

COUPLING ENERGY AND ELEMENTS IN A WARMING WORLD:  
HOW TEMPERATURE SHAPES BIOFILM ECOSYSTEM  
STRUCTURE AND FUNCTION

by

Tanner John Williamson

A thesis submitted in partial fulfillment  
of the requirements for the degree

of

Master of Science

in

Biological Sciences

MONTANA STATE UNIVERSITY  
Bozeman, Montana

July 2014

©COPYRIGHT

by

Tanner John Williamson

2014

All Rights Reserved

## ACKNOWLEDGEMENTS

Science cannot exist in a vacuum and as such I owe the success of this work to many people. I am deeply and forever indebted to my advisor Wyatt Cross for his considerable guidance and friendship over the past two years. I am very grateful to my committee members and colleagues Billie Kerans and Jill Welter for making this thesis possible. I owe special thanks to Jim Hood, Jim Junker, Eric Scholl, and Heather Bowen for their keen insight and camaraderie, and to Paula Furey for teaching me to appreciate the algae. And of course, I would not be here without Miss Allie.

## TABLE OF CONTENTS

|                                                                                                                                 |    |
|---------------------------------------------------------------------------------------------------------------------------------|----|
| 1. COUPLING ENERGY AND ELEMENTS IN A WARMING WORLD:<br>HOW TEMPERATURE SHAPES BIOFILM ECOSYSTEM<br>STRUCTURE AND FUNCTION ..... | 1  |
| Introduction.....                                                                                                               | 1  |
| Methods.....                                                                                                                    | 5  |
| Site Description.....                                                                                                           | 5  |
| Experimental Design.....                                                                                                        | 5  |
| Response Metrics .....                                                                                                          | 7  |
| Metabolism .....                                                                                                                | 7  |
| Nutrient Uptake.....                                                                                                            | 7  |
| Nitrogen Fixation .....                                                                                                         | 8  |
| Biofilm Processing.....                                                                                                         | 8  |
| Predicted Nutrient Demand.....                                                                                                  | 9  |
| Nutrient Use Efficiency .....                                                                                                   | 9  |
| Statistical Analyses .....                                                                                                      | 10 |
| Results.....                                                                                                                    | 11 |
| Treatment Effect .....                                                                                                          | 11 |
| Biomass and Community Structure .....                                                                                           | 11 |
| Metabolism .....                                                                                                                | 12 |
| Stoichiometry .....                                                                                                             | 12 |
| Nutrient Uptake and Nitrogen Fixation .....                                                                                     | 13 |
| Predicted Nutrient Demand.....                                                                                                  | 14 |
| Nutrient Use Efficiency .....                                                                                                   | 14 |
| Discussion .....                                                                                                                | 24 |
| REFERENCES CITED.....                                                                                                           | 33 |
| APPENDIX A: Additional Figures.....                                                                                             | 39 |



## LIST OF TABLES

| Table                                                                                                                                                                                                                                                                                                                                                                                                                                         | Page |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|
| 1. Physiochemical characteristics of each temperature treatment; mean treatment temperature ( $^{\circ}\text{C} \pm 1 \text{ S.D.}$ ), ammonium ( $\mu\text{g/L} \pm 1 \text{ S.D.}$ ), nitrate ( $\mu\text{g/L} \pm 1 \text{ S.D.}$ ), soluble reactive phosphorus (SRP; $\mu\text{g/L} \pm 1 \text{ S.D.}$ ), dissolved oxygen (DO; $\text{mg/L} \pm 1 \text{ S.D.}$ ), and specific conductivity ( $\mu\text{S} \pm 1 \text{ S.D.}$ )..... | 15   |
| 2. Model parameters ( $\pm\text{SE}$ ) from least means square regression of response metrics regressed against temperature. Significant p-values ( $< 0.05$ ) are bolded; marginally significant values ( $> 0.05$ and $< 0.10$ ) are denoted with an asterisk.....                                                                                                                                                                          | 16   |
| 3. Mean percentage biovolume ( $\pm 1 \text{ SD}$ ) of biofilm community functional groups across all time periods.....                                                                                                                                                                                                                                                                                                                       | 17   |
| 4. Results from a one-way ANOVA with Tukey's honest significant difference (HSD) test of pairwise temperature treatment comparisons. Significant values ( $< 0.05$ ) are bolded and marginally significant values ( $0.05-0.10$ ) are denoted with an asterisk.....                                                                                                                                                                           | 18   |

## LIST OF FIGURES

| Figure                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | Page |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|
| 1. Log <sub>e</sub> transformed biomass, chl <i>a</i> , and ecosystem metabolism rates increased across the temperature gradient. Asterisks and least squares fit displayed when significant (alpha = 0.05) .....                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           | 19   |
| 2. Carbon (C) and nitrogen (N) content showed mixed non-linear responses to temperature that generally trended towards positive, and phosphorus (P) content increased with temperature. C:N ratios did not change with temperature, and C:P and N:P ratios showed slight decreases with temperature, largely drive by relatively high C:P and N:P ratios at the coldest treatment (ambient temperature of the source stream). Asterisks displayed when significant (alpha = 0.05), error bars are $\pm 1$ SD.....                                                                                                                                                                                                                                                                                                                                                                                                           | 20   |
| 3. Rates of phosphorus (P) uptake, nitrogen (N) uptake and N <sub>2</sub> -fixation increased across the temperature gradient. The molar ratio of NH <sub>4</sub> <sup>+</sup> to SRP uptake showed weak positive response to temperature, whereas the ratio of N acquisition (N <sub>2</sub> -fixation + NH <sub>4</sub> <sup>+</sup> uptake) to SRP uptake did not change with temperature. At warmer temperatures N <sub>2</sub> -fixation supplied the majority of total N. Asterisks and least squares fit displayed when significant (alpha = 0.05), error bars are $\pm 1$ SD. If multiple dates were used in the calculation of the response metric it was noted in the upper left hand panel corner; a plus “+” indicates that multiple days/metrics were combined (e.g., NH <sub>4</sub> <sup>+</sup> uptake + N <sub>2</sub> -fixation), and a colon “:” indicates that the ratio was taken of those dates ..... | 21   |
| 4. Measured nitrogen (N) uptake fell well below predicted N demand (A), incorporating N <sub>2</sub> -fixation into a metric of total N acquisition (NH <sub>4</sub> <sup>+</sup> uptake + N <sub>2</sub> -fixation) more closely matched predicted N demand (B). Measured phosphorus (P) uptake (despite high variation) on average approximately met predicted P demand (C). .....                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | 22   |
| 5. Nitrogen (N) nutrient use efficiency (NUE; calculated with only NH <sub>4</sub> <sup>+</sup> uptake) increased across the temperature gradient (A). N NUE increased with warming, but to a lesser extent, when N <sub>2</sub> -fixation was incorporated (B). Phosphorus (P) NUE increased with warming (C). Asterisks and least squares fit displayed when significant (alpha = 0.05). If multiple dates were used in the calculation of the response metric it was noted in the upper left hand panel corner; a plus “+” indicates that multiple days/metrics were combined (e.g., NH <sub>4</sub> <sup>+</sup> uptake + N <sub>2</sub> -fixation), and a colon “:” indicates that the ratio was taken of those dates. ....                                                                                                                                                                                            | 23   |

## ABSTRACT

Freshwater ecosystems are key contributors to global fluxes of energy and materials. Within freshwater ecosystems, benthic biofilms (i.e. thin streambed mats of algae, bacteria and detrital matter) act as biogeochemical hotspots, contributing to these important fluxes. Understanding how temperature shapes the structure and function of biofilm communities, and thus the coupling of energy and material fluxes, is important to our ability to predict the effects of climate change. We cultivated stream benthic biofilm communities in experimental streamside channels under a range of warming scenarios (7.5-23.6°C) that maintained natural diel and seasonal temperature variation. We quantified autotrophic community structure, biomass, ecosystem metabolism, stoichiometry, and nutrient uptake. Biological N<sub>2</sub>-fixation was quantified as part of a concurrent study (Welter et al. *in review*). We found that temperature had strong effects on many metrics of ecosystem structure and function. Biofilm communities were dominated by cyanobacteria at all temperatures, which comprised >91% of total biovolume. Temperature had strong positive effects on biofilm biomass (2.8 to 24-fold variation) and net ecosystem productivity (44 to 317-fold variation). Temperature had minimal effects on biofilm stoichiometry; carbon:nitrogen (C:N) was constant across all temperatures, and carbon:phosphorus (C:P) declined slightly with temperature (a product of high C:P at the coldest temperature). Although ammonium uptake increased with temperature (2.8 to 6.8-fold variation), the magnitude of this response was not sufficient to meet total predicted N demand. We found that this shortfall was met by N<sub>2</sub>-fixation, particularly at warmer temperatures. In contrast, increases in dissolved SRP uptake across the thermal gradient were sufficient to meet the predicted demand. This study is one of few to isolate the effects of temperature on benthic biofilms, improving our understanding of how climate change may impact freshwater ecosystems.

# COUPLING ENERGY AND ELEMENTS IN A WARMING WORLD: HOW TEMPERATURE SHAPES BIOFILM ECOSYSTEM STRUCTURE AND FUNCTION

## Introduction

In the twentieth century global mean air temperatures rose 0.78°C as a result of anthropogenic greenhouse gas emissions (IPCC 2013). This warming has led to large changes in the structure and function of Earth's ecosystems, with demonstrated effects across broad latitudinal gradients (Parmesan and Yohe 2003). Current climate models predict that if human activities continue unchecked, global temperatures are likely to rise an additional 2.6 – 4.8°C by the end of this century (IPCC 2013). Surface air and water temperatures are strongly correlated (Pilgrim et al. 1998), and long-term records from freshwater ecosystems have begun to show clear warming trends (Adrian et al. 2009). As the effects of climate change intensify, it is anticipated that increasing temperatures will have significant direct and indirect effects on the structure and function of freshwater ecosystems (Woodward et al. 2010).

There is increasing recognition that freshwater ecosystems play an important role in global biogeochemical cycles, acting as key processors of energy and elements (Cole et al. 2007; Butman and Raymond 2011). In streams and rivers, benthic biofilms (i.e. thin streambed mats often composed of algae, bacteria, fungi and detrital matter) represent hotspots of biogeochemical activity and may contribute substantially to whole-system nutrient cycling and energy flux (Lock et al. 1984, Battin et al. 2003). For example, freshwater ecosystems contribute importantly to global carbon (C) cycles, as both sources (i.e., CO<sub>2</sub> evasion to the atmosphere) and sinks (i.e., C storage in sediments; Cole et al.

2007). These C fluxes often arise from metabolic processes, such as primary production (i.e. C fixation) and respiration (C loss as CO<sub>2</sub>). The Metabolic Theory of Ecology (MTE; Brown et al. 2004) and supporting empirical research suggest that warming will increase rates of metabolic processes (Yvon-Durocher et al. 2010a; Perkins et al. 2012). However, much uncertainty surrounds how anticipated changes in the metabolic processes that influence carbon cycling will translate to changes in the flux and storage of other biologically important elements, such as nitrogen (N) or phosphorus (P).

To a large extent, the degree of coupling between C and other important nutrients depends on the ambient availability of nutrients in the environment, and how the relative demand for nutrients changes with warming. Elevated C fixation in response to warming should drive concomitant increases in N and P demand that generally reflect the C:nutrient stoichiometry of primary producers. Thus, temperature-driven changes in primary producer stoichiometry (and demand) should be a key determinant of how coupled elemental cycles will respond to climate warming. Controlled single species studies have shown that warming can lead to increased C:nutrient stoichiometry (i.e. lower nutrient content) of primary producers (Rhee and Gotham 1981; Makino et al. 2011). Such changes have been attributed to shifting allocation of biomolecules in response to temperature (e.g., increased efficiency and reduced amounts of rRNA at warm temperatures; Sievers et al. 2004), as well as the flexible nature of autotroph cell quotas across environmental gradients (Sterner and Elser 2002). Importantly, the response of C:nutrient stoichiometry to warming may depend on ambient nutrient availability, with pronounced changes occurring in nutrient-limited environments (Frost et al. 2005; Sardans et al. 2012). In such environments, increased C:nutrient ratios may

translate into reduced relative demand for N and P, increased nutrient use efficiencies (NUE) of primary producers, and weak or less pronounced coupling of C and nutrient cycling.

In addition to species-level physiological changes, warming may lead to wholesale shifts in community structure that may also influence system-level nutrient demand. Autotrophic taxa exhibit varying temperature optima and thus experience differential responses to changing thermal regimes (Eppley 1972; Raven and Geider 1988). Long-term exposure to warming can manifest in community-level changes in species composition as temperatures exceed thermal thresholds (Eppley 1972; Hare et al. 2007). Because, primary producers show large interspecific variation in C:nutrient stoichiometry (Sterner 1995; Demars and Edwards 2007), warming-induced changes in community structure may result in shifts in bulk ecosystem stoichiometry, with important consequences for ecosystem nutrient demand, uptake and storage.

Changes in species composition can also influence the coupling of C and nutrient cycles, irrespective of changes in tissue stoichiometry. Warming may favor taxa that either alter environmental conditions or obtain nutrients from fundamentally different pools. For instance, the shrubification of arctic landscapes (e.g., transition to shrubby plants that increase shading) shifted ecosystems from nutrient to light limitation, decoupling productivity from available nutrient supply (Chapin et al. 1995). In aquatic systems, warming may similarly decouple primary production from dissolved nutrient supply by favoring N<sub>2</sub>-fixing cyanobacteria, which are thought to have higher optimal growth temperatures than other taxa (Paerl and Huisman 2008; but see Lüring et al. 2013). Thus, increased dominance of N<sub>2</sub>-fixers with warming may alter the degree to

which primary producers derive N from dissolved gaseous (i.e.,  $N_2$ ) versus mineral (i.e.,  $NH_4^+$  or  $NO_3^-$ ) forms.

Predicting how climate change may affect stream ecosystems will require a mechanistic understanding of how warming influences coupled C and nutrient cycles. Because benthic biofilms can be hotspots of biogeochemical activity, controlled warming experiments that isolate this habitat are needed. In this study, we experimentally examined the effects of warming on benthic biofilm communities in replicated streamside channels to address the following general questions: (1) how does warming alter the structure and function (e.g., metabolism, biomass, and community structure) of stream biofilms? and (2) how do these changes influence coupled C, N, and P dynamics?

We predicted that temperature would positively affect metabolic processes, enhancing rates of C fixation and respiration. We also predicted that respiration would respond more strongly to warming than C fixation, based on the different activation energies associated with cellular photosynthetic and respiratory complexes (Brown et al. 2004). Because dissolved nutrient concentrations are extremely low in our study system, we predicted that C:nutrient stoichiometry would increase with warming as a result of either physiological plasticity of individual taxa, or shifts in the relative abundance of autotrophic taxa. Finally, we predicted that N and P uptake would respond positively to warming, but to a lesser extent than C fixation (reflecting the C:nutrient stoichiometry), resulting in higher NUE of primary producers at warmer temperatures.

## Methods

### Site Description:

Our study was conducted in the Hengill region of Southwestern Iceland, a geothermally active area (64.05667 N, -21.28393 W) 30 km southeast of the capital city Reykjavik. This region contains a network of small streams and springs that vary significantly in temperature ( $\sim 6 - 100^{\circ}\text{C}$ ) despite their close spatial proximity. These wide temperature differences are the result of indirect geothermal heating of soils and bedrock, and streams consequently show little variation in solute chemistry (Friberg et al. 2009; O’Gorman et al. 2012). The dominant geology is young basalt and hyaloclastite rock formed in the late Quaternary Period (Árnason et al. 1969). Streams exhibit low dissolved N:P ratios suggesting strong N limitation (Friberg et al. 2009). The landscape is dominated by a variety of grass species, and woody vegetation is absent.

### Experimental Design:

We conducted a controlled and replicated streamside channel warming experiment using a relatively cold ( $6.7 \pm 1.7^{\circ}\text{C}$ , mean  $\pm$  S.D.; summer temperature) unnamed tributary of the Hengladalsá River with a regionally representative physiochemical profile. We achieved our temperature treatments (Table 1) with gravity-fed heat-exchanging systems (HEX;) placed in naturally occurring geothermal hot springs (Appendix A, Figure A1, also see Welter et al. [*in review*]). Briefly, a small impoundment was constructed to submerge a PVC intake that delivered cold water to the HEX system. Cold source water was split three ways. One line was routed through a stainless steel HEX ( $1.46 \text{ m}^2$  surface area) located in hot spring with a mean summer



temperature of  $\sim 30^{\circ}\text{C}$ . Cold water from another line was passed first through a similarly constructed HEX in the same hot spring and then into a second stainless steel HEX ( $0.55\text{ m}^2$  surface area) located in an adjacent hot spring (mean summer temperature  $\sim 62^{\circ}\text{C}$ ).

The third line was left unheated. Water from these three supply lines was remixed in five  $\sim 45\text{ L}$  constant head tanks, which supplied water to fifteen PVC channels (length:  $3\text{ m}$ , width:  $25.4\text{ mm}$ , height:  $40\text{ mm}$ ) arrayed in parallel on a  $1 \times 3\text{ m}$  PVC frame facing magnetic south (Appendix A, Figure A2). The  $1 \times 3\text{ m}$  PVC frame was divided into 3 blocks with 5 channels in each block, with temperature treatments randomly assigned to channels within the blocks ( $n = 5$  treatments,  $n = 3$  replicates; Appendix A, Figure A1).

Temperature was logged (Onset Computer Corp., HOBO Pendant, MA, USA) at the upstream end of each channel at 15 min intervals. Specific conductivity, nitrate ( $\text{NO}_3\text{-N}$ ), ammonium ( $\text{NH}_4^+$ ), soluble reactive phosphorus (SRP), and dissolved oxygen (DO) were monitored at the upstream end of the channel array at bimonthly intervals. Channel discharge, velocity, and depth were standardized at  $0.03 \pm 0.007\text{ L/s}$ ,  $0.24 \pm 0.09\text{ m/s}$ , and  $6 \pm 3.01\text{ mm}$  above tile surface (mean  $\pm 1\text{ S.D.}$ ), respectively. Basalt tiles ( $25 \times 25\text{ mm}$ ; Deko Tile, CA, USA;  $n \approx 110$  per channel) were deployed in each channel, and biofilm communities were allowed to naturally colonize each channel. Prior to deployment, tiles were leached for 18 days in tap water and boiled for 5 minutes (cf. Lamberti and Resh 1983). All tiles were deployed on 20 May 2013 and destructively sampled without replacement.

### Response Metrics:

Metabolism: We measured biofilm metabolism three times (30, 42, and 58 d) by measuring oxygen changes in 0.27 L clear recirculating chambers (Appendix A, Figure A2). Measurements were conducted simultaneously for all treatments within a single block, with block order chosen randomly. Four tiles were randomly selected from each treatment (surface area: 24 cm<sup>2</sup>) and placed in chambers containing sieved (250 µm) stream water. Chambers were partially submerged in the open-topped head tanks to maintain consistent incubation temperatures. We measured net ecosystem production (NEP) under ambient light conditions and ecosystem respiration (ER) in complete dark. The same tiles were used for both measurements, with water exchanged between measurements. Each metabolism measurement was paired with a tile-free chamber to correct for water column metabolism (with the exception of the 30 d measurements, when water column metabolism was measured for a single block only). Dissolved oxygen (DO) and internal chamber temperature were recorded at one-minute intervals (YSI, Pro-ODO, OH, USA). NEP and ER (mg DO m<sup>-2</sup> hr<sup>-1</sup>) were calculated as:

$$(NEP, ER) = \Delta O_2(V/S) \quad (1)$$

Where  $\Delta O_2$  is the change in DO concentration over time (mg DO L<sup>-1</sup> hr<sup>-1</sup>), V is chamber volume (L) and S is the active surface area of the tiles (m<sup>2</sup>). Gross primary production (GPP) was calculated as:  $GPP = NEP + ER$  (Bott 2006). We corrected for minimal water column metabolism by subtracting changes in DO measured in blank chambers.

Nutrient Uptake: We measured nutrient uptake rates (N and P; µg m<sup>2</sup> hr<sup>-1</sup>) in the recirculating chambers on three dates on different tiles (surface area: 24 cm<sup>2</sup>), and on

separate, but typically consecutive days. N and P (as  $\text{NH}_4\text{Cl}$  and  $\text{Na}_2\text{HPO}_4$ ) were added to a target concentration of  $\sim 50 \mu\text{g/L}$  above ambient at the beginning of the incubations. Triplicate water samples were collected at the beginning and end of incubations (typically 1.5h), filtered through  $0.45 \mu\text{m}$  glass fiber filters, and analyzed using colorimetric (P; APHA 2005) and fluorometric techniques (N; Holmes et al. 1999; Taylor et al. 2007). Areal uptake rates were calculated as the difference between nutrient mass pre- and post-incubation divided by tile surface area ( $\text{m}^2$ ) and length of incubation (hr).

Nitrogen Fixation: As part of a concurrent study (Welter et al. *in review*), rates of biological  $\text{N}_2$ -fixation were measured at three time periods that generally coincided with the aforementioned sampling schedule. Measurements were typically made on a single day at each sample period, and tile selection and handling was conducted as outlined above.  $\text{N}_2$ -fixation rates were quantified with acetylene reduction assays (see Welter et al. [*in review*] for detailed methods).

Biofilm Processing: Biofilms were scrubbed from tiles immediately after metabolism incubations with a medium bristle toothbrush into 125 ml of water. Tile slurries were homogenized, subsampled and filtered on glass fiber filters for analysis of ash free dry mass (AFDM), C, N, P content (Whatman<sup>TM</sup> GFF/F), and chlorophyll *a* (chl *a*; Andwin Scientific A/E). Nutrient incubation tiles were processed similarly, but only for AFDM and chl *a*. Chl *a* concentration was measured with a handheld fluorometer (Turner Designs, Aquafluor, CA, USA) after a 24-hour acetone extraction and expressed as chl *a* per unit area or per gram AFDM. AFDM, C, N and P filters were dried at  $55^\circ\text{C}$  for  $\geq 72$  hours and stored in a desiccator until processed. AFDM was calculated as the

difference between dry weight and ash weight retained on filters after 2 hours at 500°C. C and N content was determined with an elemental analyzer (Costech Analytical Technologies, Inc., CA, USA) at the Environmental Analytical Laboratory in the Dept. of Land Resources and Environmental Sciences at Montana State University, Bozeman, MT. P content was determined with an acid digest colorimetric technique (APHA 2005). Community composition subsamples were preserved in ~5% glutaraldehyde, centrifugally concentrated and enumerated at 400x in 0.1 ml Palmer counting cells until 30 field of view (FOV) counts per sample or  $\geq 300$  individuals was reached (Lowe and LaLiberte 2006). Algal biovolume was calculated using established taxon geometries (Sun and Lui 2003).

Predicted Nutrient Demand: We predicted nutrient demand from estimates of NEP and stoichiometry to assess how closely our measured values of nutrient acquisition met predicted demand. Predicted nutrient demand was calculated as:

$$\text{Predicted demand (N or P)} = \text{NEP moles C m}^{-2} \text{ hr}^{-1} \times (\text{N or P}): \text{C} \quad (2)$$

NEP values were converted to units of C assuming a photosynthetic quotient of 1.2 (Bott 2006). We also compared our predicted N demand estimates to total measured N acquisition, i.e. the sum of  $\text{NH}_4^+$  uptake plus  $\text{N}_2$ -fixation (Welter et al. *in review*).

Nutrient Use Efficiency: We determined nutrient use efficiencies (NUE) for N and P to assess whether temperature altered the coupling of C fixation and nutrient acquisition. NUE's were calculated as NEP per unit nutrient acquisition (Pastor and

Bridgham 1999). P NUE was calculated using measured SRP uptake rates (Equation 3 below) and N NUE was calculated with measured  $\text{NH}_4^+$  uptake (Equation 4 below) and with  $\text{NH}_4^+$  uptake plus  $\text{N}_2$ -fixation rates (Equation 5 below;  $\text{N}_2$ -fixation data from Welter et al. *in review*).

$$\text{P NUE} = \frac{\text{NEP (moles C m}^{-2} \text{ hr}^{-1})}{\text{SRP uptake (moles P m}^{-2} \text{ hr}^{-1})} \quad (3)$$

$$\text{N NUE} = \frac{\text{NEP (moles C m}^{-2} \text{ hr}^{-1})}{\text{NH}_4^+ \text{ uptake (moles N m}^{-2} \text{ hr}^{-1})} \quad (4)$$

$$\text{N NUE} = \frac{\text{NEP moles (C m}^{-2} \text{ hr}^{-1})}{\text{NH}_4^+ \text{ uptake (moles N m}^{-2} \text{ hr}^{-1}) + \text{N}_2 \text{ fixation (moles N m}^{-2} \text{ hr}^{-1})} \quad (5)$$

Statistical Analysis: All statistical analyses were performed in R (R Core Team 2013). Response metrics were  $\log_e(x)$  transformed to meet model assumptions of normality where appropriate. Data were preferentially analyzed with regression techniques, with ANOVA methods used as a ‘fall back’ when residual plots of transformed data showed evidence of non-linear responses (Cottingham et al. 2005). We found this a more conservative approach for addressing potential threshold temperature effects, thus avoiding assumptions about the shape of non-linear models. Least squares regressions were fit with the “lm” function and ANOVA’s (repeated measures [rmANOVA] and one-way) were fit with the “aov” function in the R package “stats” (R Core Team 2013). Post-hoc analyses were conducted where appropriate using Tukey’s Honest Significant Difference (Tukey HSD) tests in the R package “stats” (R Core Team

2013). All analysis were conducted at an alpha of 0.05. The block variable was non-significant for all response metrics and was thus subsequently removed to produce the most parsimonious descriptive model.

## Results

### Treatment Effect:

Consistent temperature treatments that maintained diel and seasonal variation of the source stream were achieved during the 66 d experiment (Table 1; Appendix A, Figure A3). Source water dissolved nutrient concentrations, taken from the upstream end of the channels, were not altered by the temperature treatments (rmANOVA: SRP,  $F_{5,20}=0.85$ ,  $p=0.53$ ;  $\text{NH}_4^+$ ,  $F_{5,20}=2.06$ ,  $p=0.113$ ;  $\text{NO}_3\text{-N}$ ,  $F_{5,20}=0.67$ ,  $p=0.65$ ). However, specific conductivity and dissolved oxygen (DO) decreased slightly, but significantly, at higher temperatures (Table 1; rmANOVA: specific conductivity,  $F_{5,30}=8.90$ ,  $p<0.001$ ; DO,  $F_{5,30}=86.24$ ,  $p<0.001$ ).

### Biomass and Community Structure:

Temperature had a strong positive effect on biomass and chl *a* of stream biofilms (Figure 1a, b; Table 2). Biomass (as AFDM) varied 2.8, 20 and 24-fold at 30, 42, and 58 d, respectively (Figure 1a, Table 2). Areal chl *a* concentrations varied 50, 231, and 174-fold at 30, 42 and 58 d, respectively (Figure 1b, Table 2). Temperature also had a positive effect on mass specific chl *a*, which increased 5, 14, and 4-fold with warming at the same time periods (Table 2).

Biofilm community composition was dominated by  $\text{N}_2$ -fixing cyanobacteria (largely *Nostoc* spp. and *Anabaena* spp.) in all temperature treatments. This functional

group comprised >91% of community biovolume irrespective of temperature (Table 3). The remaining functional groups included green and yellow green algae ( $2.8 \pm 8.0\%$ , mean  $\pm$  S.D.), diatoms ( $1.8 \pm 1.5\%$ ) and N<sub>2</sub>-fixing diatoms (largely *Epithemia* spp. and *Rhopalodia* spp.;  $0.5 \pm 1.4$ ; Table 3).

#### Metabolism:

Warming had strong positive effects on ecosystem metabolism throughout the experiment. At 42 d and 58 d GPP varied 51 and 55-fold, NEP varied 317 and 44-fold, and ER varied 22 and 42-fold, respectively (Figure 1c, d, e; Table 2). All of these relationships were linear once  $\log_e(x)$  transformed. While the slopes of metabolic relationships were not highly variable among dates, the intercepts typically increased with time, consistent with biomass development. GPP:ER ratios were greater than one for nearly all temperatures and time periods ( $7.0 \pm 14.2$ , mean  $\pm$  S.D.), and this ratio increased with temperature (i.e., 2.8 and 1.2-fold over the temperature range) during the final two sample periods.

#### Stoichiometry:

Temperature had idiosyncratic effects on biofilm C and N content that were generally positive during the final sampling event (Figure 2a, b). Warming led to increased C (1.3-fold; from 13.9 to 25%) and N content (1.4-fold; from 1.6 to 2.8%) at 58 d (Figure 2a, b; Table 4). At this time period C and N content was highest at the two middle temperature treatments (15.5 and 19°C), relative to the coldest (7.5 and 11.2°C) and the warmest (23.6°C), treatments. Temperature also had a positive effect on P content, which varied 2.0 to 2.5-fold across the treatments (0.10 to 0.23%; Figure 2c;

Table 4). However, the increase in P content was not linear, with P increasing rapidly between the two coldest treatments, and then leveling off at the three warmest treatments. Despite these general increases in nutrient content, warming did not significantly affect biofilm C:N ratios (Figure 2d; Table 4). In contrast, warming had a significant negative effect on biofilm C:P, and N:P ratios, but these changes were largely driven by relatively high C:P and N:P ratios in the coldest temperature treatment, i.e. the ambient temperature of the source stream (Figure 2e, f; Table 4). Warming above ambient thermal conditions produced a 1.9-fold (C:P) and 1.8-fold (N:P) decrease in stoichiometry; however, further warming led to no additional change in biofilm stoichiometry.

#### Nutrient Uptake and Nitrogen Fixation:

Warming had a significant positive effect on  $\text{NH}_4^+$  uptake on all sampling dates.  $\text{NH}_4^+$  uptake rates increased 6.8, 3.2 and 2.8-fold at 31, 44, and 65 d, respectively (Figure 3a; Table 2). The magnitude of variation in  $\text{NH}_4^+$  uptake was substantially lower than that associated with GPP (average of 53-fold increase), ER (32-fold), and NEP (180-fold). Effects of warming on SRP uptake were much less apparent, with a significant positive effect on only one date and considerable unexplained variation (Figure 3b; Table 2). Warming had a strong positive effect on the molar ratio of  $\text{NH}_4^+$  to SRP uptake on the first sampling date, when it increased 9-fold across the thermal gradient (Figure 3c; Table 2), however this relationship disappeared at later time intervals as the biofilm developed.

Temperature had a strong positive effect on rates of  $\text{N}_2$ -fixation, which varied 22, 121, and 64-fold at 41, 53, and 69 d (Figure 3d; Welter et al. *in review*). This led to a significant positive effect of warming on the ratio of  $\text{N}_2$ -fixation to  $\text{NH}_4^+$  uptake, which increased 42 and 22-fold across the temperature gradient during the final two



measurement periods (Figure 3e; Table 2). Consistent with no effect of warming on N:P stoichiometry, temperature had no effect on the total molar N acquisition to SRP uptake ratio when N<sub>2</sub>-fixation was combined with NH<sub>4</sub><sup>+</sup> uptake (N<sub>2</sub>-fixation + NH<sub>4</sub><sup>+</sup> uptake; Figure 3f; Table 2).

#### Predicted Nutrient Demand:

Using combined estimates of biofilm stoichiometry and NEP to predict N demand suggest that measured NH<sub>4</sub><sup>+</sup> uptake should scale log-linearly with a slope that is similar to NEP. Although our predicted N demand and measured NH<sub>4</sub><sup>+</sup> uptake were significantly related ( $r^2 = 0.12$ ,  $p = 0.048$ ), the majority of values fell far below the 1:1 line (Figure 4a). On average, NH<sub>4</sub><sup>+</sup> uptake only accounted for 51% of total predicted N demand, with NH<sub>4</sub><sup>+</sup> uptake accounting for a decreasing percentage of total demand as predicted demand increased. However, including N<sub>2</sub>-fixation values (Welter et al. *in review*) to produce a summed estimate of total N acquisition led to values that more closely matched predicted N demand. In this case, the sum of NH<sub>4</sub><sup>+</sup> uptake and N<sub>2</sub>-fixation explained a higher proportion of the variation in N demand ( $r^2 = 0.23$ ,  $p = 0.007$ ), and the majority of values fell near the 1:1 line (Figure 4b). With respect to P, we found that predicted P demand was on average approximately met by dissolved SRP uptake. Predicted P demand and measured SRP uptake were significantly related ( $r^2 = 0.30$ ,  $p = 0.001$ ) with the majority of values scattered near the 1:1 line (Figure 4c).

#### Nutrient Use Efficiency:

Given the relatively constant C:N stoichiometry across the thermal gradient, we expected to find little change in NUE. However, temperature had a significant effect on N

NUE which increased 97 and 15-fold across the temperature gradient during the last two measurement periods (Figure 5a: Table 2). Calculating N NUE using total N acquisition (i.e.,  $\text{N}_2$ -fixation plus  $\text{NH}_4^+$  uptake) showed that warming still led to increased N NUE, but to a lesser extent (29 and 5-fold increase; Figure 5b: Table 2). Because C:P stoichiometry decreased slightly from the ambient temperature and then remained relatively constant at warmer temperatures, we expected that P NUE would either decrease slightly or not change. Interestingly, we found that warming had a positive effect on P NUE, which increased 37-fold on 43 d (Figure 5c: Table 2).

Table 1: Physiochemical characteristics of each temperature treatment; mean treatment temperature ( $^{\circ}\text{C} \pm 1$  S.D.), ammonium ( $\mu\text{g/L} \pm 1$  S.D.), nitrate ( $\mu\text{g/L} \pm 1$  S.D.), soluble reactive phosphorus (SRP;  $\mu\text{g/L} \pm 1$  S.D.), dissolved oxygen (DO;  $\text{mg/L} \pm 1$  S.D.), and specific conductivity ( $\mu\text{S} \pm 1$  S.D.).

| Treatment | Mean temperature | $\text{NH}_4^+$ | $\text{NO}_3\text{-N}$ | SRP        | DO         | Specific conductivity |
|-----------|------------------|-----------------|------------------------|------------|------------|-----------------------|
| A         | 7.5 (2.3)        | 11.6 (7.1)      | 52.2 (44.2)            | 16.6 (7.8) | 11.5 (0.4) | 68.8 (2.6)            |
| B         | 11.2 (2.2)       | 7.0 (4.7)       | 37.0 (31.3)            | 19.0 (6.8) | 11.5 (0.4) | 67.7 (2.8)            |
| C         | 15.5 (2.3)       | 11.5 (5.5)      | 46.0 (40.6)            | 15.3 (4.0) | 11.4 (0.5) | 67.4 (2.7)            |
| D         | 19.0 (2.2)       | 7.5 (5.1)       | 45.2 (44.3)            | 18.6 (8.7) | 10.7 (0.5) | 67.4 (3.0)            |
| E         | 23.6 (2.4)       | 11.8 (7.3)      | 41.2 (43.2)            | 15.4 (7.3) | 10.0 (0.4) | 67.6 (2.8)            |

Table 2: Model parameters ( $\pm$ SE) from least means square regression of response metrics regressed against temperature. Significant p-values ( $< 0.05$ ) are bolded; marginally significant values ( $> 0.05$  and  $< 0.10$ ) are denoted with an asterisk.

| Metric                     | Units                                                            | Transformation    | Day   | Intercept    | Slope        | $p$               | $r^2$ |
|----------------------------|------------------------------------------------------------------|-------------------|-------|--------------|--------------|-------------------|-------|
| Biomass                    | g AFDM m <sup>-2</sup>                                           | $\text{Log}_e(x)$ | 30    | 0.22 (0.10)  | 0.06 (0.01)  | <b>&lt; 0.001</b> | 0.86  |
| Biomass                    | g AFDM m <sup>-2</sup>                                           | $\text{Log}_e(x)$ | 42    | -0.93 (0.21) | 0.19 (0.01)  | <b>&lt; 0.001</b> | 0.94  |
| Biomass                    | g AFDM m <sup>-2</sup>                                           | $\text{Log}_e(x)$ | 58    | -0.25 (0.13) | 0.19 (0.01)  | <b>&lt; 0.001</b> | 0.97  |
| Chl <i>a</i>               | mg m <sup>-2</sup>                                               | $\text{Log}_e(x)$ | 30    | -2.90 (0.32) | 0.24 (0.02)  | <b>&lt; 0.001</b> | 0.91  |
| Chl <i>a</i>               | mg m <sup>-2</sup>                                               | $\text{Log}_e(x)$ | 42    | -2.91 (0.29) | 0.34 (0.01)  | <b>&lt; 0.001</b> | 0.96  |
| Chl <i>a</i>               | mg m <sup>-2</sup>                                               | $\text{Log}_e(x)$ | 58    | -1.13 (0.29) | 0.30 (0.01)  | <b>&lt; 0.001</b> | 0.95  |
| Mass specific Chl <i>a</i> | μg gAFDM <sup>-1</sup>                                           | $\text{Log}_e(x)$ | 30    | -2.09 (0.31) | 0.12 (0.01)  | <b>&lt; 0.001</b> | 0.74  |
| Mass specific Chl <i>a</i> | μg gAFDM <sup>-1</sup>                                           | $\text{Log}_e(x)$ | 42    | -2.36 (0.20) | 0.17 (0.01)  | <b>&lt; 0.001</b> | 0.93  |
| Mass specific Chl <i>a</i> | μg gAFDM <sup>-1</sup>                                           | $\text{Log}_e(x)$ | 58    | -0.34 (0.35) | 0.09 (0.02)  | <b>&lt; 0.001</b> | 0.58  |
| GPP                        | mg O <sub>2</sub> m <sup>-2</sup> hr <sup>-1</sup>               | $\text{Log}_e(x)$ | 30    | 0.48 (2.57)  | 0.18 (0.12)  | 0.205             | 0.20  |
| GPP                        | mg O <sub>2</sub> m <sup>-2</sup> hr <sup>-1</sup>               | $\text{Log}_e(x)$ | 42    | -0.62 (0.64) | 0.29 (0.03)  | <b>&lt; 0.001</b> | 0.86  |
| GPP                        | mg O <sub>2</sub> m <sup>-2</sup> hr <sup>-1</sup>               | $\text{Log}_e(x)$ | 58    | 0.24 (0.37)  | 0.29 (0.02)  | <b>&lt; 0.001</b> | 0.94  |
| NEP                        | mg O <sub>2</sub> m <sup>-2</sup> hr <sup>-1</sup>               | $\text{Log}_e(x)$ | 30    | -0.81 (3.31) | 0.22 (0.15)  | 0.218             | 0.18  |
| NEP                        | mg O <sub>2</sub> m <sup>-2</sup> hr <sup>-1</sup>               | $\text{Log}_e(x)$ | 42    | -3.35 (1.17) | 0.40 (0.06)  | <b>&lt; 0.001</b> | 0.76  |
| NEP                        | mg O <sub>2</sub> m <sup>-2</sup> hr <sup>-1</sup>               | $\text{Log}_e(x)$ | 58    | 0.13 (0.33)  | 0.29 (0.01)  | <b>&lt; 0.001</b> | 0.95  |
| ER                         | mg O <sub>2</sub> m <sup>-2</sup> hr <sup>-1</sup>               | $\text{Log}_e(x)$ | 30    | 0.28 (2.68)  | 0.12 (0.13)  | 0.394             | 0.02  |
| ER                         | mg O <sub>2</sub> m <sup>-2</sup> hr <sup>-1</sup>               | $\text{Log}_e(x)$ | 42    | -0.79 (0.46) | 0.23 (0.02)  | <b>&lt; 0.001</b> | 0.86  |
| ER                         | mg O <sub>2</sub> m <sup>-2</sup> hr <sup>-1</sup>               | $\text{Log}_e(x)$ | 58    | -1.39 (0.72) | 0.29 (0.04)  | <b>&lt; 0.001</b> | 0.80  |
| N uptake                   | μg NH <sub>4</sub> <sup>+</sup> m <sup>-2</sup> hr <sup>-1</sup> | $\text{Log}_e(x)$ | 31    | 5.27 (0.47)  | 0.12 (0.03)  | <b>0.002</b>      | 0.58  |
| N uptake                   | μg NH <sub>4</sub> <sup>+</sup> m <sup>-2</sup> hr <sup>-1</sup> | $\text{Log}_e(x)$ | 44    | 6.58 (0.20)  | 0.08 (0.01)  | <b>0.000</b>      | 0.75  |
| N uptake                   | μg NH <sub>4</sub> <sup>+</sup> m <sup>-2</sup> hr <sup>-1</sup> | $\text{Log}_e(x)$ | 65    | 6.59 (0.30)  | 0.07 (0.02)  | <b>0.012</b>      | 0.51  |
| P uptake                   | μg SRP m <sup>-2</sup> hr <sup>-1</sup>                          | $\text{Log}_e(x)$ | 32    | 6.94 (0.46)  | -0.02 (0.03) | 0.531             | 0.05  |
| P uptake                   | μg SRP m <sup>-2</sup> hr <sup>-1</sup>                          | $\text{Log}_e(x)$ | 42    | 4.64 (0.67)  | 0.11 (0.04)  | <b>0.023</b>      | 0.39  |
| P uptake                   | μg SRP m <sup>-2</sup> hr <sup>-1</sup>                          | $\text{Log}_e(x)$ | 66    | 5.46 (1.27)  | 0.07 (0.07)  | 0.311             | 0.04  |
| N:P uptake                 | Molar ratio                                                      | <i>none</i>       | 31:32 | -2.18 (0.78) | 0.41 (0.04)  | <b>0.003</b>      | 0.95  |
| N:P uptake                 | Molar ratio                                                      | <i>none</i>       | 44:43 | 15.41 (3.42) | -0.39 (0.21) | 0.160             | 0.37  |
| N:P uptake                 | Molar ratio                                                      | <i>none</i>       | 65:66 | 13.8 (6.37)  | -0.41 (0.32) | 0.427             | 0.22  |

Table 2: Continued.

| Metric                            | Units       | Transformation            | Day      | Intercept     | Slope        | <i>p</i>         | <i>r</i> <sup>2</sup> |
|-----------------------------------|-------------|---------------------------|----------|---------------|--------------|------------------|-----------------------|
| N aquired:P uptake                | Molar ratio | <i>none</i>               | 41+44:43 | 11.43 (6.17)  | 0.32 (0.38)  | 0.460            | 0.07                  |
| N aquired:P uptake                | Molar ratio | <i>none</i>               | 69+65:66 | 36.04 (12.04) | -0.83 (0.61) | 0.406            | 0.29                  |
| N <sub>2</sub> -fixation:N uptake | Molar ratio | <i>none</i>               | 41:44    | -1.15 (0.51)  | 0.13 (0.03)  | <b>0.022</b>     | 0.81                  |
| N <sub>2</sub> -fixation:N uptake | Molar ratio | <i>none</i>               | 69:65    | -0.84 (0.51)  | 0.16 (0.03)  | <b>0.013</b>     | 0.86                  |
| N NUE <sup>1</sup>                | Molar ratio | <i>Log<sub>e</sub>(x)</i> | 30       | -0.55 (1.89)  | 0.12 (0.09)  | 0.405            | 0.29                  |
| N NUE <sup>1</sup>                | Molar ratio | <i>Log<sub>e</sub>(x)</i> | 42       | -2.81 (0.85)  | 0.28 (0.05)  | <b>0.012</b>     | 0.87                  |
| N NUE <sup>1</sup>                | Molar ratio | <i>Log<sub>e</sub>(x)</i> | 58       | 0.47 (0.14)   | 0.16 (<0.01) | <b>&lt;0.001</b> | 0.98                  |
| N NUE <sup>2</sup>                | Molar ratio | <i>Log<sub>e</sub>(x)</i> | 42       | -2.36 (0.94)  | 0.21 (0.05)  | <b>0.035</b>     | 0.75                  |
| N NUE <sup>2</sup>                | Molar ratio | <i>Log<sub>e</sub>(x)</i> | 58       | 0.68 (0.43)   | 0.09 (0.02)  | <b>0.042</b>     | 0.72                  |
| P NUE                             | Molar ratio | <i>Log<sub>e</sub>(x)</i> | 30       | -0.62 (2.58)  | 0.22 (0.12)  | 0.337            | 0.48                  |
| P NUE                             | Molar ratio | <i>Log<sub>e</sub>(x)</i> | 42       | 0.08 (1.15)   | 0.23 (0.07)  | <b>0.465</b>     | 0.70                  |
| P NUE                             | Molar ratio | <i>Log<sub>e</sub>(x)</i> | 58       | 2.93 (0.75)   | 0.12 (0.03)  | 0.184            | 0.83                  |

<sup>1</sup>N NUE calculated with NH<sub>4</sub><sup>+</sup> uptake values only. <sup>2</sup>N NUE calculated with the sum of NH<sub>4</sub><sup>+</sup> uptake and N<sub>2</sub>-fixation (from Welter et al. *in review*). If multiple dates were used in the calculation of the response metric it was noted in the Day column; a plus “+” indicates that multiple days/metrics were combined (e.g., NH<sub>4</sub><sup>+</sup> uptake + N<sub>2</sub>-fixation), and a colon “:” indicates that the ratio was take of those dates. N = Nitrogen; P = Phosphorus; GPP = Gross primary productivity; NEP = Net ecosystem productivity; ER = Ecosystem respiration; NUE = Nutrient use efficiency

Table 3: Mean percentage biovolume (±1 SD) of biofilm community functional groups across all time periods.

|           | Cyanobacteria | Diatoms   | N <sub>2</sub> -fixing diatoms | Green and yellow green algae |
|-----------|---------------|-----------|--------------------------------|------------------------------|
| Treatment | --            | --        | --                             | --                           |
| A         | 98.2 (2.3)    | 1.6 (2.3) | 0.02 (0.07)                    | 0.04 (0.08)                  |
| B         | 97.9 (1.7)    | 1.1 (0.4) | NA                             | 0.8 (1.6)                    |
| C         | 98.5 (1.0)    | 1.2 (1.0) | 0.02 (0.03)                    | 0.2 (0.3)                    |
| D         | 91.7 (14.8)   | 2.4 (1.7) | 0.7 (1.6)                      | 5.1 (13.3)                   |
| E         | 91.6 (8.5)    | 2.9 (1.6) | 1.3 (2.0)                      | 4.0 (7.2)                    |

Table 4: Results from a one-way ANOVA with Tukey's honest significant difference (HSD) test of pairwise temperature treatment comparisons. Significant values (<0.05) are bolded and marginally significant values (0.05-0.10) are denoted with an asterisk.

| Metric | Units       | Day | Df   | Treatment |                  | Tukey HSD pairwise comparisons |                  |                  |                  |              |              |              |       |        |              |
|--------|-------------|-----|------|-----------|------------------|--------------------------------|------------------|------------------|------------------|--------------|--------------|--------------|-------|--------|--------------|
|        |             |     |      | F-stat    | P-value          | A-B                            | A-C              | A-D              | A-E              | B-C          | B-D          | B-E          | C-D   | C-E    | D-E          |
| C      | Percent     | 30  | 4,10 | 2.48      | 0.111            | 0.334                          | 0.141            | 0.109            | 0.537            | 0.969        | 0.927        | 0.993        | 1.000 | 0.838  | 0.751        |
| C      | Percent     | 42  | 4,10 | 3.73      | <b>0.042</b>     | 0.925                          | 0.998            | 0.247            | 0.372            | 0.985        | 0.076*       | 0.122        | 0.162 | 0.252  | 0.998        |
| C      | Percent     | 58  | 4,10 | 14.44     | <b>&lt;0.001</b> | 0.936                          | <b>0.003</b>     | <b>0.001</b>     | 0.190            | <b>0.010</b> | <b>0.002</b> | 0.504        | 0.821 | 0.122  | <b>0.024</b> |
| N      | Percent     | 30  | 4,10 | 0.66      | 0.633            | 0.654                          | 0.776            | 0.713            | 0.970            | 0.999        | 1.000        | 0.935        | 1.000 | 0.981  | 0.961        |
| N      | Percent     | 42  | 4,10 | 3.19      | 0.062*           | 1.000                          | 1.000            | 0.213            | 0.250            | 1.000        | 0.197        | 0.232        | 0.200 | 0.236  | 1.000        |
| N      | Percent     | 58  | 4,10 | 26.84     | <b>&lt;0.001</b> | 0.491                          | <b>&lt;0.001</b> | <b>&lt;0.001</b> | <b>0.040</b>     | <b>0.001</b> | <b>0.001</b> | 0.440        | 1.000 | 0.007* | <b>0.007</b> |
| P      | Percent     | 30  | 4,10 | 14.80     | <b>&lt;0.001</b> | 0.357                          | <b>0.002</b>     | <b>0.002</b>     | <b>0.001</b>     | <b>0.035</b> | <b>0.031</b> | <b>0.011</b> | 1.000 | 0.929  | 0.948        |
| P      | Percent     | 42  | 4,10 | 4.85      | <b>0.019</b>     | 0.956                          | 0.738            | <b>0.016</b>     | 0.264            | 0.980        | <b>0.046</b> | 0.589        | 0.108 | 0.872  | 0.402        |
| P      | Percent     | 58  | 4,10 | 30.35     | <b>&lt;0.001</b> | <b>&lt;0.001</b>               | <b>&lt;0.001</b> | <b>&lt;0.001</b> | <b>&lt;0.001</b> | 0.759        | 0.934        | 0.995        | 0.993 | 0.552  | 0.782        |
| C:N    | Molar ratio | 30  | 4,10 | 4.50      | <b>0.024</b>     | 0.997                          | 0.075*           | 0.060*           | 0.248            | 0.122        | 0.098*       | 0.377        | 1.000 | 0.921  | 0.868        |
| C:N    | Molar ratio | 42  | 4,10 | 0.33      | 0.845            | 0.814                          | 0.994            | 0.982            | 0.917            | 0.955        | 0.980        | 0.999        | 1.000 | 0.992  | 0.998        |
| C:N    | Molar ratio | 58  | 4,10 | 0.23      | 0.912            | 0.994                          | 0.999            | 0.987            | 1.000            | 1.000        | 0.896        | 0.992        | 0.936 | 0.998  | 0.989        |
| C:P    | Molar ratio | 30  | 4,10 | 6.13      | <b>0.009</b>     | 0.091*                         | <b>0.015</b>     | <b>0.013</b>     | <b>0.021</b>     | 0.760        | 0.718        | 0.870        | 1.000 | 0.999  | 0.998        |
| C:P    | Molar ratio | 42  | 4,10 | 1.84      | 0.197            | 0.349                          | 0.275            | 0.183            | 0.474            | 1.000        | 0.988        | 0.999        | 0.998 | 0.991  | 0.944        |
| C:P    | Molar ratio | 58  | 4,10 | 4.07      | <b>0.032</b>     | <b>0.024</b>                   | 0.263            | 0.561            | 0.091*           | 0.534        | 0.246        | 0.904        | 0.966 | 0.945  | 0.663        |
| N:P    | Molar ratio | 30  | 4,10 | 3.50      | <b>0.048</b>     | 0.209                          | 0.070*           | 0.064*           | 0.084*           | 0.945        | 0.927        | 0.970        | 1.000 | 1.000  | 1.000        |
| N:P    | Molar ratio | 42  | 4,10 | 5.31      | <b>0.014</b>     | 0.159                          | <b>0.028</b>     | <b>0.012</b>     | 0.233            | 0.783        | 0.481        | 0.999        | 0.980 | 0.636  | 0.350        |
| N:P    | Molar ratio | 58  | 4,10 | 9.07      | <b>0.002</b>     | <b>0.002</b>                   | 0.084*           | 0.118            | <b>0.008</b>     | 0.129        | 0.092*       | 0.798        | 0.999 | 0.550  | 0.430        |

C = Carbon, N = Nitrogen, P = Phosphorus

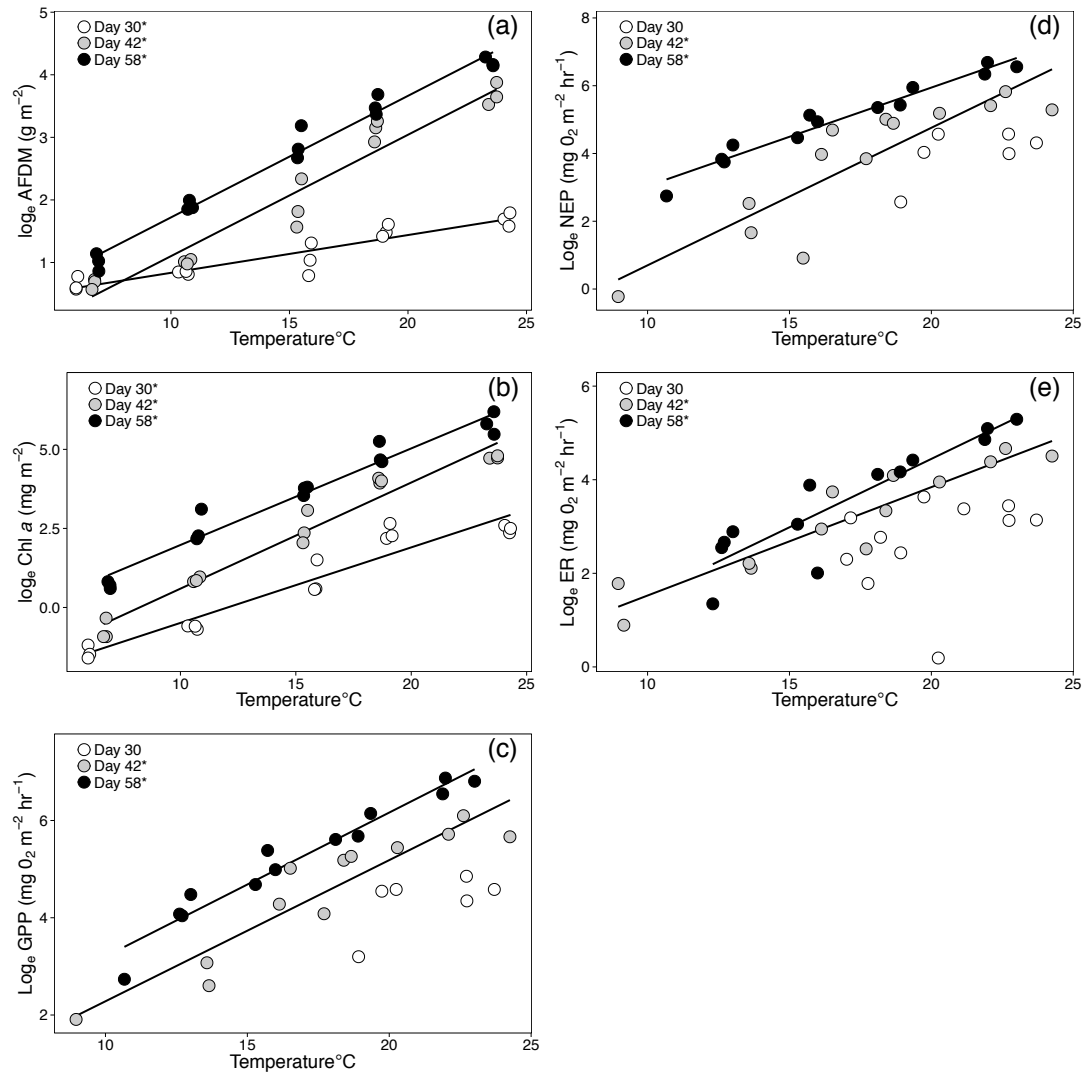


Figure 1:  $\text{Log}_e$  transformed biomass, chl  $a$ , and ecosystem metabolism rates increased across the temperature gradient. Asterisks and least squares fit displayed when significant ( $\alpha = 0.05$ ).

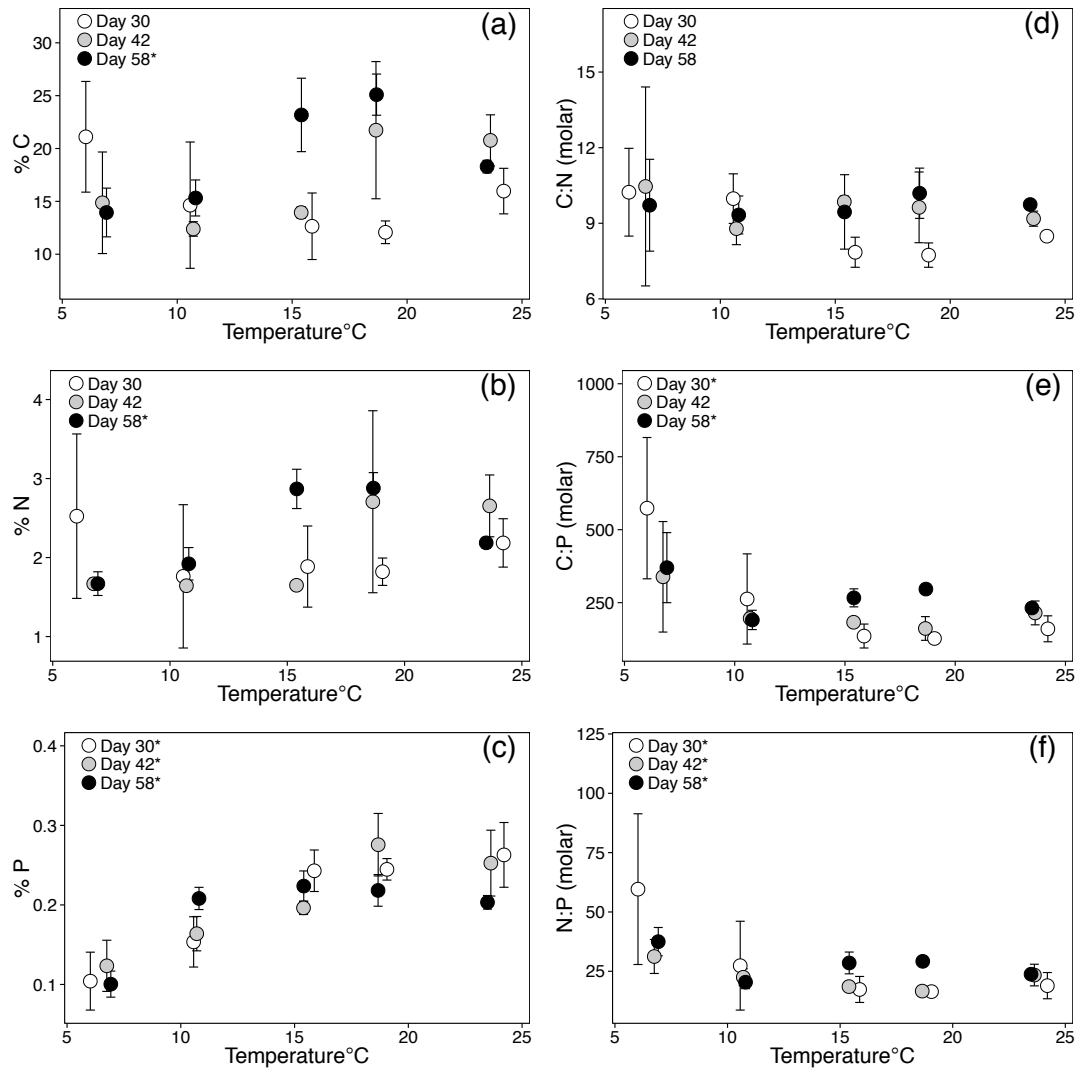


Figure 2: Carbon (C) and nitrogen (N) content showed mixed non-linear responses to temperature that generally trended towards positive, and phosphorus (P) content increased with temperature. C:N ratios did not change with temperature, and C:P and N:P ratios showed slight decreases with temperature, largely drive by relatively high C:P and N:P ratios at the coldest treatment (ambient temperature of the source stream). Asterisks displayed when significant ( $\alpha = 0.05$ ), error bars are  $\pm 1$  SD.

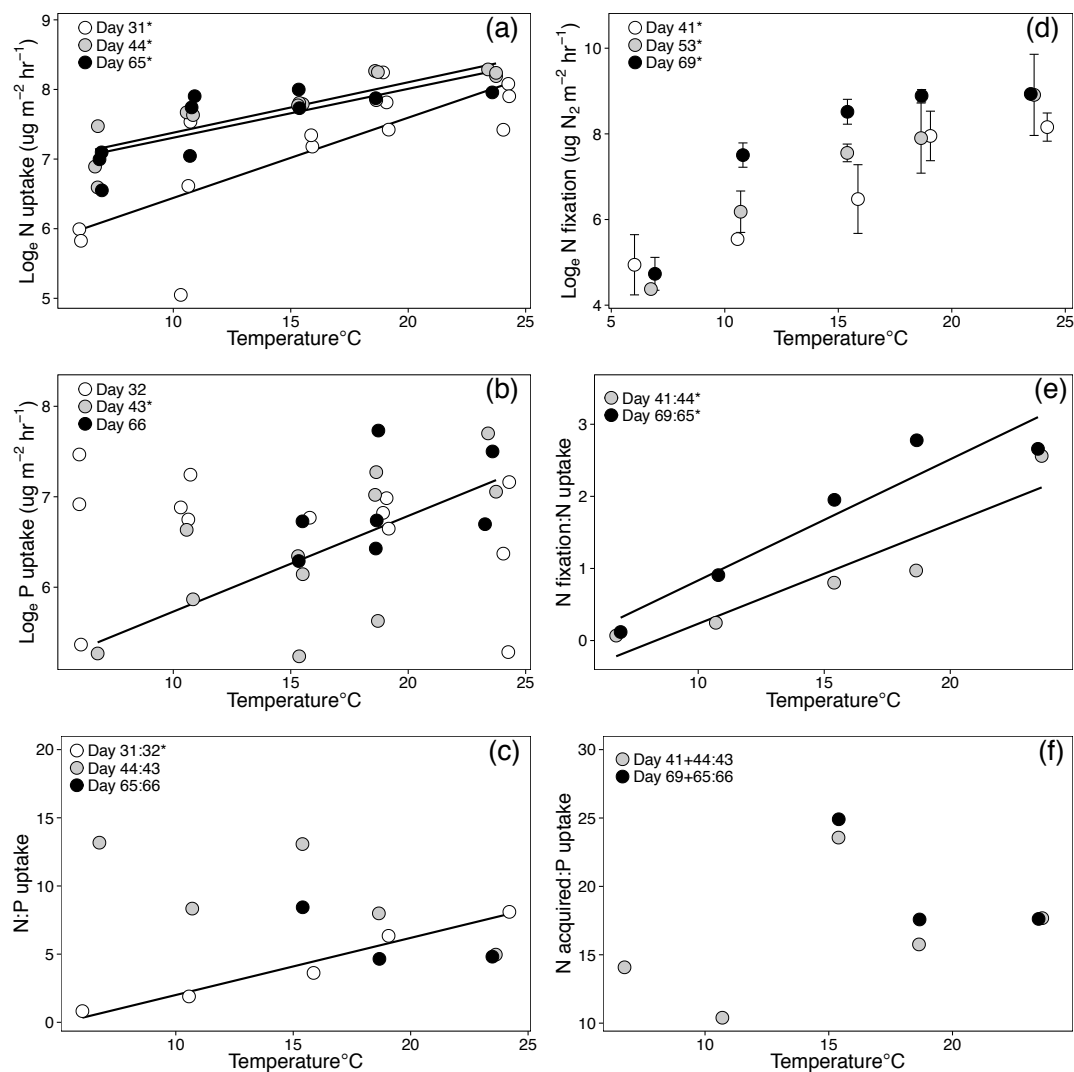


Figure 3: Rates of phosphorus (P) uptake, nitrogen (N) uptake and N<sub>2</sub>-fixation increased across the temperature gradient. The molar ratio of NH<sub>4</sub><sup>+</sup> to SRP uptake showed weak positive response to temperature, whereas the ratio of N acquisition (N<sub>2</sub>-fixation + NH<sub>4</sub><sup>+</sup> uptake) to SRP uptake did not change with temperature. At warmer temperatures N<sub>2</sub>-fixation supplied the majority of total N. Asterisks and least squares fit displayed when significant (alpha = 0.05), error bars are ±1 SD. If multiple dates were used in the calculation of the response metric it was noted in the upper left hand panel corner; a plus “+” indicates that multiple days/metrics were combined (e.g., NH<sub>4</sub><sup>+</sup> uptake + N<sub>2</sub>-fixation), and a colon “:” indicates that the ratio was taken of those dates.



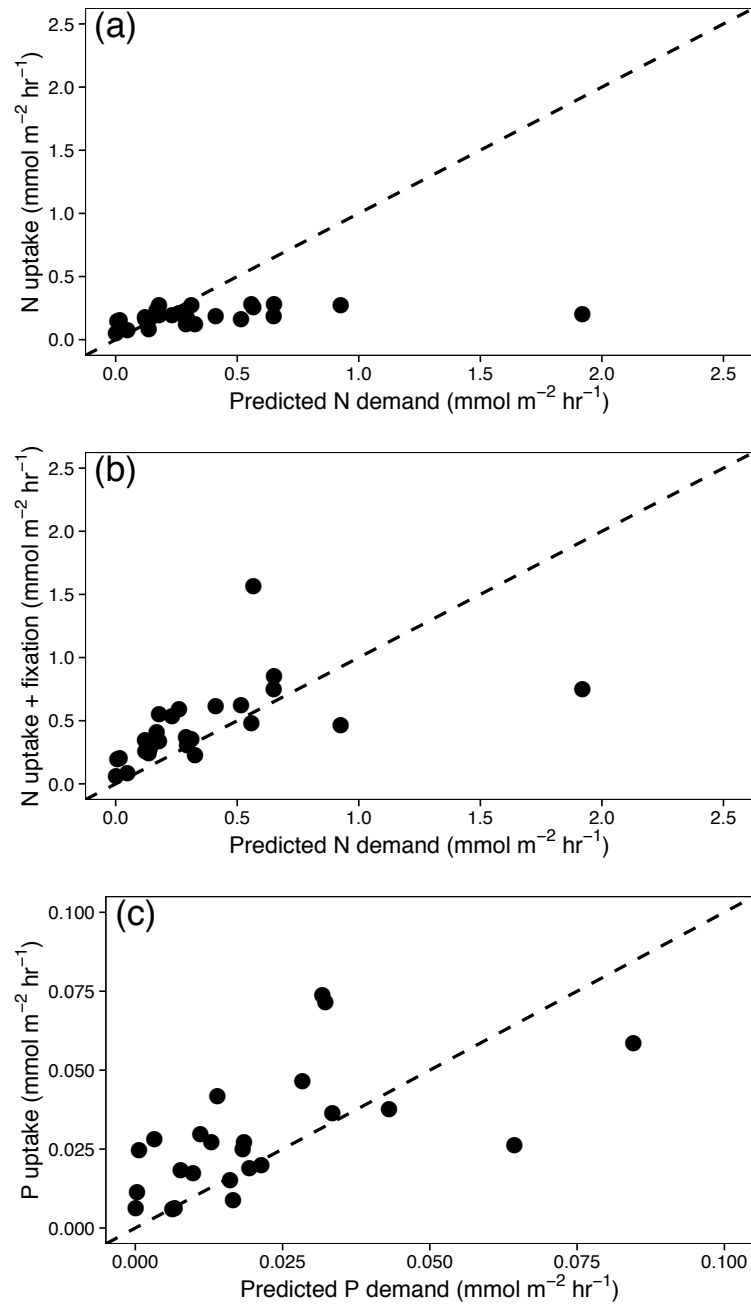


Figure 4: Measured nitrogen (N) uptake fell well below predicted N demand (A), incorporating  $\text{N}_2$ -fixation into a metric of total N acquisition ( $\text{NH}_4^+$  uptake +  $\text{N}_2$ -fixation) more closely matched predicted N demand (B). Measured phosphorus (P) uptake (despite high variation) on average approximately met predicted P demand (C).

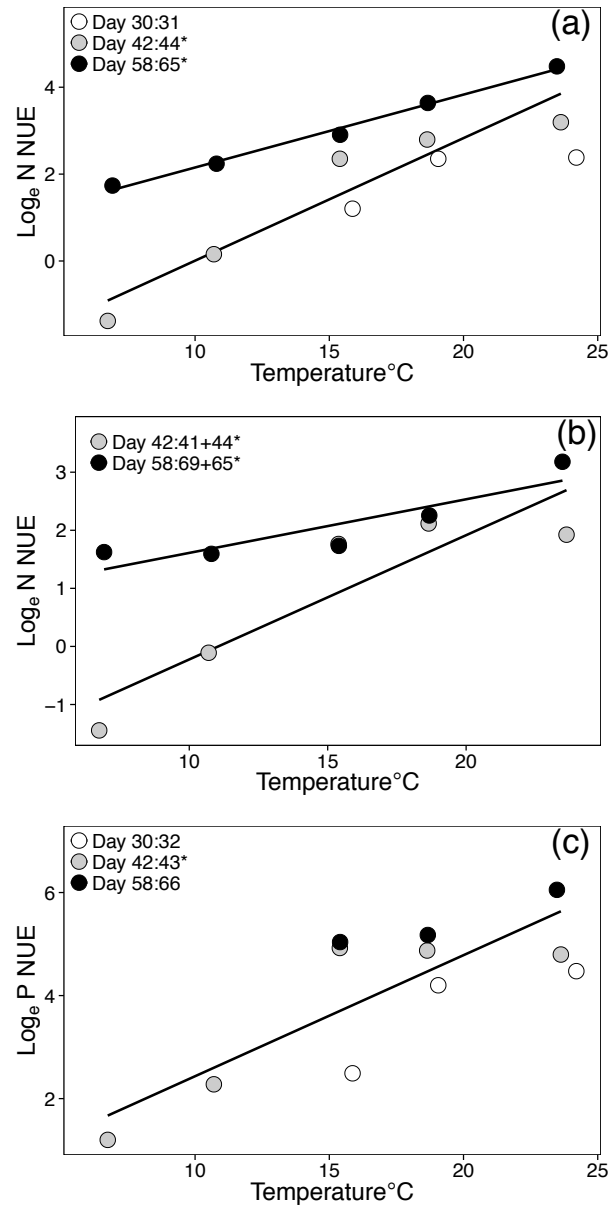


Figure 5: Nitrogen (N) nutrient use efficiency (NUE; calculated with only  $\text{NH}_4^+$  uptake) increased across the temperature gradient (A). N NUE increased with warming, but to a lesser extent, when  $\text{N}_2$ -fixation was incorporated (B). Phosphorus (P) NUE increased with warming (C). Asterisks and least squares fit displayed when significant ( $\alpha = 0.05$ ). If multiple dates were used in the calculation of the response metric it was noted in the upper left hand panel corner; a plus “+” indicates that multiple days/metrics were combined (e.g.,  $\text{NH}_4^+$  uptake +  $\text{N}_2$ -fixation), and a colon “:” indicates that the ratio was taken of those date

## Discussion

Anticipating and responding to the complex effects of climate change is a central challenge for ecologists. Because biofilm communities contribute significantly to the flux of energy and materials in freshwater ecosystems, experiments that isolate the effects of warming on these communities are critical for understanding system-level responses to climate change. We found that warming enhanced metabolic processes and biomass accumulation of biofilms, with a >24-fold increases in these characteristics over a 3-fold increase in temperature. Contrary to our predictions, warming had little effect on biofilm C:N stoichiometry, and dissolved uptake of ammonium was apparently not sufficient to meet total N demand. We showed that this shortfall in N demand could be accounted for by incorporating a new source of N (i.e., N<sub>2</sub>-fixation), underscoring the important roles of species composition and functional traits in driving responses of coupled carbon-nutrient cycles to environmental warming. Our study is among the few to explicitly isolate thermal effects on stream biofilms, and thus provides critical insight into how climate warming may alter the structure and function of these habitats, particularly under low nutrient conditions.

It is well known that temperature modulates metabolic processes of autotrophic organisms through changes in enzymatic reaction rates (DiNicola 1996; Gillooly et al. 2001). The temperature dependence of reactions governing metabolism, such as photosynthesis and respiration, can be expressed through the activation energy (AE) of each process. Cellular respiration has a higher effective AE (~0.67 eV) than photosynthesis (~0.32 eV), and we might expect these sub-cellular patterns to manifest at the ecosystem level (Brown et al. 2004; Yvon-Durocher et al. 2010a). Consistent with

previous findings (Yvon-Durocher et al. 2010a; Demars et al. 2011; Perkins et al. 2012), we measured a strong positive effect of warming on rates of ecosystem metabolism (GPP, NEP, and ER). However, the temperature dependencies of these metabolic rates were considerably amplified relative to predictions based on subcellular AEs (GPP: mean 2.1 eV, ER: 1.7 eV; Welter et al. *in review*). Welter et al. (*in review*) attributed this amplified response to increased resource availability (i.e. addition of 'new' N via increased rates of N<sub>2</sub>-fixation) with warming. Increasing N<sub>2</sub>-fixation would have reduced N limitation, allowing an amplified metabolic response to warming. Here, the dominance of one functional group, cyanobacteria, led to temperature dependencies that would have been difficult to predict based on theory. These results illustrate how strongly species traits can influence ecosystem function, and underscores the need to further explore the linkages between community structure and ecosystem function (Webb et al. 2010).

Metabolic theory predicts that standing stock biomass should decline with warming, if resource supply rates remain constant (Yvon-Durocher et al. 2010b). Given temperature invariant resources we would expect respiration to respond more strongly to warming than photosynthesis, producing declining biomass. Many previous warming experiments support this theory, demonstrating reduced biomass of primary producers at higher temperatures (Yvon-Durocher et al. 2010b; Dossena et al. 2012; Shurin et al. 2012; *but see* Baulch et al. 2005). In contrast, we found a large increase in biomass of biofilms across the thermal gradient, a pattern that was consistent across all sampling dates. This strong biomass response may be explained by changing resource availability across the thermal gradient, i.e. increased N via N<sub>2</sub>-fixation at warmer temperatures. Increasing resource availability may promote strong photosynthetic responses to

warming, which is consistent with the increasing NEP and the high (i.e., above one) GPP:ER ratios. Such deviations from theoretic predictions demonstrate the challenges inherent in scaling sub-cellular processes to whole ecosystems, and interactions between temperature and resource availability may prove a fruitful direction for future work (Anderson-Teixeira et al. 2008; Cross et al. *in review*).

We predicted that C:nutrient stoichiometry would increase with temperature, driven by cellular-level physiological adjustments and/or shifts towards taxa with higher C:nutrient stoichiometry. In contrast, we found that biofilm C:N stoichiometry remained relatively constant across all temperatures, and C:P stoichiometry decreased slightly with warming, despite the relatively low concentrations of dissolved N and P. Our community composition analysis can shed light on this pattern, as cyanobacteria dominated benthic biovolume at all temperatures. These taxa can access dissolved N<sub>2</sub> gas and maintain relatively high productivity and tissue N content by increasing rates of N<sub>2</sub>-fixation. Further, N<sub>2</sub>-fixers typically have high P requirements, which may result from the energy intensive high adenosine triphosphate (ATP) cost of N<sub>2</sub>-fixation (Vitousek et al. 2002). Dissolved P is relatively abundant (i.e., low dissolved N:P) in our study area (O'Gorman et al. 2012), which, in conjunction with the high P requirements of N<sub>2</sub>-fixers, may explain why C:P stoichiometry decreased slightly with warming. These results are consistent with recent work by De Senerpont Domis et al. (2014), who found that C:P stoichiometry of algal communities did not substantially increase with temperature in P rich conditions.

Although warming had relatively little effect on biofilm C:nutrient ratios, we found a 2-3-fold increase in P content, and a less pronounced (and idiosyncratic) increase in C and N across the thermal gradient. In addition to the generally high nutrient content

of N<sub>2</sub>-fixers (as outlined above), this pattern may be explained by temperature-induced changes in primary producer growth rates. Like many metabolic processes, growth rates are positively influenced by temperature, and increased growth can lead to elevated tissue nutrient content (e.g., Elser et al. 2000). The growth rate hypothesis (GRH; Elser et al. 1996) posits that with increasing growth, organisms must allocate additional resources to P-rich ribonucleic acid (RNA) and, to a lesser extent, N-rich proteins necessary for growth (Elser et al. 2000). This may explain why P content clearly increased with warming, whereas trends in C and N content were less clear. Interestingly, our findings are contrary to a recent synthesis of experimental studies demonstrating that warming reduces tissue N and P content of cold-acclimated poikilothermic organisms by ~30-50% (Woods et al. 2003). It may be that species were able to establish across the thermal gradient according to their respective thermal optimums, potentially avoiding the downward trends in nutrient content that cold acclimated species demonstrate. This illustrates the large uncertainty regarding how thermal acclimation may interact with ambient nutrient availability to modulate the elemental content of organisms at longer time scales.

Consistent C:N stoichiometry and increased N content across the thermal gradient suggest that biofilms were able to acquire sufficient N to meet demand across a broad range of metabolic rates. If NH<sub>4</sub><sup>+</sup> was the only potential source of N, we would expect to see greatly increased rates of NH<sub>4</sub><sup>+</sup> uptake with warming. We found that although NH<sub>4</sub><sup>+</sup> uptake increased with warming, the rate of increase was considerably lower than that of GPP or NPP, suggesting that NH<sub>4</sub><sup>+</sup> uptake was not sufficient to meet N demand. Further, our analysis of predicted N demand (based on NEP and biofilm stoichiometry)

demonstrated that  $\text{NH}_4^+$  uptake only accounted for an average of 51% of total N demand. This missing N may be explained by N acquisition via  $\text{N}_2$ -fixation. Welter et al. (*in review*) found that rates of  $\text{N}_2$ -fixation increased substantially across the temperature gradient, with temperature dependencies that were more consistent with those of NEP. When N acquisition through  $\text{N}_2$ -fixation and  $\text{NH}_4^+$  uptake were summed, these values more closely matched predicted N demand, suggesting that  $\text{N}_2$ -fixation contributes significantly to total N supplied to biofilms. We also found that  $\text{N}_2$ -fixation supplied an increasing proportion of total N under warmed conditions. At the coldest treatment  $\text{N}_2$ -fixation supplied ~9% of total N, whereas this increased to ~72% in the warmest treatment, effectively shifting communities from dissolved  $\text{NH}_4^+$  uptake to  $\text{N}_2$ -fixation as the primary N source. These data showcase the important role  $\text{N}_2$ -fixation plays in alleviating, or reducing, N limitation. This further illustrates the importance of including community trait characteristics when predicting how coupled biogeochemical cycles will respond to climate change (Webb et al. 2010; Finzi et al. 2011).

The important role of biological  $\text{N}_2$ -fixation has been demonstrated in many aquatic ecosystems, yet the fate of this fixed N and the potential consequences for aquatic food webs remain unclear (Howarth et al. 1988; Marcarelli et al. 2008). We found that community composition was dominated by  $\text{N}_2$ -fixing cyanobacteria (*Nostoc* spp. and *Anabaena* spp.), consistent with the low dissolved N:P ratio of the source water (i.e. likely N limitation; Levine and Schindler 1999; van de Wall et al. 2010). Dominance of cyanobacteria may have significant food web consequences because many of these taxa are considered a poor quality food resource for consumers (Gulati and Demott 1997) and have been associated with decreased energy transfer between trophic levels (Filstrup et

al. 2014). At smaller spatial scales the inedibility of cyanobacteria may reduce the transfer of energy and elements to higher trophic levels, creating trophic “dead-ends”. However, at larger spatial scales cyanobacteria may contribute to dissolved nutrient pools through cellular release or microbial pathways that decompose and mineralize nutrient rich cyanobacteria tissue (Gilbert and Bronk 1994). These contributions to dissolved nutrient pools could potentially fuel autotrophic growth in downstream reaches. This may translate to longitudinal gradients of  $N_2$ -fixation, with high fixation rates in nutrient poor headwater streams that decline with increasing stream order. The consequences of such spatial patterns of  $N_2$ -fixation remain poorly understood and warrant further study.

Contrary to predictions, we found that SRP uptake was generally not related to temperature. This is surprising considering the increased P content and slight decline in C:P ratios across the temperature gradient. The lack of a strong SRP uptake response may be due to relatively low demand for P (i.e., low dissolved N:P ratios) in Hengill streams (Friberg et al. 2009; O’Gorman et al. 2012). SRP uptake was generally high across all temperatures and sample periods, and our estimates of predicted P demand suggest that uptake of dissolved P roughly met, and often exceeded, P demand across all temperatures. These findings may not be entirely surprising when one considers that P is not a limiting nutrient. Following the Liebig model of nutrient limitation (Liebig 1855), we might expect SRP uptake rates to increase only when N is no longer limiting, a threshold we likely did not reach. This is consistent with recent work, which found that limiting nutrients can control acquisition rates of non-limiting nutrients (Perini and Bracken 2014). Again, this illustrates the importance of ambient nutrient availability in shaping the nutrient content and stoichiometric response to warming.



We found an increase in N NUE across the temperature gradient, despite the relative temperature invariance of biofilm C:N ratios. Such a result may arise from increased NUE of individuals (i.e., physiological changes; Baligar et al. 2001), species level community shifts towards more N efficient taxa (Hiremath and Ewel 2001), or other sources of N that we did not account for. While our data cannot address the possibilities of changing physiology of individual taxa or taxon-specific efficiencies, it is possible that unmeasured sources of N uptake could contribute to the observed increases in NUE. For instance, if  $\text{NO}_3$  represents a substantial contribution to total N uptake, particularly at high temperatures, our estimates of NUE would artificially increase metrics of NUE. We measured mineral uptake as  $\text{NH}_4^+$ , the form of N typically most available to aquatic autotrophs (Reddy 1983; Reay et al. 1999), but complementary studies from the Hengill region have shown that  $\text{NO}_3$  uptake can account for a temperature dependent ~25-40% of total dissolved N acquisition (Rasmussen et al. 2011; Demars et al. 2011). However, including estimated  $\text{NO}_3$  uptake in estimates of N NUE (assuming  $\text{NO}_3$  uptake  $\approx$ 25% of dissolved N acquisition at low temperatures and  $\approx$ 40% at warm temperature) does not significantly alter the positive relationship between temperature and N NUE (34 and 5.6-fold increases in N NUE at the last two time periods;  $p < 0.05$ ). We also found that P NUE increased with warming. Similarly to N, increasing P NUE could arise from changing cellular physiology, shifts in species level community structure, or could be an artifact of the loosely coupled relationship between production and SRP uptake.

There are important assumptions embedded in our calculations of both NUE and predicted nutrient demand that may influence our interpretations. We calculated NUE and predicted demand from estimates of NEP, consistent with previous methods (Pastor and

Bridgham 1999). We choose NEP, instead of GPP, as it provides a more conservative estimate of predicted nutrient demand. A potential issue with this approach is that it presumes the stoichiometry of new growth is comparable to existing biomass, and that gross nutrient acquisition is equal to net incorporation of nutrients in biomass. Nonetheless, our intention was not to develop a method that precisely predicts nutrient demand, but rather to understand if our measured values of nutrient acquisition realistically represented ecosystem fluxes. Further, using the more conservative estimate of NEP, instead of GPP, reduces predicted demand by ~20%, but does not substantially alter our interpretations.

Anticipating and understanding how climate change will affect ecosystems will require a broad array of research techniques that draw from theory, observation and experimentation. Mesocosm experiments can provide valuable insight into how ecosystems may respond to climate change because they allow us to elucidate mechanisms that may otherwise be obscured in observation studies of natural systems (Stewart et al. 2013). Indeed, much of the power of mesocosm experiments lies in their high degree of control and replication. However, these attributes may come at the cost of realism and potential scope of inference. We clearly showed that temperature had a strong influence on benthic biofilm structure, function and coupled elemental cycles. However, much uncertainty surrounds how these results may translate to broader spatial extents and across longer timescales, e.g., years-centuries (Osmond et al. 2004). Recent complementary research across a natural gradient of temperature in whole streams suggests that our results may reflect the trends (if not the magnitude) of warming responses. For example, increasing metabolism and nutrient uptake with warming is

consistent with previous work, though we found a more strongly amplified metabolic response (Demars et al. 2011; Rasmussen et al. 2011; Perkins et al. 2012; Welter et al. *in review*). Additionally, preliminary findings show that biofilm stoichiometry is remarkably consistent across the natural thermal gradient (J. R. Junker and W. F. Cross *unpublished data*), and that N<sub>2</sub>-fixation becomes increasingly important at warmer temperatures (Welter et al. *in review*). These patterns lend credence to the value of our relatively small-scale experiments, while underscoring the need for comparison with long-term warming manipulations and observational studies that utilize existing natural thermal gradients (such as those found in Hengill and other geothermal regions; O’Gorman et al. 2014).

Irrespective of scaling challenges, we clearly demonstrate that warming can strongly influence aquatic ecosystems. Our findings suggest that freshwater processing of C will increase with warming, due in part to enhanced metabolic processes. However, our findings suggest that how this will translate to other elemental cycles (e.g., N and P) will depend upon numerous site-specific characteristics. For example, we showed that the trait characteristics of dominant functional groups can strongly influence how C:nutrient coupling responds to warming. In addition, ambient nutrient availability will also influence how warming shapes C:nutrient coupling. This showcases the need to incorporate species functional traits, community structure, and ambient resource availability with metabolic theory in order to understand how climate change will alter fluxes of energy and elements in aquatic ecosystems.

## REFERENCES CITED

- Adrian, R. et al. (2009). Lakes as sentinels of climate change. *Limnology and Oceanography* 54:6
- American Public Health Association (APHA) (2005). Standard methods for the examination of water and wastewater. 21<sup>st</sup> edition
- Anderson-Teixeira, K.J. et al. (2008). Amplified temperature dependence in ecosystems developing on the lava flows of Mauna Loa, Hawai'i. *Proceedings of the National Academy of Sciences* 105:1
- Árnason, B., P. Theodorsson, S. Björnsson, & K. Saemundsson. (1969). Hengill, a high temperature thermal area in Iceland. *Bulletin of Volcanology* 33:245-259
- Baligar, V.C. et al. (2001). Nutrient use efficiency in plants. *Communications in Soil Science and Plant Analysis* 32:7
- Battin, T.J. et al. (2003). Contributions of microbial biofilms to ecosystem processes in stream mesocosms. *Nature* 426
- Baulch, H.M. *et al.* (2005). Effects of warming on benthic communities in a boreal lake: implication of climate change. *Limnology and Oceanography* 50:5
- Bott, T.L. (2006). Primary production and community respiration. Chapter 28 in: *Methods in Stream Ecology* 2<sup>nd</sup> Ed. Elsevier
- Brown, J.H. *et al.* (2004). Toward a metabolic theory of ecology. *Ecology* 85:7
- Butman, D. and P.A. Raymond (2011). Significant efflux of carbon dioxide from streams and rivers in the United States. *Nature Geoscience*
- Chapin III, F.S. et al. (1995). Response of arctic tundra to experimental and observed changes in climate. *Ecology* 76:3
- Cole, J.J. et al. (2007). Plumbing the global carbon cycle: Integrating inland waters into the terrestrial carbon budget. *Ecosystems* 10
- Cottingham et al. (2005). Knowing when to draw the line: designing more informative ecological experiments. *Frontiers in Ecology and the Environment* 3:3
- Cross, W.F. et al. (in review). Interactions between temperature and nutrients at physiological to ecosystem scales. *Global Change Biology*.
- Demars, B.O.L. and A.C. Edwards (2007). Tissue nutrient concentrations in freshwater

aquatic macrophytes: high inter-taxon differences and low phenotypic response to nutrient supply. *Freshwater Biology* 52

- Demars, B.O.L. et al. (2011). Temperature and the metabolic balance of streams. *Freshwater Biology* 56
- De Senerpont Domis, L.N. et al. (2014). Community stoichiometry in a changing world: combined effects of warming and eutrophication on phytoplankton dynamics. *Ecology* 95:6
- DiNicola, D.M. (1996) Periphyton responses to temperature at different ecological levels. In *Algal Ecology* Ed. R.J. Stenvenson, M.L. Bothwell, R.L. Lowe. Academic Press
- Dossena, M. et al. (2012). Warming alters community size structure and ecosystem functioning. *Proceedings of the Royal Society* 279
- Elser, J.J. et al. (1996). Organism size, life history, and N:P stoichiometry. *BioScience* 46:9
- Elser, J.J. et al. (2000). Biological stoichiometry from genes to ecosystems. *Ecology Letters* 3
- Eppley, R.W. (1972). Temperature and phytoplankton growth in the sea. *Fisheries Bulletin* 70:4
- Filstrup, C.T. et al. (2014). Cyanobacteria dominance influences resource use efficiency and community turnover in phytoplankton and zooplankton communities. *Ecology Letters*
- Finzi, A.C. et al. (2011). Responses and feedbacks of coupled biogeochemical cycles to climate change: examples from terrestrial ecosystems. *Frontiers in Ecology and the Environment* 9:1
- Friberg, N. et al. (2009) Relationships between structure and function in streams contrasting in temperature. *Freshwater Biology* 54
- Frost, P.C. et al. (2005). Are you what you eat? Physiological constraints on organismal stoichiometry in an unbalanced world. *Oikos* 109
- Gilbert, P.M. and D.A. Bronk (1994). Release of dissolved organic nitrogen by marine diazotrophic cyanobacteria, *Trichodesmium spp.* *Applied and Environmental Microbiology*
- Gillooly, J.F. et al. (2001). Effects of size and temperature on metabolic rate. *Science* 293

- Gulati, R.D. and W.R. Demott (1997). The role of food quality for zooplankton: remarks on state-of-the-art, perspectives and priorities. *Freshwater Biology* 38
- Hare, C.E. et al. (2007). Consequences of increased temperature and CO<sub>2</sub> for phytoplankton community structure in the Bering Sea. *Marine Ecology Progress Series* 352
- Hiremath, A.J. and J.J. Ewel (2001). Ecosystem nutrient use efficiency, productivity, and nutrient accrual in model tropical communities. *Ecosystems* 4
- Holmes, R.M, et al. (1999). A simple and precise method for measuring ammonium in marine and freshwater ecosystems. *Canadian Journal of Fisheries and Aquatic Science* 56
- Howarth, R.W. et al. (1998). Nitrogen fixation in freshwater, estuarine, and marine ecosystems. 1. Rates and importance. *Limnology and Oceanography* 33:4
- IPCC (2013). Summary for Policymakers. In: *Climate Change 2013: the Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press
- Lamberti, A., and V.H. Resh (1983). Geothermal effects on stream benthos: separate influences of thermal and chemical components on periphyton and macroinvertebrates. *Canadian Journal of Fisheries and Aquatic Science*, 40:1995-2009.
- Levine, S.N. and D.W. Schindler (1999). Influence of nitrogen to phosphorous supply ratios and physiochemical conditions on cyanobacteria and phytoplankton species composition in the Experimental Lakes Area, Canada. *Canadian Journal of Fisheries and Aquatic Science* 56
- Liebig, J.V. (1855). *Agricultural chemistry with special reference to the late researches made in England*. Walton and Maberly
- Lock, M.A., et al. (1984). River epilithon: toward a structural-functional model. *Oikos* 42
- Lowe, R.L. and G.D. LaLiberte (2006). Benthic stream algae: distribution and structure. Chapter 28 in: *Methods in Stream Ecology* 2<sup>nd</sup> Ed. Elsevier
- Lüring, M. et al. (2013). Comparison of cyanobacterial and green algal growth rates at different temperatures. *Freshwater Biology* 58
- Makino, W. et al. (2011). Stoichiometric effects of warming on herbivore growth: experimental test with plankters. *Ecosphere* 2:7
- Marcarelli, A.M. et al. (2008). Is in-stream N<sub>2</sub> fixation an important N source for benthic

- communities and stream ecosystems. *Journal of the North American Benthological Society* 27:1
- O’Gorman, E.J.O. et al. (2012). Impacts of warming on the structure and functioning of aquatic communities: individual- to ecosystem-level responses. *Advances in Ecological Research*.
- O’Gorman, E.J.O. et al. (2014). Climate change and geothermal ecosystems: natural laboratories, sentinel systems, and future refugia. *Global Change Biology* 20
- Osmond, B. et al. (2004). Changing the way we think about global change research: scaling up in experimental ecosystem science. *Global Change Biology* 10
- Paerl, H.W. and J. Huisman (2008). Blooms like it hot. *Science* 340
- Parmesan, C and G. Yohe (2003). A globally coherent fingerprint of climate change impacts across natural systems. *Nature*. 421
- Pastor, J. and S. D. Bridgham (1999). Nutrient efficiency along nutrient availability gradients. *Oecologia* 118
- Perini, V. and M.E.S. Bracken (2014). Nitrogen availability limits phosphorus uptake in an intertidal macroalga. *Oecologia* 175
- Perkins, D. M. et al. (2012) Consistent temperature dependence of respiration across ecosystems contrasting in thermal history. *Global Change Biology*. 1365-2486
- Pilgrim, J.M. (1998). Stream temperature correlations with air temperatures in Minnesota: Implications for climate warming. *Journal of the American Water Resources Association* 34:5
- Rasmussen, J.J. et al. (2011). Stream ecosystem properties and processes along a temperature gradient. *Aquatic Ecology* 45
- Raven, J.A and R.J. Geider (1988). Temperature and algal growth. *New Phytology* 110
- R Core Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <http://www.R-project.org/>
- Reay, D.S. et al. (1999). Temperature dependence of inorganic nitrogen uptake: reduced affinity for nitrate at suboptimal temperatures in both algae and bacteria. *Applied and Environmental Microbiology* 65:6
- Reddy, K.R. (1983). Fate of nitrogen and phosphorus in a waste-water retention reservoir contacting aquatic macrophytes. *Journal of Environmental Quality* 12:1

- Rhee, G. and I.J. Gotham (1981). The effect of environmental factors on phytoplankton growth: Temperature and the interactions of temperature with nutrient limitation. *Limnology and Oceanography* 26:4
- Sardans, J. et al. (2012). The C:N:P stoichiometry of organisms and ecosystems in a changing world: a review and perspectives. *Perspective in Plant Ecology, Evolution, and Systematics* 14
- Shurin, J.B. et al. (2012). Warming shifts top-down and bottom-up control of pond foodweb structure and function. *Philosophical Transactions of the Royal Society* 367
- Sievers, A. et al. (2004). The ribosome as an entropy trap. *Proceedings of the National Academy of Sciences* 101:21
- Sterner, R.W. (1995). Elemental stoichiometry of species in ecosystems. In: *Linking Species and Ecosystems*. Chapman and Hall
- Sterner, R.W. and J.J Elser (2002) *Ecological stoichiometry: the biology of elements from the molecules to the biosphere*. Princeton University Press
- Stewart, R.I.A et al. (2013). Mesocosm experiments as a tool for ecological climate change research. *Advances in Ecological Research* 48
- Sun, J. and D. Liu (2003). Geometric models for calculating cell biovolume and surface area for phytoplankton. *Journal of Plankton Research* 25:11
- Taylor, B.W. et al (2007). Improving the flourometric ammonium method: matrix effects, background florescence, and standard additions. *Journal of the North American Benthological Society* 26:2
- van de Waal, D.B. et al (2010). Climate-driven changes in the ecological stoichiometry of aquatic ecosystems. *Frontiers in Ecology and the Environment* 8:3
- Vitousek, P.M. et al. (2002). Towards an ecological understanding of biological nitrogen fixation. *Biogeochemistry* 57:58
- Webb, C.T. et al. (2010). A structured and dynamic framework to advance traits-based theory and prediction in ecology. *Ecology Letters* 13
- Welter, J.R. et al. (in review). Does N<sub>2</sub>-fixation amplify the temperature dependence of biofilm metabolism? *Ecology Letters*
- Woods, H.A. et al. (2003). Temperature and the chemical composition of poikilothermic organisms. *Functional Ecology* 17



Woodward, G., D. M. Perkins, L. E. Brown (2010). Climate change and freshwater ecosystems: impacts across multiple levels of organization. *Philosophical Transactions of the Royal Society* 365

Yvon-Durocher, G. *et al.* (2010a). Warming alters the metabolic balance of ecosystems. *Philosophical Transactions of the Royal Society* 365

Yvon-Durocher, G. *et al.* (2010b). Warming alters the size spectrum and shifts the distribution of biomass in aquatic ecosystems. *Global Change Biology* 17:4

APPENDIX A

ADDITIONAL FIGURES

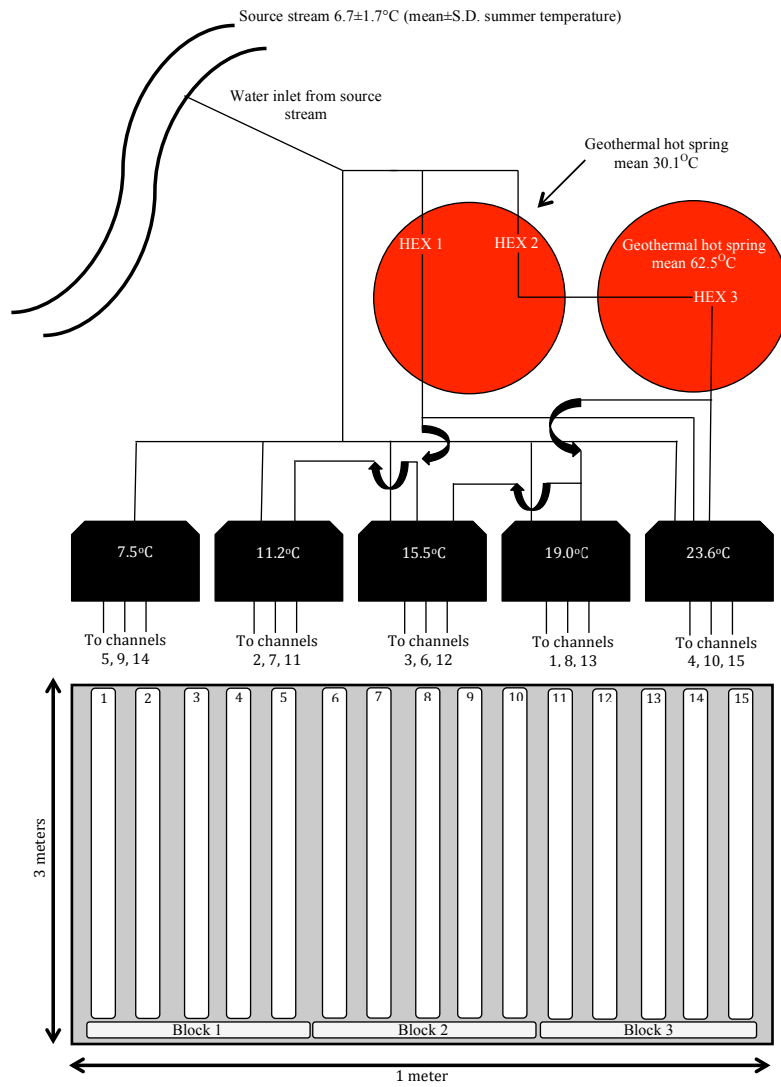


Figure A1: Experimental design schematic

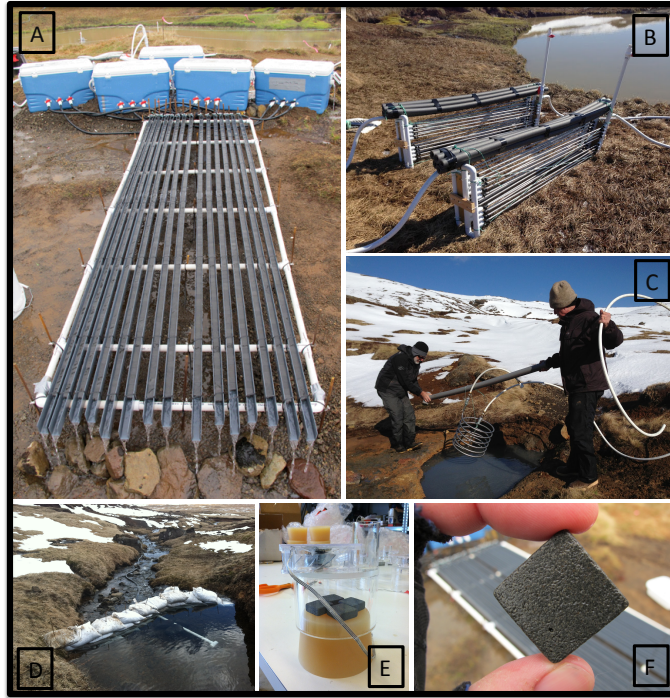


Figure A2: Experimental streamside channel array with constant head mix tanks in the background (modified coolers) (A); HEX 1 and HEX 2 prior to deployment in the geothermal hot spring (B); A.D. Huryn and P.W. Johnson deploying HEX 3 in geothermal hot spring (C); Small impoundment serving as inlet supply to the system (D); 0.27 L chamber used for nutrient uptake and metabolism measurements (E); 25 x 25 mm basalt tiles used to culture biofilms (D). Photo credits: (A, D, E) T. J. Williamson, (B, C, F) J. P. Benstead

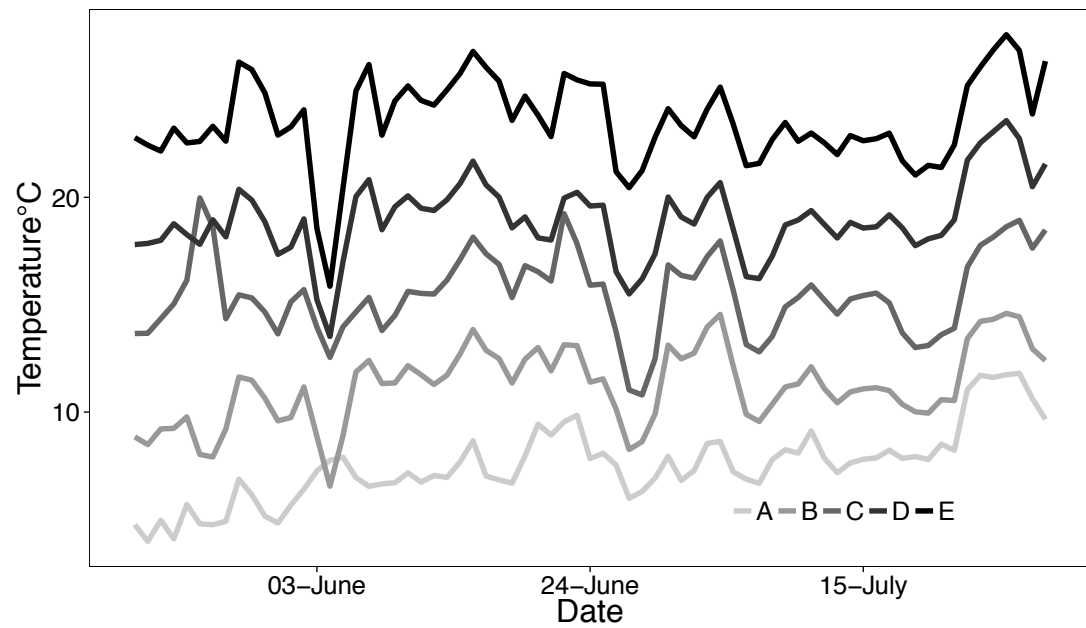


Figure A3: Daily mean time series of the 5 temperature treatments.