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

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Abstract

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

Introduction

Global climate change is currently altering the ecological structure and functioning of arctic landscapes. Warmer temperatures and modified precipitation patterns affect key biophysical features of high latitude ecosystems, including the length of growing seasons and the magnitude and timing of soil frost and snowmelt (ACIA 2004). In turn, these conditions have led to greater terrestrial productivity (Mao et al. 2016), shifts in the spatial distribution of trees and shrubs (Xu et al. 2013), altered seasonal hydrology (Déry et al. 2005), and changes in the mobilization and export of soil resources (McClelland et al. 2007). While research on climate-sensitivity in the arctic has overwhelmingly emphasized terrestrial ecosystems, the close connection between running waters and the catchments they drain make it likely that these observed changes on land also influence the productivity of streams and rivers (Davis et al. 2013). Yet predicting how running waters will respond to climate change at high latitudes remains a major challenge and requires that we understand how concurrent alterations to thermal, light, flow, and resource regimes interact to shape patterns of biological activity. To this end, our study explores how these interacting factors influence stream biofilm productivity along a climate gradient in arctic Sweden.

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

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

Climate change in the artic will shift a number of these physical and chemical drivers, and stream biofilm responses will ultimately depend on how these factors interact. For example, warmer temperatures are very likely to elevate rates of biological activity in northern streams (Friberg et al. 2009, Hood et al. 2017), yet these responses may be constrained or amplified by concurrent changes to light and/or resource supply (Cross et al. 2015). Similarly, while arctic streams are often well lit and can support comparatively high rates of gross primary production (GPP; Huryn et al. 2014), the encroachment of riparian zones by trees and shrubs may constrain algal growth through shading (cf. Warren et al. 2016), regardless of changes in temperature and/or resource availability. Finally, observed increases (McClelland et al. 2007) or decreases (Lucas et al. 2016) in the loading of dissolved organic carbon (DOC) and nutrients to arctic streams also have the potential to alter biofilm processes. Indeed, these ecosystems are often oligotrophic (Peterson et al. 1983, Gudmundsdottir et al. 2011), and fertilization experiments have shown clear ecosystem responses to enrichment (Peterson et al. 1993). However, such experiments to date have addressed the role of nutrient availability in isolation, and the significance of resource limitation in the face of other factors that potentially exert stronger and/or interactive influences on biofilm production (e.g., light, temperature, and disturbance) remains poorly understood (Kendrick & Huryn 2015).

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

Materials and methods

Study site

We used 12 streams in northern Sweden to investigate how resource availability influences biofilm accrual and activity. Four streams each drain sub-catchments dominated by one of three arctic landscape units: heath-tundra, birch forest, or coniferous forest (“stream types”, Fig. 1). The streams are located some 200 km above the Arctic Circle and are distributed along a regional vegetation gradient ranging from 540 m.a.s.l in the tundra catchments to about 340 m.a.s.l in the coniferous, with birch forest catchments in the middle of this gradient. Climate varies along this gradient from continental in the coniferous to maritime in the tundra. Accordingly, precipitation increases from ca. 490 mm yr^{-1} in the coniferous catchments to 840 mm yr^{-1} in the tundra catchments, while annual temperature is relatively unchanged around -1.7 °C (climate data, SMHI 2016). Total atmospheric N deposition (wet+dry) is <1 kg N ha^{-1} y^{-1} (Bergström et al. 2013). Annual growing season length is about 100 days yr^{-1} and it is considerably longer in the coniferous catchments compared to tundra (Karlsen et al. 2008). Snow depth has increased in the area over the last climatic period (Kohler et al. 2006) but the duration of ice cover in Lake Torneträsk has decreased (Callaghan et al. 2010). Discontinuous permafrost exists in the surroundings of our study area although highly connected to peat soils (Åkerman & Johansson 2008, Gisnås et al. 2017) and thus unlikely to be present in our studied catchments. All studied streams are headwaters with catchment areas less than 6 km^2; stream depth varied between 10 to 50 cm during the study period and stream width was always less than 3m (S1). Spot measurements of flow velocity (EM flow meter, Valeport, Devon, U.K.) were relatively stable over time, with an average of 0.23 (±0.02 standard error, hereafter SE) m s^{-1} across stream type and season.

Resource limitation experiment

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

2009). Accordingly, in this study, rates of respiration were, on average, thirteen times higher on organic than inorganic surfaces. Both inorganic and organic NDS were enriched with 0.5 M $NaNO_3$ (N treatment), 0.5 M KH_2PO_4 (P treatment), both (NP treatment), or unamended agar (A treatment). For the organic NDS, we also added 0.5M $C_2H_3NaO_2$ (acetate, C treatment) and a combination of all resources (CNP treatment). Four replicates of each treatment (16 inorganic and 24 organic NDS in total per stream) were randomly attached with cable ties to a stainless steel L-bar, placed underwater in main channels at a depth of approximately 20 cm. NDS were deployed for 19 days, which is the recommended time period for maintaining elevated rates of diffusion and enrichment (Tank et al. 2006, Bernhardt & Likens 2004, and Lang et al. 2004 for additional information about diffusion rates). Upon removal, we placed surfaces individually in 50 mL Falcon centrifuge tubes filled with unfiltered stream water. At retrieval, the depth of the NDS was more variable, from 5-40 cm. Samples were stored refrigerated (4°C) until analyses the following day.

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

Metabolic rates were calculated as the difference in DO between start and finish of incubations, correcting for any DO change in the controls, and presented per surface area (μg O_2 cm^{-2} h^{-1}).

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

Physical and chemical parameters

During all NDS deployment periods, we recorded light and temperature data every hour using HOBO pendant loggers (Onset Computer Corporation, Borne, U.S.A.). We attached one or two loggers to each set of NDS and converted lux to photosynthetically active radiation (PAR; using a conversion factor of 0.0185 according to Thimijan & Heins 1983). Light data are presented as daily photon flux (mol photons m^{-2} day^{-1}) and percent time during deployment above a threshold (100 µmol photons m^{-2} s^{-1}) that has been shown to be important to photosynthesis in streams (Hill et al. 2009). We measured water velocity and concentrations of nitrate (NO_3^-), ammonium (NH_4^+), soluble reactive phosphate (SRP) and DOC at beginning and end of each deployment period. Dissolved inorganic nitrogen (DIN) was calculated by summing NO_3^- and NH_4^+. Finally, DOC:DIN ratio was calculated based on molar mass. Samples for water chemistry were filtered in the field (0.45 µm Millex HA filter, Millipore) and either frozen before analysis (for nutrients) or analyzed unfrozen after acidification with 6 M hydrochloric acid (for DOC). NO_3-N (ISO 13395:1996; Method G-384-08 Rev. 2), NH_4-N (ISO 11732:2005; Method G-171-96 Rev. 12) and SRP (ISO 6878:2004; Method G-297-03 Rev. 1) were analyzed colorimetrically using a SEAL Analytical AutoAnalyzer 3 (SEAL Analytical, Mequon, WI, U.S.A); DOC was analyzed on a Shimazdu TOC-VcPH total organic carbon analyzer.

Chl-a analysis

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

The BenthoTorch measures an area of 1.1 cm^2 and we systematically measured three separate locations on each NDS surface to produce a representative mean Chl-a number for each surface.

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

Statistical analyses

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

Results

Stream physico-chemical characteristics

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

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

Nutrient limitation experiment

Biofilm activity and biomass accrual on unamended NDS

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

8.24 µg O_2 cm^{-2} h^{-1} and CR from 0.50 to 5.41 µg O_2 cm^{-2} h^{-1}. GPP and CR on these different surfaces were closely correlated across sites and seasons (r=0.76, $p<0.01$, n=34) and were uniformly highest in birch streams during July (Fig. S3).

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

Response to resource additions

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

We used the response ratio of N (RR_N) to assess secondary controls by light availability, temperature, DIN, DOC and DOC:DIN ratio on biofilm activity and biomass. We focused only on N because responses to N treatment were significant in all stream-types and response variables, while there were diverse responses to CNP, NP and P. For Chl-a, multiple regression models based on all sites indicated that RR_N was enhanced in warmer and lighter conditions and depressed by higher DIN concentrations (r^2=0.46, $p<0.01$ for inorganic surfaces and r^2=0.37, $p<0.01$ for organic surfaces). In these models, all variables were significant ($p<0.05$) except for DIN in the model for Chl-a on organic surfaces (full models including AIC selection in S4). Based on the multiple regression models on CR and GPP, the RR_N was greatest with higher DOC 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 significant). This means that the RR_N of both CR and GPP was positively related to the 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). Chl-a on the other hand was not related to DOC:DIN ratio in the stream water. The average RR_N (±SE) pooling all sites was 1.7 (±0.09) for Chl-a on inorganic surfaces, 3.0 (±0.18) for Chl-a on organic surfaces, 1.6 (±0.06) for GPP, and 2.2 (±0.11) for CR.

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

Discussion

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

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

Nutrient limitation of autotrophs

Biofilm Chl-a and GPP were strictly N limited in all stream-types (tundra, birch and coniferous catchments), with secondary limitation of P (i.e., where the NP treatment was significantly higher than N) only for Chl-a in tundra and coniferous streams. These results contrast with studies of nutrient limitation in the North American arctic, which have emphasized the importance of P at short (Peterson et al. 1983) and long (Slavik et al. 2004) temporal scales. However, N limitation has been observed for phytoplankton in sub-alpine lakes of arctic Sweden (Bergström et al. 2013), as well in streams (Burrows et al. 2015) and lakes (Bergström et al. 2008) of boreal Sweden. The persistence of N limitation along our gradient, despite variable climatic and catchment characteristics, is not surprising given low DIN concentrations observed across sites and seasons (average 12 µg DIN L^{-1}). By comparison, DIN concentrations in the P limited Kuparuk River (AK, USA) were reported as high as 80 µg N L^{-1} during summer (Peterson et al. 1993, Slavic et al. 2004), with similar values observed for nearby tributaries (Benstead et al. 2005). However, consistent N limitation across our sites does not match predictions based on the molar DIN:SRP ratios, which were routinely high enough to suggest P limitation (average ratio: 39 ±5 SE). One potential reason for this discrepancy is that SRP only accounts for ~50% of total P (TP, SLU monitoring data from Abiskojokka, 2000-2013) in our study area, and indeed DIN:TP has been shown to be the more predictive ratio for understanding phytoplankton nutrient limitation (Morris & Lewis 1988, Ptacnik et al. 2010). Thus, we are perhaps missing some portion of the bioavailable P pool associated with dissolved organic matter (DOM, e.g., Soares et al. 2017). Regardless, the effects of N addition were clear, and predictions of aquatic nutrient limitation from N:P ratios often do not match results from bioassays in more oligotrophic systems (Levine & Whalen 2001, Tank & Dodds 2003, and Ogbebo et al. 2009).

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

Nutrient limitation of heterotrophs

Primary N limitation was also observed for CR in all stream-types and throughout the study period. CR was secondarily limited by P in all stream types (i.e., NP treatment was significantly higher than N treatment) and by C in coniferous streams. Interestingly, these results suggest that nutrient limitation was strong enough to override the effects of adding labile carbon alone, even in tundra streams where DOC concentrations were <2 mg C L^{-1}. Similar heterotrophic nutrient limitation and lack of response to C addition has been observed in Canadian arctic lakes with extremely low DOC concentrations (Granéli et al. 2004), as well as in boreal streams (Franke et al. 2013). By contrast, strong and persistent C limitation to heterotrophs has been reported throughout the year in boreal streams with a large, but relatively recalcitrant DOC pool (Burrows et al. 2017), as well as in North American streams with higher nutrient concentrations compared to our streams (Olapade & Leff 2005). Notably, there have been very few studies of resource limitation of heterotrophs in arctic freshwaters (e.g. only 3 out of 104 studied systems in our literature summary).

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

Climate change effects in resource limited ecosystems

Resource limitation was sufficiently strong in all streams that spatial and temporal gradients in temperature, light, and DOC only emerged as important factors after we experimentally satisfied nutrient demand. Similar constraints on biofilm response to stream temperature change have been reported along a geothermal gradient in Icelandic (Friberg et al. 2009). Together, these results suggest that how climate change effects N and P supply to arctic streams operates as the key constraints on how they will respond to other changing habitat properties. There are, however, conflicting observations related to how climate change may alter nutrient concentrations in arctic streams. For example, in some parts of the North American and West Siberian arctic, riverine DIN concentrations have shown increases in response to permafrost degradation (Frey et al. 2007, Bowden et al. 2008, and Abbott et al. 2015). By contrast, DIN export has declined over the last 30 years in several northern Swedish rivers including in Abiskojokka, located near our sites (Lucas et al. 2016). In fact, for Abiskojokka, the average growing season DIN concentration has declined from ca. 30 to less than 15 μg N L^{-1} over this period, and future projections for this region suggests these trends in concentration may persist (Teutschbein et al. 2017). In this region, where permafrost is not continuous, such declines in DIN concentration and export are consistent with increasing terrestrial nutrient demand and retention linked to greater plant productivity (Xu et al. 2013), tree line expansion (Van Bogaert et al. 2011) and/or increased shrub abundance (Sturm et al. 2001). Overall, differences in the balance between terrestrial 'greening' as an N sink and permafrost thaw as an N source may underlie this variation in long-term stream nutrient trends observed across the arctic.

Where increasing nutrient concentrations coincide with warming, arctic stream biofilm productivity is very likely to increase as well. However, results from this and other arctic studies (e.g., Huryn et al. 2005) emphasize that such responses will also depend upon how catchment variables mediates other abiotic factors that constrain biological activity. In this context, our results suggest the strongest responses to warmer temperatures will be in tundra streams, where co-occurring increases in nutrient supply, in the absence of light limitation, would elevate autotrophic and heterotrophic activity. Similar temperature dependence of nutrient use, particularly early in the growing season, has been observed in other subarctic lakes (Bergström et al. 2013) and tundra streams (Rasmussen et al. 2010). At more forested sites, however, we show that light limitation may act as an additional control over autotrophic processes, even if resource supply and temperature are both elevated. In the long-term, tundra streams may also experience more frequent light-limitation if riparian vegetation shifts from heath to shrubs and tree lines continue to encroach (Xu et al. 2013). In addition to these drivers, constraints to biotic activity imposed by physical disturbances related to stream bed movement (Parker & Huryn 2013) and antecedent flow regimes (Kenrdick & Huryn 2015) are also very likely to modify patterns of stream productivity, irrespective of changes in temperature and nutrient supply.

Finally, our results suggest that trends toward oligotrophication of the Fennoscandian arctic (e.g., Lucas et al. 2016, Huser et al. 2018) may result in decreased stream productivity, regardless of warming, unless there are major changes in internal nutrient cycling. Theory predicts that N_2 fixing microbes should become increasingly competitive under such N limited conditions (Vitousek & Howarth 1991), and this processes may be upregulated by warmer temperatures (Grimm & Petrone 1997). For example, Welter et al. (2015) showed that increasing temperature (from 7 to 23 °C) led to dramatic increases in rates of N_2 fixation among experimental streams in Iceland. Moreover, in a whole-stream experiment at this same location, Hood et al. (2017) showed that warming (by 4 °C) led to greater primary productivity, which was enabled by a combination of elevated rates of N mineralization, together with species shifts that led to increased N use efficiency, and possibly higher rates of N_2 fixation. However, these Icelandic streams are relatively high in P (SRP: 15-19 µg/L), and in more oligotrophic systems, N fixation may be limited by the availability of P and/or other trace elements (Horne & Carmiggelt 1975). Regardless, despite fundamentally different experimental approaches, we come to similar conclusions about the overriding importance of nutrient limitation for understanding climate change effects in arctic streams. Ultimately, predicting how arctic streams will respond to climate change requires understanding about how shifts in the net nutrient balance of terrestrial landscapes will interact with multiple physical habitat factors to either enhance or constrain rates of aquatic productivity.

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

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

Figure captions

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

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

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

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

Figure 5. (a) Ambient Chl-a on inorganic surfaces, Community Respiration (CR), and Gross Primary Production (GPP) in relation to temperature in tundra streams. (b) Ambient Chl-a on inorganic surfaces and GPP in relation to % time during the deployment above a light threshold (100 µmol photons m^{-2} s^{-1}) in coniferous streams. (c) Response 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 addition in relation to temperature in tundra streams. All regression lines are significant at $p<0.05$. (d) Response ratio 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 the deployment above light threshold (100 µmol photons m^{-2} s^{-1}) in coniferous streams. Regression in panel d assumes log-relationship due to expected saturating function of light. Lines are significant at p=0.018 (Chl-a) and p=0.058 (GPP). All panels include data from June, July, and August.

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

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

Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article.

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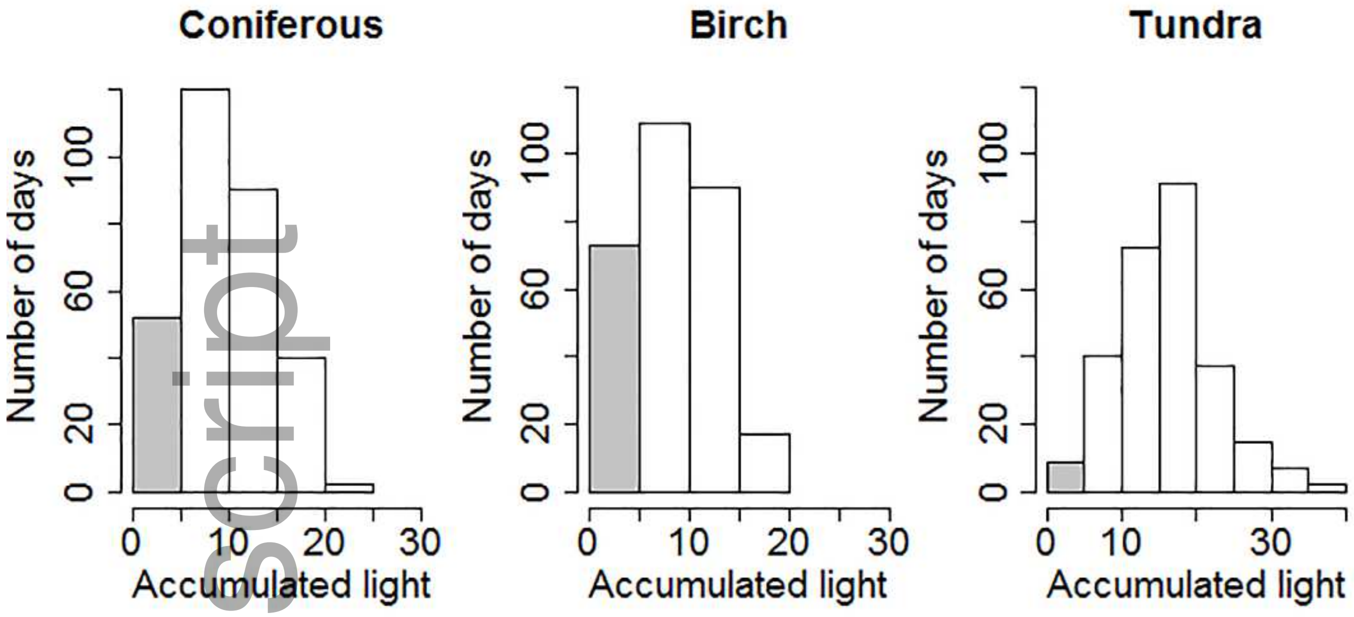

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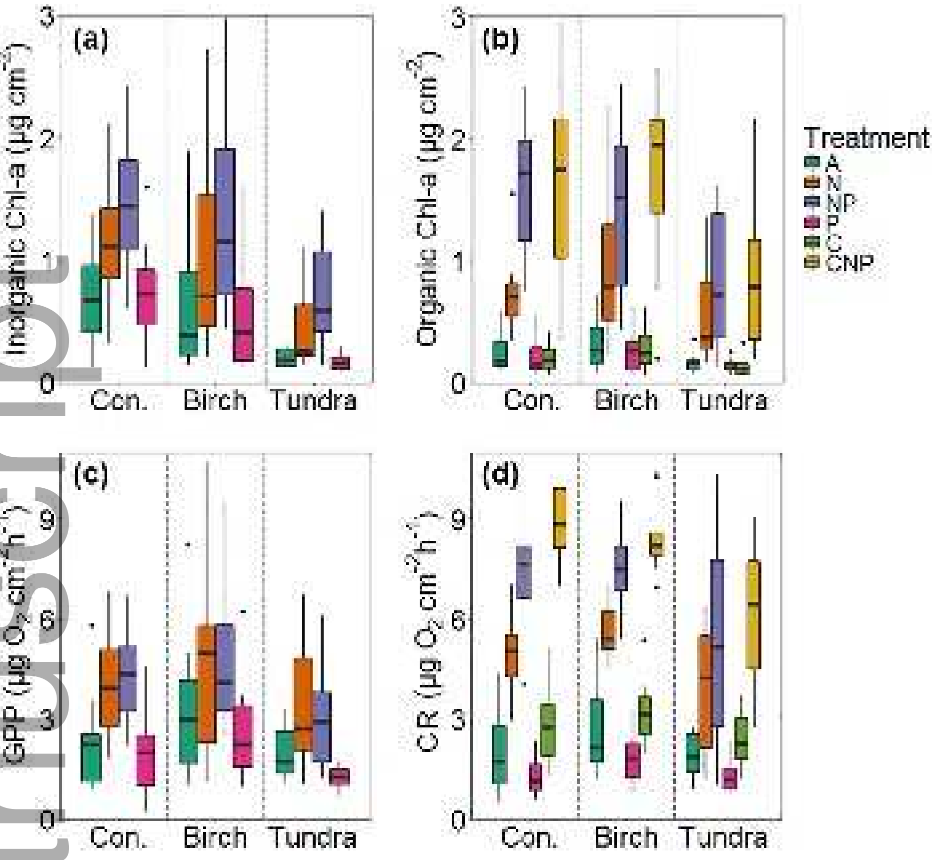

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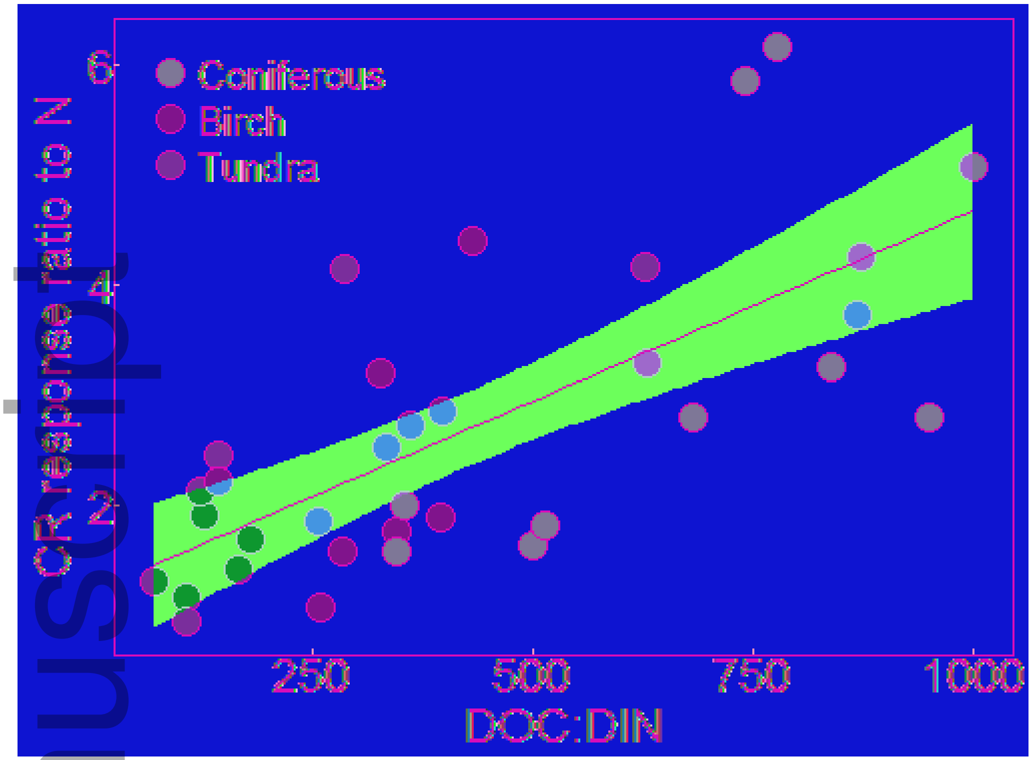

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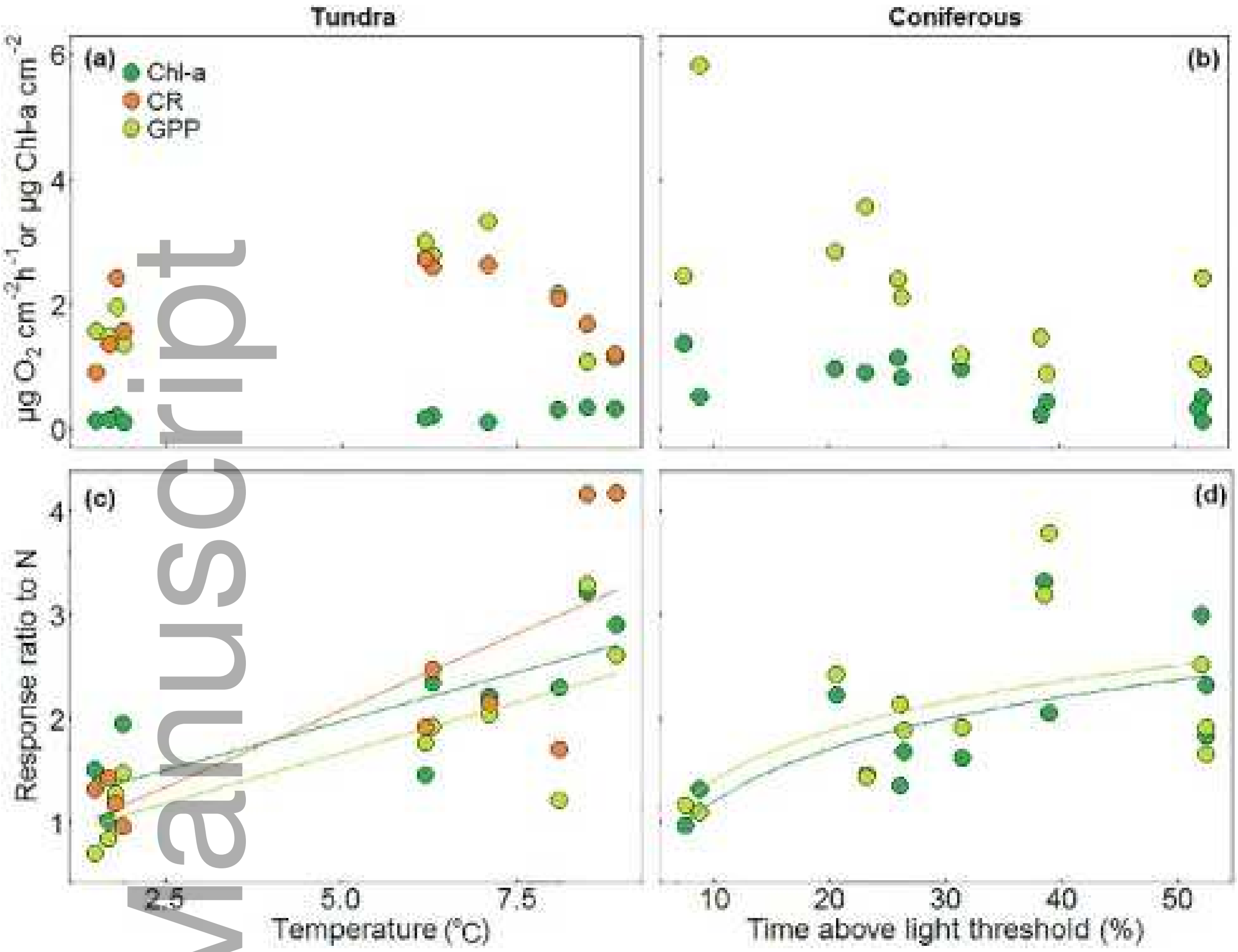

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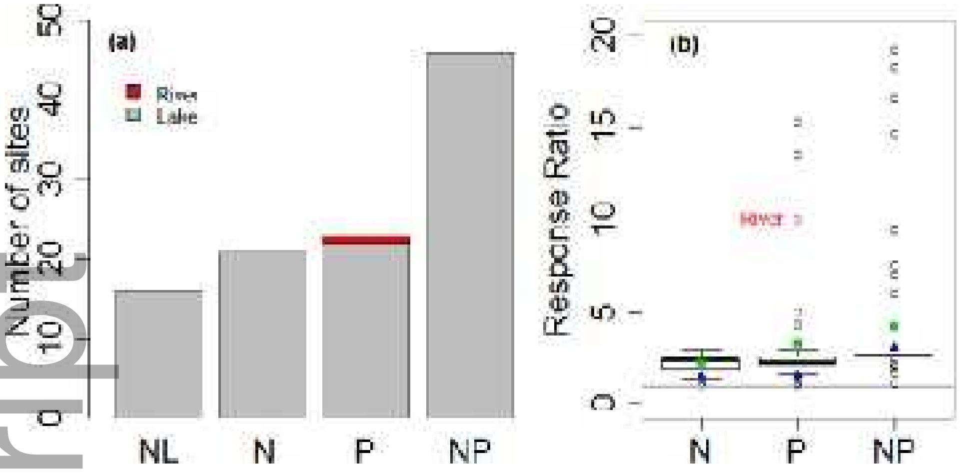

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