient concentrations compared
400 to our streams (Olapade & Leff 2005). Notably, there have been very few studies of resource
401 limitation of heterotrophs in arctic freshwaters (e.g. only 3 out of 104 studied systems in our
402 literature summary).

403
404 While we did not see strong effects of adding labile C, we did find that DOC loading relative to
405 background DIN (i.e., DOC:DIN ratio) across our gradient correlated closely with patterns of
406 heterotrophic RR_N . Specifically, this result suggests that inputs of organic matter relative to DIN
407 determines the strength of heterotrophic N demand and limitation. On one hand, this relationship
408 is consistent with the general idea that C availability can constrain N use in ecosystems, for
409 example, by regulating rates of immobilization (Taylor & Townsend 2010). On the other, the
410 relationship between CR RR_N and DOC:DIN suggests that the heterotrophic use of terrestrial
411 DOM by stream biofilms across this gradient of catchments is facilitated by the availability of
412 inorganic nitrogen. Wickland et al. (2012) highlighted a similar relationship, showing that that
413 relative availability of DIN (i.e., DOC:DIN) was the principle constraint on microbial DOC
414 degradation in the Yukon River of Alaska. The DOC:DIN ratio across our streams (range 70-950)
415 was even higher than those reported for the Yukon (6.5-589), suggesting the potential for even
416 stronger N limitation to DOM degradation. Similar nutrient constraints on DOM use, but with P
417 as the proximal limiting nutrient, was recently shown in eastern more streams in Alaska,
418 particularly during snowmelt (Mutschlecner et al. 2018). Together with these observations, our
419 results suggest that regardless of whether terrestrial resource inputs increase or decrease in the
420 future, any changes in the relative concentrations of DOC and nutrients could have important
421 implications for arctic stream biofilms and their capacity to utilize terrestrial organic matter.

422
423 Climate change effects in resource limited ecosystems

424 Resource limitation was sufficiently strong in all streams that spatial and temporal gradients in
425 temperature, light, and DOC only emerged as important factors after we experimentally satisfied
426 nutrient demand. Similar constraints on biofilm response to stream temperature change have been
427 reported along a geothermal gradient in Icelandic (Friberg et al. 2009). Together, these results
428 suggest that how climate change effects N and P supply to arctic streams operates as the key

429 constraints on how they will respond to other changing habitat properties. There are, however,
430 conflicting observations related to how climate change may alter nutrient concentrations in arctic
431 streams. For example, in some parts of the North American and West Siberian arctic, riverine
432 DIN concentrations have shown increases in response to permafrost degradation (Frey et al.
433 2007, Bowden et al. 2008, and Abbott et al. 2015). By contrast, DIN export has declined over the
434 last 30 years in several northern Swedish rivers including in Abiskoajokka, located near our sites
435 (Lucas et al. 2016). In fact, for Abiskoajokka, the average growing season DIN concentration has
436 declined from ca. 30 to less than 15 $\mu\text{g N L}^{-1}$ over this period, and future projections for this
437 region suggests these trends in concentration may persist (Teutschbein et al. 2017). In this region,
438 where permafrost is not continuous, such declines in DIN concentration and export are consistent
439 with increasing terrestrial nutrient demand and retention linked to greater plant productivity (Xu
440 et al. 2013), tree line expansion (Van Bogaert et al. 2011) and/or increased shrub abundance
441 (Sturm et al. 2001). Overall, differences in the balance between terrestrial ‘greening’ as an N sink
442 and permafrost thaw as an N source may underlie this variation in long-term stream nutrient
443 trends observed across the arctic.

444
445 Where increasing nutrient concentrations coincide with warming, arctic stream biofilm
446 productivity is very likely to increase as well. However, results from this and other arctic studies
447 (e.g., Huryn et al. 2005) emphasize that such responses will also depend upon how catchment
448 variables mediates other abiotic factors that constrain biological activity. In this context, our
449 results suggest the strongest responses to warmer temperatures will be in tundra streams, where
450 co-occurring increases in nutrient supply, in the absence of light limitation, would elevate
451 autotrophic and heterotrophic activity. Similar temperature dependence of nutrient use,
452 particularly early in the growing season, has been observed in other subarctic lakes (Bergström et
453 al. 2013) and tundra streams (Rasmussen et al. 2010). At more forested sites, however, we show
454 that light limitation may act as an additional control over autotrophic processes, even if resource
455 supply and temperature are both elevated. In the long-term, tundra streams may also experience
456 more frequent light-limitation if riparian vegetation shifts from heath to shrubs and tree lines
457 continue to encroach (Xu et al. 2013). In addition to these drivers, constraints to biotic activity
458 imposed by physical disturbances related to stream bed movement (Parker & Huryn 2013) and

459 antecedent flow regimes (Kenrdick & Huryrn 2015) are also very likely to modify patterns of
460 stream productivity, irrespective of changes in temperature and nutrient supply.

461
462 Finally, our results suggest that trends toward oligotrophication of the Fennoscandian arctic (e.g.,
463 Lucas et al. 2016, Huser et al. 2018) may result in decreased stream productivity, regardless of
464 warming, unless there are major changes in internal nutrient cycling. Theory predicts that N₂
465 fixing microbes should become increasingly competitive under such N limited conditions
466 (Vitousek & Howarth 1991), and this processes may be upregulated by warmer temperatures
467 (Grimm & Petrone 1997). For example, Welter et al. (2015) showed that increasing temperature
468 (from 7 to 23 °C) led to dramatic increases in rates of N₂ fixation among experimental streams in
469 Iceland. Moreover, in a whole-stream experiment at this same location, Hood et al. (2017)
470 showed that warming (by 4 °C) led to greater primary productivity, which was enabled by a
471 combination of elevated rates of N mineralization, together with species shifts that led to
472 increased N use efficiency, and possibly higher rates of N₂ fixation. However, these Icelandic
473 streams are relatively high in P (SRP: 15-19 µg/L), and in more oligotrophic systems, N fixation
474 may be limited by the availability of P and/or other trace elements (Horne & Carmiggelt 1975).
475 Regardless, despite fundamentally different experimental approaches, we come to similar
476 conclusions about the overriding importance of nutrient limitation for understanding climate
477 change effects in arctic streams. Ultimately, predicting how arctic streams will respond to climate
478 change requires understanding about how shifts in the net nutrient balance of terrestrial
479 landscapes will interact with multiple physical habitat factors to either enhance or constrain rates
480 of aquatic productivity.

481

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489

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731

732 Table 1. Mean \pm SE for stream chemical and physical parameters (average of 4 streams in each stream type, except only 2 tundra streams in August). * Daily
 733 photon flux (DPF) is presented as the accumulated photons in $\text{mol m}^{-2} \text{day}^{-1}$. Light % represents percent time of deployment time above $100 \mu\text{mol photons m}^{-2} \text{s}^{-1}$.
 734 DOC:DIN ratio is calculated based on molar mass and detection limit for SRP was $0.1 \mu\text{g L}^{-1}$.

	June 2015			July 2015			August 2014		
	Coniferous	Birch	Tundra	Coniferous	Birch	Tundra	Coniferous	Birch	Tundra
Temp. ($^{\circ}\text{C}$)	6.3 \pm 0.9	4.0 \pm 0.3	1.7 \pm 0.1	7.4 \pm 1.3	8.5 \pm 0.4	6.9 \pm 0.4	7.7 \pm 0.8	8.1 \pm 0.3	8.7 \pm 0.2
DPF (*)	11.3 \pm 1.8	7.1 \pm 0.4	19.4 \pm 2.1	8.1 \pm 2.0	10.0 \pm 0.7	17.0 \pm 2.7	9.1 \pm 1.3	6.4 \pm 1.3	23.6 \pm 0.1
Light (%)	43.8 \pm 5.1	25.5 \pm 4.4	51.9 \pm 2.2	30.7 \pm 9.5	40.1 \pm 3.6	45.8 \pm 4.0	20.2 \pm 4.4	10.9 \pm 3.4	36.4 \pm 5.8
DOC (mg L^{-1})	7.0 \pm 0.6	3.5 \pm 0.5	1.0 \pm 0.1	4.6 \pm 0.7	2.4 \pm 0.4	0.8 \pm 0.1	5.8 \pm 0.4	2.6 \pm 0.4	2.3 \pm 1.1
SRP ($\mu\text{g L}^{-1}$)	1.1 \pm 0.5	0.4 \pm 0.1	0.3 \pm 0	2.2 \pm 1.0	0.8 \pm 0.2	0.4 \pm 0.1	1.1 \pm 0.1	1.0 \pm 0.1	0.7 \pm 0.1
DIN ($\mu\text{g L}^{-1}$)	11.0 \pm 0.8	12.0 \pm 1.8	10.7 \pm 0.9	13.0 \pm 2.0	9.8 \pm 2.1	7.0 \pm 0.1	8.0 \pm 1.0	9.1 \pm 3.3	5.5 \pm 0.8
DOC:DIN	746 \pm 44	341 \pm 8	112 \pm 20	427 \pm 46	298 \pm 33	142 \pm 13	786 \pm 70	460 \pm 151	457 \pm 170

735 **Figure captions**

736 Figure 1. (a) Location of study streams from west to east (blue circles=tundra, green circles=birch and red
737 circles=coniferous). The large lake is Torneträsk, below is the village of Abisko. Right panels: (b) tundra stream, (c)
738 birch forest stream and (d) coniferous forest stream.

739
740 Figure 2. Estimated accumulated light ($\text{mol photons m}^{-2} \text{ day}^{-1}$) in the different stream types (coniferous, birch and
741 tundra). Grey bars represent number of days below light saturation, $5 \text{ mol photons m}^{-2} \text{ day}^{-1}$, for autotrophic activity
742 (indicated by Hill et al. 2009). Averaged accumulated light by deployment period and stream type is available in S2.
743

744 Figure 3. Response of chlorophyll-a concentration (Chl-a) on inorganic (a) and organic (b) substrates, as well as
745 gross primary production (GPP) on inorganic surface (c) and community respiration (CR) on organic surface (d) to
746 nutrient addition across sites; coniferous (Con.), birch, and tundra. A = unamended treatment. Error bars represent
747 95% confidence interval and dots are outliers. Results for individual streams are available in S5 and ANOVA tables
748 with F and p values are available in S3.

749
750 Figure 4. Response ratio to N (RR_N) of community respiration (CR) on organic surfaces in relation to the DOC:DIN
751 ratio in coniferous (red), birch (green) and tundra (blue) streams (including June, July and August data). Linear
752 regression: $p < 0.05$, $r^2 = 0.49$, $RRN = 1.223 + 0.0035 * \text{DOC.DIN}$, gray area represents 95% confidence interval.
753

754 Figure 5. (a) Ambient Chl-a on inorganic surfaces, Community Respiration (CR), and Gross Primary Production
755 (GPP) in relation to temperature in tundra streams. (b) Ambient Chl-a on inorganic surfaces and GPP in relation to %
756 time during the deployment above a light threshold ($100 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$) in coniferous streams. (c) Response
757 ratio of Chl-a on inorganic surfaces (dark green, $r^2=0.63$), GPP (light green, $r^2=0.53$), and CR (orange, $r^2=0.59$) to N
758 addition in relation to temperature in tundra streams. All regression lines are significant at $p < 0.05$. (d) Response ratio
759 of Chl-a on inorganic surfaces (dark green, $r^2=0.39$) and GPP (light green, $r^2=0.25$) to N in relation to % time during
760 the deployment above light threshold ($100 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$) in coniferous streams. Regression in panel d
761 assumes log-relationship due to expected saturating function of light. Lines are significant at $p=0.018$ (Chl-a) and
762 $p=0.058$ (GPP). All panels include data from June, July, and August.

763 Figure 6. Literature summary of resource limitation work in arctic and subarctic freshwaters based on different
764 combinations of the search criteria; arctic/subarctic + bioassay/nutrient limitation in Web of Science. We searched
765 exclusively for studies that tested N and P separately. Statistics for treatment effects were always available; however,
766 response ratios were in many occasions extracted from figures. Presented NP limitation means N+P treatment was
767 significantly higher than the corresponding unamended bioassay; we were not able to extract information about NP
768 relative to N / P treatments alone. Note that only one study (Granéli et al. 2004) with 4 lakes, studied heterotrophic
769 responses. (a) Number of lakes with nutrient limitation, NL= no response to treatment. Altogether, we found 8
770 studies including assays from a total of 103 lakes (Brutemark et al. 2006, Granéli et al. 2004, Hogan et al. 2014,
771 Levine & Whalen 2001, Ogbebo et al. 2009, Bergström et al. 2013, Symons et al. 2012) and only a single arctic

772 river, the Kuparuk River, (Peterson et al. 1983). (b) Boxplots show response ratios (RR) to treatment from studies
773 included in panel a. Error bars show SE. Horizontal, grey line represents no limitation (i.e., $RR = 1$). Green squares
774 represent average RR of each treatment from this literature survey (boxplots show medians). Blue triangles represent
775 a global average RR of each treatment from freshwater systems calculated from Elser et al. (2007). Note that the
776 present study results are not included in the figure.

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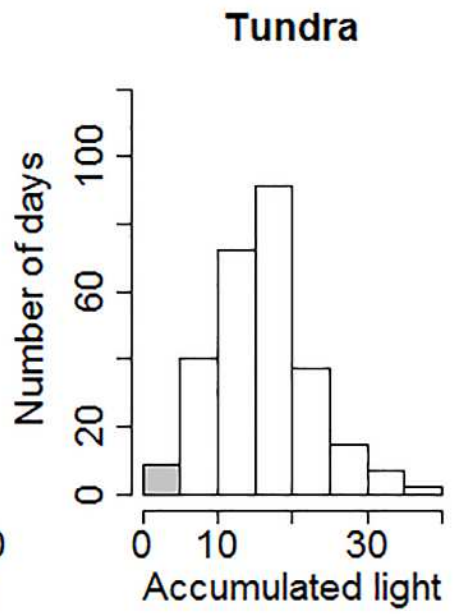
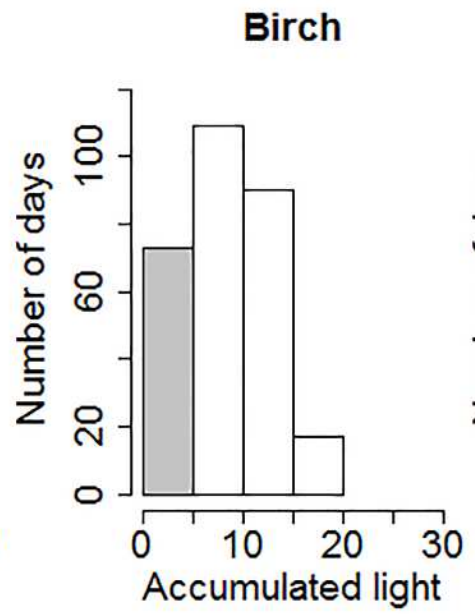
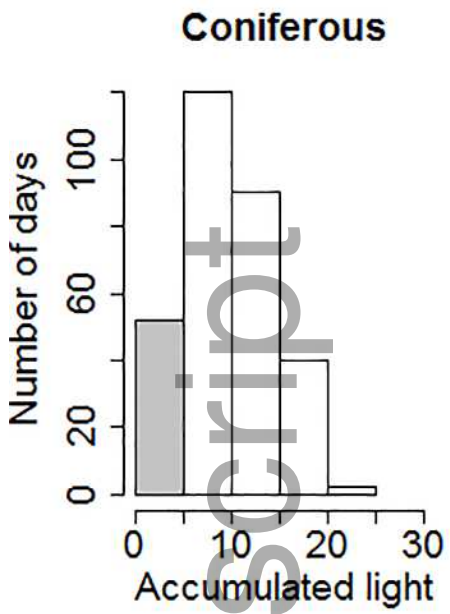
787 **Supporting Information**

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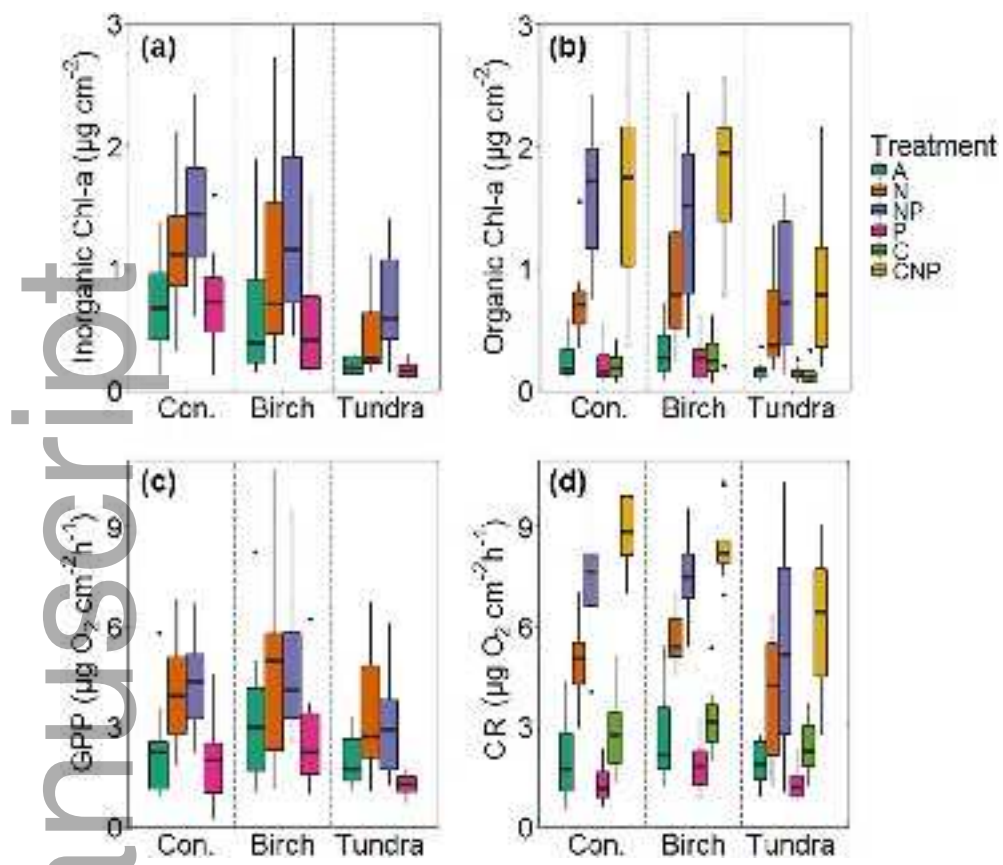


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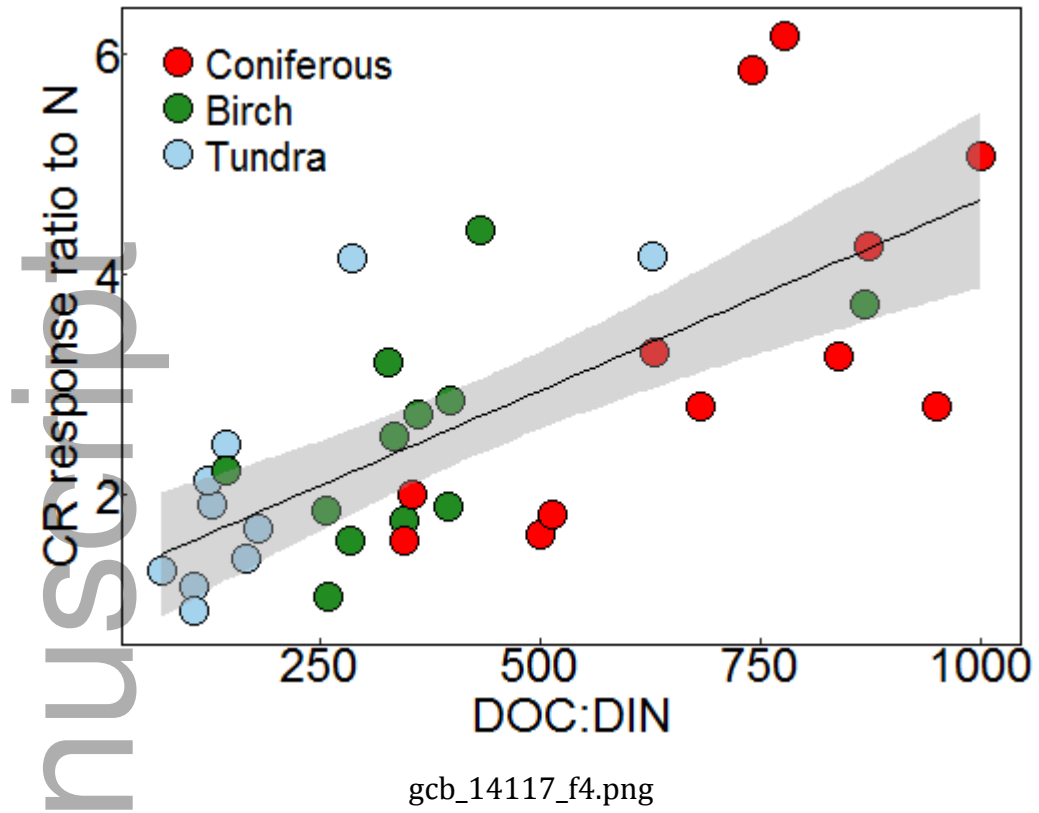


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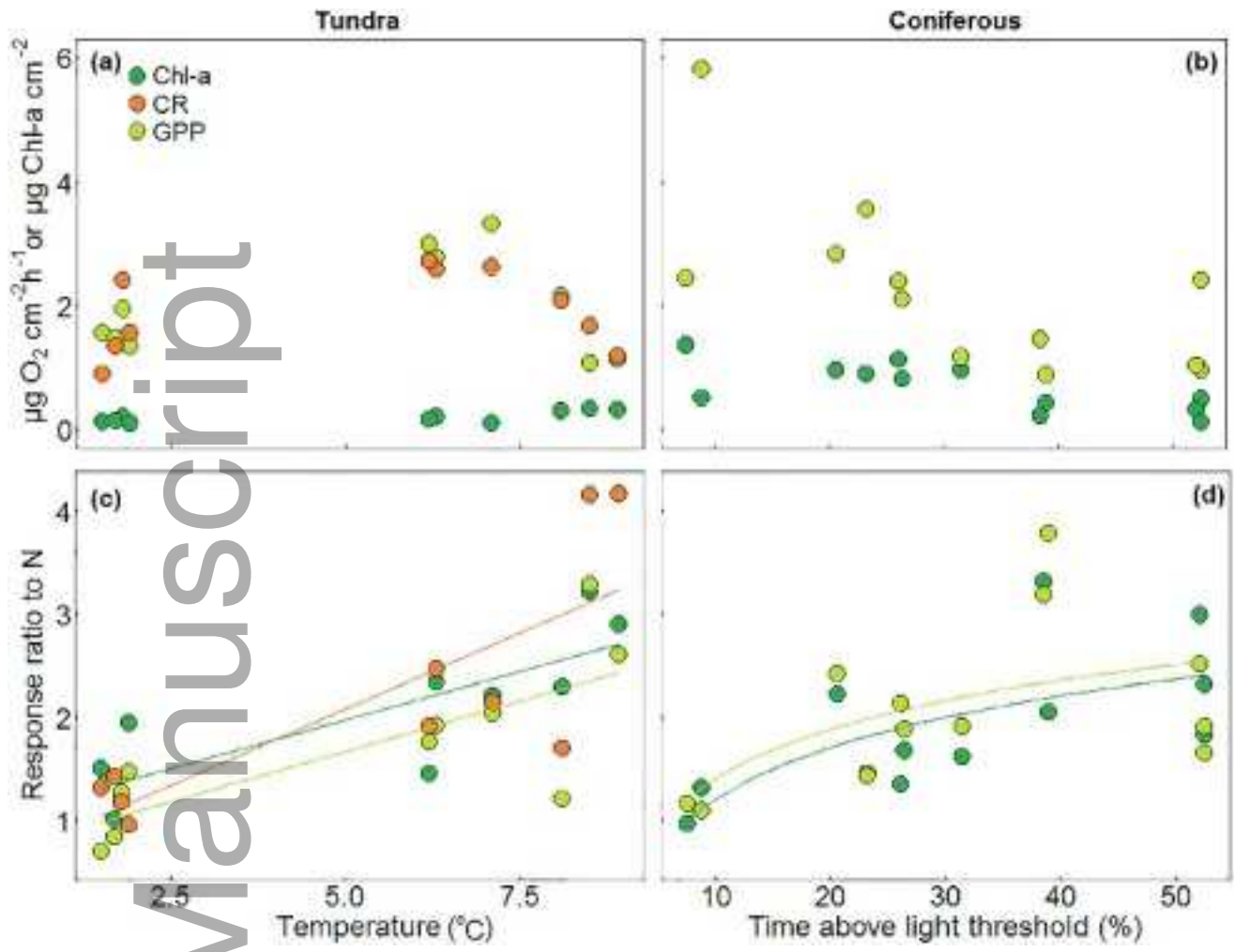
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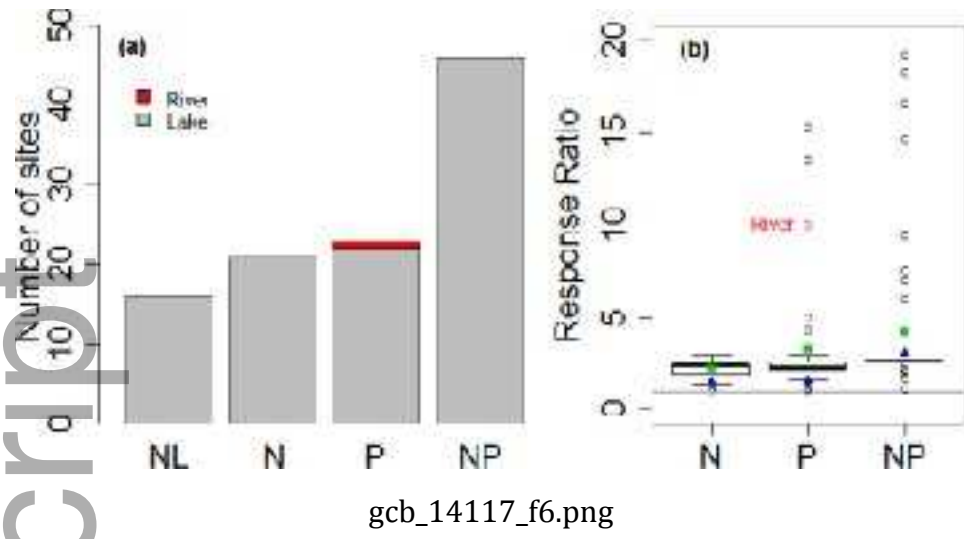
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