Factors influencing benthic primary production and consumer resource use in the context of Lake Mývatn's ecosystem variability

By

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ABSTRACT

Determining the mechanisms influencing ecosystem variability can be challenging, especially when complex interactions are involved. Ecosystems with high temporal variability facilitate understanding these mechanisms because they provide repeated opportunities to observe different ecological conditions. Lake Mývatn is a relatively simple system that shows substantial year-to-year variation in many ecological properties; thus, it is well-suited for examining how ecological components interact as a cause and consequence of this variation. Two examples of Mývatn's variability include population fluctuations of its dominant consumer, *Tanytarsus gracilentus*, and shifts in the allocation of primary production between benthic and pelagic habitats. These forms of variability are potentially connected, as benthic algae are *T. gracilentus*' main food source. I explore factors affecting benthic primary production and resource use of benthic consumers in the context of Mývatn's ecological variability.

Resource limitation for *T. gracilentus* poses the question of whether abiotic factors limit their food availability. Thus, in Chapter 1, I investigate benthic algal responses to nutrient enrichment. Benthic algae were not nutrient limited; rather, nitrogen transiently inhibited primary production and caused community composition shifts. Productivity did not differ among nutrient treatments at the end of the experiment, suggesting that compensatory compositional shifts achieved similar ecological function. In Chapter 2, I analyzed the partitioning of primary production between benthic and pelagic habitats in Mývatn. This study highlights the intra- and interannual variation in Mývatn's autotrophic structure, the competitive effects of cyanobacteria on in situ benthic production, and the value of environmental monitoring for understanding the balance between benthic and pelagic production. In Chapter 3, I examined interactions between

T. gracilentus and benthic productivity using carbon stable isotope analysis (a proxy for primary production) of archived specimens. Results from this study provide new, supportive evidence that consumer-resource interactions underly *T. gracilentus* population fluctuations in Mývatn. Chapter 4 explores the patterns in abundance and resource use between *T. gracilentus* and Mývatn's second most abundant consumer, *Chironomus islandicus*. These ecologically similar taxa show synchronous population fluctuations and coexist in sediment habitats. Results from this study demonstrate their differences in resource use, suggesting that resource partitioning may facilitate their co-occurrence at high abundances.

INTRODUCTION

Ecosystem variability is influenced by external drivers and internal dynamics (Weathers et al. 2013). In many systems, natural or anthropogenically induced environmental variation affects ecosystem processes and structure (Vitousek 1994, Beard et al. 2005), such that external drivers can impact temporal ecosystem variability. For example, Finger et al. (2013) demonstrate that changes to external nutrient loading and meteorological variability can control lake primary productivity at decadal and interannual time scales. However, in other cases, internal dynamics may more strongly affect ecosystem variability than environmental factors (Krebs et al. 1995, Vahtera et al. 2007, Yoshida et al. 2008, Søndergaard et al. 2016). For instance, internal processes related to nutrient flux and transformations exert more control on interannual variation in nutrient availability than external loading in the Baltic Sea (Vahtera et al. 2007).

Detecting mechanisms that contribute to ecosystem variability can be challenging, especially when they involve complex interactions among multiple ecological components (Stow et al. 1998). One method for understanding these mechanisms includes whole-ecosystem experiments, in which researchers perturb the system to examine how different components respond and interact with one another (Carpenter et al. 1995). This manipulative approach has been particularly useful for understanding how ecosystems respond to factors associated with environmental change (e.g., eutrophication, land-use change, chemical pollutants) and/or management strategies to mitigate these effects (e.g., re-oligotrophication, biomanipulation) (Likens et al. 1970, Schindler et al. 1973, Hall et al. 1980, Carpenter et al. 2001, Jeppesen et al. 2002, Lathrop et al. 2002). Another method involves observing temporal trends in a particular ecosystem (Rodriguez et al. 2003) or comparing multiple ecosystems amongst each another

(Kratz et al. 1997, Rosenthal et al. 2006), which can distinguish factors that alter ecosystem function and structure. In particular, natural ecosystem variability provides repeated opportunities to observe a system in different ecological conditions and examine what components may interact as a cause and consequence of this variation. Thus, ecosystems with high temporal variability are particularly valuable for untangling how internal dynamics impact ecosystem function.

Lake Mývatn is well-suited for examining how internal dynamics control ecosystem function for two primary reasons. First, several aspects of Mývatn's ecology show substantial year-to-year variation, which provides the opportunity to examine how interactions among multiple components contribute to and respond to these ecosystem changes. In particular, this dissertation focuses on the temporal variability in the abundance of Mývatn's dominant consumer and in the partitioning of primary production between benthic and pelagic habitats. Second, Mývatn has a relatively simple food web structure; thus, detecting how one ecological component affects other components should be easier than in a more complex ecosystem.

Lake Mývatn is located in northeastern Iceland (65°40'N 17°00'W), in an area characterized by a subarctic climate. The lake is located in a rain shadow of a large glacier, Vatnajökull, which contributes to relatively low rainfall and high solar radiation (Jónasson 1979). The Mývatn area is relatively sparsely populated, although the land surrounding the lake is agriculturally developed for growing hay and grazing sheep (Einarsson et al. 2004). The lake's watershed consists of permeable, basaltic soil and heathland vegetation. Mývatn is relatively large and is split between a main south basin (28.2 km^2) and a smaller north basin (8.5 km^2), which are connected by a narrow channel. The main basin has average depth of 2.3 m and a maximum depth of 4.2 m (Jónasson 1979). In the north basin, the average natural depth is 1 m,

but diatomite dredging from the 1967 to 2004 created depressions > 5 m deep (Einarsson et al. 2004). With the exception of these deep depressions, the lake remains mixed during the ice-free period, and water temperatures largely fluctuate with air temperatures. Mývatn is naturally eutrophic due to nutrient-rich groundwater springs that enter the lake along its eastern shore (Ólafsson 1979a). External phosphorus inputs are high, and nutrient inputs to the lake have relatively low N:P ratios (6:1 and 1:1 in the north and south basin, respectively) (Ólafsson 1979a). Internal loading of nutrients from the sediment to the water column is potentially high due to large nutrient pools in the interstitial water (Gíslason et al. 2004). Together, high nutrient inputs and long daylength during the summer growing season contribute to Mývatn's high productivity.

The most notable example of Mývatn's variability is the dramatically fluctuating abundance of its main consumer. The English translation of Mývatn is "midge lake," and chironomid (Chironomidae: Diptera) larvae dominate secondary production. The most prevalent species, *Tanytarsus gracilentus*, undergoes large fluctuations in abundance that span 4-5 orders of magnitude, with irregular population crashes occurring every 4.5-9.5 years. *T. gracilentus* population fluctuations have substantial effects on other aspects of the ecosystem. For example, silken tubes constructed by *T. gracilentus* larvae stabilize the sediment and provide physical structure to benthic habitats (Ólafsson and Paterson 2004). This ecosystem engineering affects community structure of benthic Cladocera, such that some species are positively associated with high *T. gracilentus* abundance and others become abundant when midge abundance is low (Einarsson and Örnólfsdóttir 2004, Webert et al. 2017). Additionally, most of the roughly 35 other species of chironomids fluctuate in synchrony with *T. gracilentus* (Gardarsson et al. 2004). Emergent adult midges provide an allochthonous resource pulse to the adjacent landscape, such

that the population abundance of *T. gracilentus* can also influence community structure and ecosystem processes in terrestrial food webs (Hoekman et al. 2011, 2019, Gratton et al. 2017).

The most likely cause underlying *T. gracilentus* population fluctuations is the consumer-resource interaction between larvae and their dominant food source, benthic diatoms. *T. gracilentus* wing lengths (i.e., a proxy for resource availability) decline in the generations leading to a population crash, but there is no relationship between the abundances of *T. gracilentus* and potential predators (Einarsson et al. 2002). Additionally, a strong relationship between midge abundance (fossilized head capsules) and diatoxanthin (a pigment produced by diatoms) was detected in a paleoecology study (Einarsson et al. 2016). Moreover, a consumer-resource model that predicts alternative dynamical states between a stable high point and a high-amplitude cycle closely matches the complex patterns in *T. gracilentus* abundance observed over multiple decades (Ives et al. 2008). Conversely, *T. gracilentus* fluctuations are asynchronous with those in a nearby lake, suggesting that population dynamics at Mývatn are not driven by external climatic or environmental factors (Einarsson et al. 2004; Ives et al. 2008). In short, resource availability strongly affects *T. gracilentus* population fluctuations, and *T. gracilentus* is a keystone species in the Mývatn ecosystem. Therefore, understanding the dynamics of the whole system requires characterizing the controls on benthic primary production.

Another example of Mývatn's ecosystem variability is the partitioning of primary production between benthic and pelagic habitats. Mývatn's shallow depth promotes high benthic primary production, which can contribute a majority of total lake productivity (Einarsson et al. 2004). However, intermittent phytoplankton blooms, predominantly *Dolichospermum* spp. cyanobacteria, can also substantially contribute to total production (Jónasson and Adalsteinsson 1979, Phillips 2020). While cyanobacteria blooms are a natural part of Mývatn's ecology, their

timing, severity, and spatial extent are highly variable. In some years, a dense bloom spreads throughout much of the lake, but in other years, the lake largely remains relatively clear, such that benthic algae likely dominate total production (Einarsson et al. 2004). This variation in the relative contributions by benthic and pelagic primary producers likely has substantial effects on other ecosystem processes. For instance, while benthic pathways dominate energy flow in Mývatn (Jónasson 1979), shading by cyanobacteria could reduce benthic productivity and, consequently, affect energy availability in benthic habitats. Moreover, like other systems (Jansson 1980, Hansson 1988), benthic algae appear to control nutrient flux from Mývatn's sediment to the water column (Thorbergsdóttir and Gíslason 2004), such that reductions in their photosynthetic activity could impact nutrient transport and cycling.

Compared to *T. gracilentus* population fluctuations, the causal factors contributing to the variation in cyanobacteria bloom intensity at Mývatn are less clear and may include external and internal drivers. Previous discussions have linked early ice-off with earlier cyanobacteria bloom formation and proliferation throughout the lake (Jónasson and Adalsteinsson 1979). Due to its shallow depth, Mývatn is susceptible to wind-driven mixing and sediment resuspension (Ólafsson 1979b), which is associated with internal phosphorus loading in other lakes (Søndergaard et al. 1992). In particular, early summer wind events contribute to high phosphate release in Mývatn (Einarsson et al. 2004). While meteorological drivers associated with ice off and wind events may influence cyanobacteria bloom dynamics at Mývatn, neither explanation is likely the single causal factor. For example, 2014 had an unusually early ice-off date (March 26; Árni Einarsson, unpublished data) compared to 2015 (May 17), but the cyanobacteria bloom in 2015 appeared to spread throughout Mývatn earlier than in 2014 (Chapter 2, Figure 3). Additionally, in two consecutive years that experienced early summer sediment resuspension and

associated phosphate release, a cyanobacteria bloom only developed in one of the years (Einarsson et al. 2004). Lastly, high *T. gracilentus* densities should theoretically decrease cyanobacteria bloom occurrence because larval tube-building and bioturbation respectively stabilize and oxygenate the sediment (Ólafsson and Paterson 2004, Holker et al. 2015), thereby mitigating nutrient release (especially phosphate). However, in some years, severe cyanobacteria blooms have failed to develop when midge abundance is relatively low (e.g., 2019), while extensive cyanobacteria blooms have occurred in years with high midge abundance (e.g., 2014). Thus, internal dynamics potentially interact with external drivers to influence variation in the partitioning of primary production in Mývatn.

Much of the work presented in this dissertation explores factors affecting benthic primary productivity and resource use of benthic consumers in the context of Mývatn's high natural ecosystem variability. Resource limitation as a contributing factor for *T. gracilentus* population fluctuations poses the question of whether abiotic factors (e.g., nutrients) control benthic primary production. In Chapter 1, I examine whether nutrients (nitrogen and phosphorus) limit benthic primary producers. In particular, I hypothesized that nitrogen might be limiting because of the low N:P ratios in Mývatn and previous measurements suggesting high nitrogen assimilation by benthic algae (Thorbergsdóttir and Gíslason 2004). Measuring benthic algal biomass, primary production, and composition allowed me to examine relationships among these components in response to nutrient enrichment. Benthic primary production was not nutrient limited; rather, nitrogen resulted in a transient inhibition of benthic primary production and also a shift in community composition. At the end of the experiment, primary production did not differ among nutrient treatments. Results from this experiment suggest that nutrient-driven taxonomic shifts can result in community compositions that achieve similar ecological function. While this

experiment did not detect nutrient limitation of benthic primary producers, it represents a snapshot response to nutrient enrichment under admittedly unnatural conditions. Thus, it does not necessarily rule out the potential importance of nutrient-algae interactions in influencing temporal variability of benthic primary production.

Chapter 2 also examines factors influencing benthic primary production in Mývatn. In an observational study based on routine monitoring data, I estimate the relative contribution of benthic and pelagic primary production across seven years. In three years of the study, cyanobacteria blooms spread throughout much of the lake and reduced the benthic production by limiting benthic light availability. Measurements of the maximum potential productivity rates by benthic algae showed variation across and within years. Using this observed natural variation, I explored how maximum potential benthic productivity rates can influence the response of total production during a shift from benthic-dominated production toward pelagic-dominated production. Results from this study illustrate the substantial variation in how primary production is partitioned between habitats in Mývatn and highlights the value of ecological monitoring for understanding temporal dynamics in the balance between benthic and pelagic production.

In Chapter 3, I examine consumer-resource interactions between *T. gracilentus* and benthic productivity using carbon isotope analysis of archived specimens collected from 1977 to 2015. Because *T. gracilentus* is an herbivore with reliance on benthic resources, their $\delta^{13}C$ values are interpreted as a proxy for benthic primary production. A state-space model that estimated interactions within and between *T. gracilentus* abundance and $\delta^{13}C$ values produced results that were consistent with a consumer-resource interaction. ^{13}C-enrichment, indicating high productivity, was associated with increased *T. gracilentus* abundance; and high *T. gracilentus* abundance was associated with ^{13}C-depletion, consistent with benthic productivity declines.

These results provide new, supportive evidence that consumer-resource interactions drive *T. gracilentus* population fluctuations and demonstrates potential applications of stable isotope analyses for reconstructing consumers-resource dynamics.

In Chapter 4, I examine the patterns in abundance and resource use between coexisting benthic consumers. *Chironomus islandicus* is the second-most abundant benthic consumer in Mývatn, and its population undergoes fluctuations that are synchronous with those of *T. gracilentus* (Gardarsson et al. 2004). *T. gracilentus* and *C. islandicus* are ecologically similar taxa and co-occur in the benthos; this poses the question of whether resource use overlap and potential interspecific competition influence both species' population fluctuations. Larval abundances of the two taxa were positively correlated at small spatial scales, such that high *T. gracilentus* larval abundances were also associated with high *C. islandicus* larval abundances. Stable carbon isotope analysis revealed interspecific differences in resource use. These results suggest that *T. gracilentus* and *C. islandicus* partition resources at the spatial scale of individual midge larvae, which may in part explain their coexistence at high densities. Overall, these projects illustrate some of the interactions that are linked to Mývatn's ecosystem variability.

References

Beard, K. H., K. A. Vogt, D. J. Vogt, F. N. Scatena, A. P. Covich, R. Sigurdardottir, T. G. Siccama, and T. A. Crowl. 2005. Structural and functional responses of a subtropical forest to 10 years of hurricanes and droughts. Ecological Monographs 75:345–361.

Carpenter, S. R., S. W. Chisholm, C. J. Krebs, D. W. Schindler, and R. F. Wright. 1995. Ecosystem experiments. Science 269:324–327.

Carpenter, S. R., J. J. Cole, J. R. Hodgson, J. F. Kitchell, M. L. Pace, D. Bade, K. L. Cottingham, T. E. Essington, J. N. Houser, and D. E. Schindler. 2001. Trophic cascades, nutrients, and lake productivity: whole-lake experiments. Ecological Monographs 71:163–186.

Einarsson, Á., A. Gardarsson, G. M. Gíslason, and A. R. Ives. 2002. Consumer – resource interactions and cyclic population dynamics of Tanytarsus gracilentus (Diptera: Chironomidae). Journal of Animal Ecology 71:832–845.

Einarsson, Á., U. Hauptfleisch, P. R. Leavitt, and A. R. Ives. 2016. Identifying consumer-resource population dynamics using paleoecological data. Ecology 97:361–371.

Einarsson, Á., and B. E. Örnólfsdóttir. 2004. Long-term changes in benthic Cladocera populations in Lake Myvatn, Iceland. Aquatic Ecology 38:253–262.

Einarsson, Á., G. Stefánsdóttir, H. Jóhannesson, J. S. Ólafsson, G. M. Gíslason, I. Wakana, G. Gudbergsson, and A. Gardarsson. 2004. The ecology of Lake Myvatn and the River Laxa: Variation in space and time. Aquatic Ecology 38:317–348.

Finger, D., W. Alfred, and P. Bossard. 2013. Effects of oligotrophication on primary production in peri-alpine lakes. Water Resources Research 49:4700–4710.

Gardarsson, A., Á. Einarsson, G. M. Gíslason, T. Hrafnsdóttir, H. R. Ingvason, E. Jónsson, and J. S. Ólafsson. 2004. Population fluctuations of chironomid and simuliid Diptera at Myvatn in

1977-1996. Aquatic Ecology 38:209–217.

Gíslason, S. R., E. S. Eiríksdóttir, and J. S. Ólafsson. 2004. Chemical composition of interstitial water and diffusive fluxes within the diatomaceous sediment in Lake Myvatn, Iceland. Aquatic Ecology 38:163–175.

Gratton, C., D. Hoekman, J. Dreyer, and R. D. Jackson. 2017. Increased duration of aquatic resource pulse alters community and ecosystem responses in a subarctic plant community. Ecology 98:2860–2872.

Hall, R. J., G. E. Likens, S. B. Fiance, and G. R. Hendrey. 1980. Experimental acidification of a stream in the Hubbard Brook Experimental Forest , New Hampshire. Ecology 61:976–989.

Hansson, L.-A. 1988. Effects of competitive interactions on the biomass development of planktonic and periphytic algae in lakes. Limnology and Oceanography 33:121–128.

Hoekman, D., J. Dreyer, R. D. Jackson, P. A. Townsend, and C. Gratton. 2011. Lake to land subsidies : Experimental addition of aquatic insects increases terrestrial arthropod densities. Ecology 92:2063–2072.

Hoekman, D., M. A. McCary, J. Dreyer, and C. Gratton. 2019. Reducing allochthonous resources in a subarctic grassland alters arthropod food webs via predator diet and density. Ecosphere 10:e02593.

Holker, F., M. J. Vanni, J. J. Kuiper, C. Meile, H.-P. Grossart, P. Stief, R. Adrian, A. Lorke, O. Delwig, A. Brand, M. Hupfer, W. M. Mooij, G. Nutzmann, and J. Lewandowski. 2015. Tube-dwelling invertebrates: tiny ecosystem engineers have large effects in lake ecosystems. Ecological Monographs 85:333–351.

Ives, A. R., A. Einarsson, V. A. A. Jansen, and A. Gardarsson. 2008. High-amplitude fluctuations and alternative dynamical states of midges in Lake Myvatn. Nature 452:84–87.

Jansson, M. 1980. Role of benthic algae in transport of nitrogen from sediment to lake water in a shallow clearwater lake. Archiv für Hydrobiologie 89:101–109.

Jeppesen, E., J. P. Jensen, M. Søndergaard, E. Jeppesen, J. P. Jensen, and M. Søndergaard. 2002. Response of phytoplankton , zooplankton, and fish to re-oligotrophication: An 11 year study of 23 Danish lakes. Aquatic Ecosystem Health & Management 5:31–43.

Jónasson, P. M. 1979. The Lake Mývatn ecosystem, Iceland. Oikos 32:289–305.

Jónasson, P. M., and H. Adalsteinsson. 1979. Phytoplankton Production in Shallow Eutrophic Lake Mývatn, Iceland. Oikos 32:113–138.

Kratz, T. K., K. E. Webster, C. J. Bowser, J. J. Magnuson, and B. J. Benson. 1997. The influence of landscape position on lakes in northern Wisconsin. Freshwater Biology 37:209–217.

Krebs, C. J., S. Boutin, R. Boonstra, A. R. E. Sinclair, J. N. M. Smith, M. R. T. Dale, K. Martin, and R. Turkington. 1995. Impact of Food and Predation on the Snowshoe Hare Cycle. Science 269:1112–1115.

Lathrop, R. C., S. R. Carpenter, and F. Collins. 2002. Stocking piscivores to improve fishing and water clarity : a synthesis of the Lake Mendota biomanipulation project:2410–2424.

Likens, G. E., F. H. Bormann, N. M. Johnson, D. W. Fisher, and R. S. Pierce. 1970. Effects of forest cutting and herbicide treatment on nutrient budgets in the Hubbard Brook watershed-ecosystem. Ecological Monographs 40:23–47.

Ólafsson, J. 1979a. The chemistry of Lake Mývatn and River Laxa. Oikos 32:82–112.

Ólafsson, J. 1979b. Physical Characteristics of Lake Mývatn and River Laxá. Oikos 32:38–66.

Ólafsson, J. S., and D. M. Paterson. 2004. Alteration of biogenic structure and physical properties by tube-building chironomid larvae in cohesive sediments. Aquatic Ecology 38:219–229.

Phillips, J. S. 2020. Time-varying responses of lake metabolism to light and temperature. Limnology and Oceanography 65:652–666.

Rodriguez, C. F., E. Becares, and M. Fernandez-Alaez. 2003. Shift from clear to turbid phase in Lake Chozas (NW Spain) due to the introduction of American red swamp crayfish (Procambarus clarkii). Hydrobiologia 506:421–426.

Rosenthal, S. K., S. S. Stevens, and D. M. Lodge. 2006. Whole-lake effects of invasive crayfish (Orconectes spp.) and the potential for restoration. Canadian Journal of Fisheries and Aquatic Sciences 63:1276–1285.

Schindler, D. W., H. Kling, R. V. Schmidt, J. Prokopowich, V. E. Frost, R. A. Reid, and M. Capel. 1973. Eutrophication of Lake 227 by addition of phosphate and nitrate: the second, third, and fourth years of enrichment, 1970, 1971, and 1972. J. Fish. Res. Board Canada 30:1415–1440.

Søndergaard, M., P. Kristensen, and E. Jeppesen. 1992. Phosphorus release from resuspended sediment in the shallow and wind-exposed Lake Arreso, Denmark. Hydrobiologia 228:91–99.

Søndergaard, M., S. E. Larsen, L. S. Johansson, T. L. Lauridsen, and E. Jeppesen. 2016. Ecological classification of lakes: Uncertainty and the influence of year-to-year variability. Ecological Indicators 61:248–257.

Stow, C. A., S. R. Carpenter, K. E. Webster, and T. M. Frost. 1998. Long-term environmental monitoring: some perspectives from lakes. Ecological Applications 8:269–276.

Thorbergsdóttir, I. M., and S. R. Gíslason. 2004. Internal loading of nutrients and certain metals in the shallow eutrophic Lake Myvatn, Iceland. Aquatic Ecology 38:191–207.

Vahtera, E., D. J. Conley, B. G. Gustafsson, H. Kuosa, H. Pitkänen, O. P. Savchuk, T.

Tamminen, M. Viitasalo, M. Voss, N. Wasmund, and F. Wulff. 2007. Internal ecosystem feedbacks enhance nitrogen-fixing cyanobacteria blooms and complicate management in the Baltic Sea. AMBIO: A Journal of the Human Environment 36:186–194.

Vitousek, P. M. 1994. Beyond global warming: ecology and global change. Ecology 75:1861–1876.

Weathers, K. C., D. L. Strayer, G. E. Likens. 2012. Fundamentals of Ecosystem Science. Academic Press.

Webert, K. C., C. M. Herren, Á. Einarsson, M. Bartrons, U. Hauptfleisch, and A. R. Ives. 2017. Midge-stabilized sediment drives the composition of benthic cladoceran communities in Lake Mývatn, Iceland. Ecosphere 8:e01659.

Yoshida, M., T. Yoshida, A. Kashima, Y. Takashima, and N. Hosoda. 2008. Ecological dynamics of the toxic bloom-forming cyanobacterium Microcystis aeruginosa and its cyanophages in freshwater. Applied and Environmental Microbiology 74:3269–3273.

CHAPTER 1

Responses of benthic algae to nutrient enrichment in a shallow lake: linking community production, biomass, and composition

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Running title: Benthic algal response to nutrients

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SUMMARY

1. Understanding how nutrient limitation affects algal biomass and production is a long-standing interest in aquatic ecology. Nutrients can influence these whole-community characteristics through several mechanisms, including shifting community composition. Therefore, incorporating the joint responses of biomass, taxonomic composition, and production of algal communities, and relationships among them, is important for understanding effects of nutrient enrichment.

2. In shallow subarctic Lake Mývatn, benthic algae compose a majority of whole-lake production, support high secondary production, and influence nutrient cycling. Given the importance of these ecosystem processes, the factors that limit benthic algae have a large effect on the function and dynamics of the Mývatn system.

3. In a 33-day nutrient enrichment experiment conducted in Lake Mývatn, we measured the joint responses of benthic algal biomass, primary production, and composition to nitrogen (N) and phosphorus (P) supplementation. We enriched N and P using nutrient-diffusing agar overlain by sediment, with three levels of N and P that were crossed in a factorial design.

4. We found little evidence of community-wide nutrient limitation, as chlorophyll-*a* concentrations showed a negligible response to nutrients. Gross primary production (GPP) was unaffected by P and inhibited by N enrichment after 10 days, although the inhibitory effect of N diminished by day 33.

5. In contrast to biomass and primary production, community composition was strongly affected by N and marginally affected by P, with some algal groups increasing and others decreasing with enrichment. The taxa with the most negative and positive responses to N enrichment were Fragilariaceae and *Scenedesmus*, respectively.

6. The abundance of particular algal groups, based on standardized cell counts, was related to GPP measured at the end of the experiment. *Oocystis* was negatively associated with GPP but was unaffected by N or P, while Fragilariaceae and *Scenedesmus* were positively associated with GPP but had opposite responses to N. As a result, nutrient-induced compositional shifts did not alter GPP.

7. Overall, our results show that nutrient enrichment can have large effects on algal community composition while having little effect on total biomass and primary production. Our study suggests that nutrient-driven compositional shifts may not alter the overall ecological function of algal communities if (i) taxa have contrasting responses to nutrient enrichment but have similar effects on ecological processes, and/or (ii) taxa that have strong influences on ecological function are not strongly affected by nutrients.

INTRODUCTION

Nutrients, especially nitrogen (N) and phosphorus (P), commonly limit aquatic primary producers (Elser et al., 2007; Francoeur, 2001). While nutrient limitation is often described as a community-level characteristic that influences total biomass and production, algal taxa within a community may display contrasting responses to enrichment due to differences in nutrient uptake rates, utilization, and requirements for maximum growth rates (Bothwell, 1989; Litchman, Klausmeier, Schofield, & Falkowski, 2007; Tilman, 1977). Consequentially, compositional shifts often accompany changes in nutrient conditions, with potential impacts on community biomass and production. Since nutrient-driven responses of algal biomass and production may be mediated by nutrient-induced compositional shifts, understanding these three components of the

community (biomass, production, and composition) is important to clarify mechanisms behind whole-community responses to nutrients.

Whole-community biomass, often using chlorophyll or biovolume as a proxy, is a commonly measured response in nutrient enrichment studies (Borchardt, 1996; Francoeur, 2001). However, compositional shifts can drive changes in community biomass. For instance, increased benthic algal biomass under nutrient enrichment may be due almost exclusively to the increase of one or two algal taxa (Carrick & Lowe, 1988; Fairchild, Lowe, & Richardson, 1985). Alternatively, even if a taxon's abundance increases due to nutrient enrichment, it may not have a large effect on whole-community biomass if its relative abundance, in terms of proportion of total biovolume, is low (Fairchild et al., 1985). Compensatory biomass changes may also occur, such that nutrient-induced compositional shifts may support alternate phytoplankton communities of similar biomass, resulting in no significant nutrient effects on overall biomass (Vrede et al., 2009). Consequentially, whole-community biomass patterns may not be fully understood without assessing the responses of individual taxa.

In addition to community biomass, nutrient enrichment can also affect rates of primary production; these effects can be indirect, acting through the effects of nutrient addition on biomass, biomass-specific production rates, and community composition. Observational studies demonstrate a positive relationship between biomass and production (Cole, Cloern, & Alpine, 1986; Loeb & Reuter, 1981), and concurrent increases in area-specific production rates and algal biomass can follow experimental nutrient enrichment (Rosemond, 1993). Furthermore, enhanced production through increased biomass can occur without changing per capita metabolic rates (Marcarelli, Bechtold, Rugenski, & Inouye, 2009), possibly because biomass can continue to accrue beyond nutrient concentrations that saturate cellular growth rates (Bothwell, 1989). As

nutrient limitation can reduce photosynthetic capacity, impair protein synthesis, and decrease chlorophyll pigments (Young & Beardall, 2003), increased nutrient availability can alleviate this physiological stress and enhance photosynthetic and growth rates of taxa in the existing community. Thus, nutrient enrichment can also influence production by altering biomass-specific production rates, even when the total biomass of the community does not change. For example, Greenwood & Rosemond (2005) observed increased algal productivity without a corresponding change in biomass or composition in a two-year enrichment experiment. Finally, compositional shifts may mediate nutrient-driven effects on production (Guasch et al., 1995; Marcarelli & Wurtsbaugh, 2006), since different algal taxa have different photosynthetic rates (Edwards, Thomas, Klausmeier, & Litchman, 2015; Underwood et al., 2005). For example, in several studies, *Stigeoclonium tenue* increased in abundance in terms of cell counts and/or biovolume under N and P enrichment (Fairchild et al., 1985; Marks & Lowe, 1989; Ozersky et al., 2018), but because this species has relatively low biomass-specific productivity compared to other algae (e.g., diatoms) (Rosemond & Brawley, 1996), this compositional shift could potentially reduce production compared to a community with similar overall biomass but an alternate assemblage. Similarly, increased density of small taxa with high growth rates may not manifest in observable changes in whole-community biovolume (Marks & Lowe, 1993), but if nutrient enrichment favors these taxa, this could increase productivity. Finally, multiple mechanisms may simultaneously influence production under nutrient enrichment. For example, increased benthic production may be affected by both a compositional shift toward taxa with higher photosynthetic capacities and an increase in overall community biomass (Guasch et al., 1995), which highlights the interrelatedness of biomass, primary production, and composition.

Our objective was to determine the effect of sediment nutrient enrichment on benthic algal biomass, primary production, and composition in shallow Lake Mývatn located in northeastern Iceland. Many lakes worldwide are shallow (Cael, Heathcote, & Seekell, 2017; Karlsson et al., 2009; Wetzel, 2001), and benthic algae play important ecological roles in these systems (Vadeboncoeur, Peterson, Vander Zanden, & Kalff, 2008; Vadeboncoeur, Vander Zanden, & Lodge, 2002). Thus, understanding the factors controlling benthic algal growth, production, and community composition is important for understanding ecosystem processes across a global scale. While Mývatn's benthic production can be episodically limited by light (Phillips et al., 2019), it is unknown whether benthic algae are nutrient limited. There have been no direct tests of benthic nutrient limitation in Mývatn, but Thorbergsdóttir & Gíslason (2004) found that NH_4^+ fluxes were consistently from the water column toward the sediment, suggesting high assimilation by benthic algae. Furthermore, while the lake receives high nutrient inputs from springs, the low N:P molar ratio of these inputs (Ólafsson, 1979) suggests the possibility of benthic N-limitation (Thorbergsdóttir & Gíslason, 2004). Because the response to nutrients may differ across enrichment levels and N:P concentrations, we used a gradient of no, low, and high supplementation of N and P. We predicted that community-wide biomass and production would respond to N supplementation. We investigated community structure because we were interested in whether nutrient-driven community-level responses (i.e., production and biomass) were associated with compositional shifts of different taxa.

METHODS

Study System

Lake Mývatn is a shallow, naturally eutrophic lake located in northeastern Iceland (65°40'N 17°00'W) with a surface area of 37 km^2 and a tundra-subarctic climate (average water temperature from June-August is approximately 12°C). The lake's main south basin has an average depth of 2.3 m and a maximum depth of 4.2 m (Jónasson, 1979). Water enters Mývatn from springs on the east side of the lake, and these waters are naturally nutrient rich, with annual inputs of 1.5 g P m^{-2} y^{-1}, 1.4 g N m^{-2} y^{-1}, and 340 g Si m^{-2} y^{-1} (Ólafsson, 1979). The nutrient inputs into the lake have a molar N:P ratio of approximately 2.8:1, and the nutrient inputs from the springs into the south basin have an N:P ratio of 1.1:1 (Ólafsson, 1979). Primary production is high, with estimates of 220 g C m^{-2} y^{-1}, and benthic production can contribute a majority of annual productivity (Jónasson & Adalsteinsson, 1979). Diatoms (especially Fragilariaceae) that grow on the sediment dominate benthic production. In some regions of the lake, mats of filamentous green algae (*Cladophora glomerata* (L.) Kütz and *Aegagropila linnaei* Kütz) also contribute to benthic primary production. Phytoplankton production varies within and between years, and dense blooms of the N-fixing cyanobacterium *Dolichospermum* spp. (basionym: *Anabaena*; Wacklin, Hoffmann, & Komarek, 2009) intermittently occur. Benthic algae support large populations of midges (Diptera: Chironomidae) that may exceed densities of 200,000 m^{-2} and account for more than 90% of secondary biomass production (Lindegaard & Jónasson, 1979). The midges in turn support higher trophic levels, including fish and waterfowl. The midge species undergo large population fluctuations that have irregularly timed peaks and crashes (Einarsson et al., 2004), and these fluctuations are thought to be driven by consumer-resource interactions between larvae of the dominant midge species, *Tanytarsus gracilentus* (Holmgren), and benthic diatoms (Einarsson, Gardarsson, Gíslason, & Ives, 2002; Einarsson, Hauptfleisch, Leavitt, & Ives, 2016; Ives, Einarsson, Jansen, & Gardarsson, 2008). Therefore, characterizing

the controls on benthic algal production is necessary for understanding the dynamics of the whole system.

Ambient conditions

Ambient physiochemical conditions are routinely measured at a monitoring station in the middle of Mývatn's south basin (Ives, 2013). Water temperature at 1-m depth at this station is recorded by a sonde (Hydrolab DS5X, Hach Company, Loveland, Colorado, USA). Approximately every 10 days, water samples are collected for measuring total N and total P concentrations, and water column characteristics (i.e., pH, dissolved oxygen, light) are measured at 0.5-m intervals. Hourly solar irradiance data are recorded by a local weather station. A regression model based on the routine light profiles and solar irradiance at the time of the profile was used to estimate light levels just below the water's surface from solar irradiance data.

Experimental Design and Sampling

We measured the biomass, primary production, and taxonomic composition of benthic algae in Mývatn across N and P gradients using experimental microcosms, which consisted of 1-L clear, plastic containers (10.4 cm diameter × 16 cm height; *sensu* Herren et al., 2017). Nutrients were added to the microcosm sediment using nutrient-enriched, slow-diffusing agar, prepared according to the methods of Tank, Bernot, & Rosi-Marshall (2006). Three levels for both N and P were crossed in a factorial design (9 treatment combinations) with 4 replicates each for a total of 36 microcosms. Nitrogen treatment levels were control (agar with no enrichment), medium (concentration of 0.1 M NH_4Cl in the agar), and high (0.5 M NH_4Cl). P treatment levels

were control, medium (0.0065 M KH_2PO_4), and high (0.033 M KH_2PO_4). We assessed benthic algae responses to NH_4^+ enrichment rather than NO_3^- because NH_4^+ is the dominant source of inorganic N in the interstitial water of Mývatn (Gíslason, Eiríksdóttir, & Ólafsson, 2004) and sediments in general (Steinman, Abdimalik, Ogdahl, & Oudsema, 2016). Furthermore, the ratio of dissolved inorganic carbon (DIC) consumption and gross benthic dissolved oxygen production in Mývatn is consistent with algae using NH_4^+ rather than NO_3^- as an N source (Thorbergsdóttir & Gíslason, 2004). Medium and high treatments represent 100- and 500-fold enrichments of ambient N and P concentrations, respectively, from interstitial water in Mývatn's sediment (0.001 M NH_4 and 0.000065 M PO_4; Gíslason et al., 2004). These enrichment concentrations were not designed to represent realistic nutrient inputs, and we acknowledge that both medium-N and high-N treatments are much higher than ambient NH_4^+ interstitial concentrations. However, a concentration of 0.5 M NH_4^+ is commonly used in experiments employing nutrient-diffusing agar to assess nutrient limitation (Fairchild et al., 1985; Reisinger, Tank, & Dee, 2016; Steinman et al., 2016; Tank & Dodds, 2003; Tank et al., 2006; Vizza et al., 2018) to attempt to provide a source of supplemental nutrients over the duration of the experiment. The molar N:P ratios are the same for the medium-N × medium-P and high-N × high-P nutrient treatments, and they match the NH_4:PO_4 ratios (15.4:1) reported by Gíslason et al. (2004). Each microcosm received 100 mL of agar cut into 1-cm cubes. A mesh-covered plastic ring that fit snugly against each microcosm's inner wall was placed over the top of agar cubes to prevent them from floating.

We collected sediment for the experiment from the routine monitoring site in the middle of Mývatn's south basin (3.3 m deep) using Kajak corers on 28 June 2016. As this area was free of macrophytes, our study focused on the algal community living on the sediment (epipelon). The top 7 cm of sediment from multiple cores was homogenized and sieved through 125-µm

mesh to remove macroinvertebrates while retaining the associated epipelic communities. The sediment settled in a cool, dark location for 84 h before being added to the microcosms. We covered the agar cubes and mesh-covered plastic ring in each microcosm with 200 mL of sieved sediment (sediment height 4.5 cm). The bottom portion of sediment in the microcosms was excluded from light exposure with multiple layers of black tape, but the top 2 cm of sediment adjacent to the sides of the microcosms were exposed to light. A schematic of this setup can be found in the Supplemental Information (Figure S1). Microcosms were secured onto 3 flat metal racks which each held 12 microcosms. Each rack had a complete block of 9 N and P treatment combinations, and the remaining microcosms were haphazardly distributed among the 3 racks. While setting up the experiment, one replicate of the high N × control P was lost. The racks were deployed on the bottom of the lake at 1.75 m depth in a shallow bay near the southern shore of the lake on 4 July 2016 (day 0 of the experiment).

We measured microcosm metabolism and sediment characteristics twice during the field experiment. On days 10 and 33, we measured net ecosystem production (NEP) and community respiration (RESP) with paired light and dark incubations of each microcosm, during which we measured the change in dissolved oxygen (DO) levels. We randomly determined the order of dark and light incubations for a rack of microcosms. Dark conditions were achieved by applying fitted black tarp coverings on top of each microcosm. We measured DO concentration (mg O_2 L^{-1}) of the water column of each microcosm at the start and end of each incubation with a ProODO Probe (YSI, Yellow Springs, Ohio, USA). During the incubations lasting 3-6 h, microcosms were sealed with a water-tight lid, and care was taken to remove all air bubbles when lids were applied. Water in the microcosms was left static during the incubations, but we gently mixed the water column with the probe before taking DO measurements. Several times during incubations,

we recorded photosynthetically active radiation (PAR) with a light sensor (Li-192 Quantum Underwater Sensor, Li-COR, Lincoln, Nebraska, USA) at different depths and regressed ln-transformed PAR against depth to estimate light attenuation coefficients (k_D) at the experimental site. We used the k_D coefficients and the estimated light levels just below the water's surface to calculate PAR availability during the experimental incubations and deployment. NEP was calculated as the hourly change in evolved O_2 (mg O_2 h^{-1}) during light incubations, and RESP corresponded to the rate of oxygen consumption (mg O_2 h^{-1}) during dark conditions. Under the assumption that RESP is equal under light and dark conditions, we calculated gross primary production (GPP) as the summed magnitudes of NEP and RESP. Microcosms were incubated at a depth of 1.75 m, except for the second set of day 10 incubations (a dark incubation for two racks and a light incubation for one rack), which were done onshore due to abrupt, poor weather conditions. Incubation location (in lake vs. onshore) did not influence day 10 NEP ($t_{28.26} = -0.66$, $p = 0.515$), but RESP was higher for incubations onshore than in the lake ($t_{25.49} = -9.51$, $p < 0.001$), likely due to differences between water and air temperatures. However, this effect had no interaction with N or P, and the distribution of microcosm nutrient treatments between onshore and in-lake incubation locations was relatively balanced for dark incubations. One of the medium-N × medium-P replicate microcosms was lost on day 33 during the incubations.

Based on observations from day 10 incubations in the field experiment (see RESULTS: *Ecosystem Metabolism*), we set up a supplemental laboratory experiment to investigate whether the nutrient effects on production were reproducible among experiments. The lab experiment methods and results, which are broadly similar to the field experiment, are described in the Supplemental Information.

We collected sediment from each microcosm on days 10 and 33 of the experiment by haphazardly pipetting roughly 1 mL of sediment from 5 points distributed across the sediment surface of each microcosm. We used a 0.5 mL subsample of this sediment sample to measure benthic chlorophyll-*a* concentrations (CHL). Sediment samples for CHL were extracted in 100% methanol for 24 h in the dark and measured using a fluorometer (AquaFluor, Turner Designs, San Jose, California, USA). On day 33 we observed green bands of sediment (~1 cm thick) adjacent to the exterior walls of some microcosms and roughly 2 cm below the sediment surface, which had been exposed to light during the experiment because it was above the black shading. We sampled this sediment by pipetting a total volume 2 mL from 5 locations adjacent to the wall of each microcosm. Subsamples of both the surface and subsurface sediment (0.5 mL each) from day 33 were stored in separate 3.7-mL glass vials with water and preserved with Lugol's solution. To characterize algal composition, sample vials were gently inverted, and a 65-µL subsample was placed onto a microscope slide with a cover slip. Identification was completed with a compound microscope at 400× magnification. Cell composition was quantified by recording all cells occurring along a transect across the slide width, and two transects on a single slide were counted for each sample. Besides three diatom groups (Fragilariaceae, *Navicula* spp., *Epithemia* spp.), most taxa were rare and were grouped as either 'other large diatoms' (length >40 µm) or 'other small diatoms.' Diatoms were also scored as alive or dead based on whether their chloroplasts were fully expanded. Surface and subsurface samples were examined separately from each microcosm, and the cell counts were summed from the two samples per microcosm for subsequent analysis.

Nutrient Release Rates

To examine differences in nutrient release from sediment-containing agar across treatments, we set up additional microcosms as in the field experiment containing a subset of the nutrient treatment combinations (control N × control P, medium N × medium P, and high N × high P), with two replicates each. These microcosms were maintained indoors, and unlike the experimental microcosms which were open to the lake water column, the nutrient release units were closed systems. Consequentially, the nutrient concentrations in these release rate units are likely quite different from those overlaying the sediment in the field experiment, and these concentrations may influence flux measurements. Nonetheless, our intention was that the flux rates would provide a comparison among our nutrient treatments. Release rates were measured three times (4, 14, and 32 d after setup). For each release rate measurement, 'initial' and 'final' samples were collected (24-28 h apart) from the water overlying the sediment, filtered (0.45 µm) into 20-mL polyethylene bottles, and frozen. The sampled water was replenished between day 4, 14, and 32 release rate measurements. Water samples from the day 14 measurements were analyzed with the phenol hypochlorite technique for ammonia (O'Dell, 1993a) and the antimony-phospho-molybdate technique for soluble reactive phosphorus (SRP) (O'Dell, 1993b) using a segmented flow autoanalyzer (Flow Solution FS 3100, OI Analytical, College Station, Texas, USA), and ammonium and phosphate samples from days 4 and 32 were analyzed using ion chromatography (Dionex 2100 and 1100 Ion Chromatography, Thermo Scientific, Waltham, Massachusetts, USA). Since analysis methods differed across the dates of release rate measurements, we focus our comparison of nutrient diffusion rates from the sediment to water column among nutrient treatments for each time point. Release rates were calculated by converting the difference between the final and initial solute concentrations to a flux rate of

solutes by accounting for the volume of water in each microcosm, area of the sediment surface (81 cm^2), and time between initial and final samples.

Statistical Analysis

We used linear mixed models (LMMs) to examine effects of N and P enrichment on CHL, GPP, NEP, and RESP. For each response variable, we included N and P enrichment, time, and all two-way interactions as fixed effects. N and P were treated as factors (control, medium, high) rather than as numeric concentrations to better account for potential nonlinearities that have been observed in previous nutrient enrichment studies across multiple levels of N and P (Marks & Lowe, 1993). The models were fit using restricted maximum likelihood (REML), including rack and microcosm identity as random effects to account for blocking and the fact that each microcosm was sampled twice. Response variables CHL, GPP, NEP, and RESP were ln-transformed to meet normality assumptions. P-values from the LMMs were calculated from F-tests with the Kenward-Roger approximation. We report the p-values for the full version of each model, rather than first removing non-significant variables from the model to avoid "selective inference" (Taylor & Tibshirani, 2015) which occurs when removing model predictor terms artifactually increases the explanatory power of non-significant terms on a response variable (Freedman, 1983). However, we also performed type II F-tests to investigate the influence of the main effects without considering higher-order interactions, and we present these results in the Supplemental Information. The interpretations from type III and II tests are qualitatively the same, except for RESP, in which a main effect became significant when not considering non-significant interactions (i.e., Type II F-test results), which we indicate in the results. In order to

investigate the relationship between biomass and production, we used an LMM to determine if CHL influenced GPP (see Supplemental Information for details).

We quantified variation in algal composition across N and P concentrations using two complementary methods: redundancy analysis (RDA) and multilevel linear models (MLMs). (MLMs and LMMs are mathematically equivalent, but we use "MLM" for the community composition analyses to distinguish them from the analyses of CHL, GPP, NEP, and RESP.) For both community analyses, we standardized (z-scored) cell counts for each algal group as well as N and P concentrations by subtracting their respective means and dividing by their standard deviations. All community composition analyses were based on standardized cell counts rather than other metrics (i.e., biovolume). As RDA maximizes variation along predetermined axes of interest, this analysis allowed us to examine how closely community composition was associated with nutrient treatments. We used partial Mantel tests to assess the effects of N and P concentrations on composition over 2000 permutations; to investigate the effect of either N or P, the RDA was performed while permuting the nutrient of interest and keeping the other constant.

In the MLMs, the standardized cell counts of algal groups were regressed against fixed effects of N and P concentrations. The first MLM had random effects that allowed different responses to N and P enrichment for each algal group (Bartrons et al., 2015; Jackson, Turner, Pearson, & Ives, 2012) and a random effect for each microcosm. Significant variation in taxa-specific responses to nutrient treatments implies shifts in community composition (Jackson et al., 2012). To determine whether the treatment effects influenced algal composition, we used a likelihood ratio test (LRT) to compare the full model to a model without the random effect of an N or P slope for each algal group; a significant random effect of the slope implies that the response to N or P varies significantly among taxa. Although this MLM gives a test for variation

among groups in their responses to N and P and provides a taxon-specific coefficient, it is difficult to estimate confidence in the random taxon-specific slopes. Therefore, we used a second MLM that treated algal groups as categorical variables and included interactions of taxon-specific slopes with N and P as fixed effects (rather than random effects as in the first MLM); the model also included a random effect for microcosm. We performed a parametric bootstrap on the second MLM over 2000 simulations and used the output to calculate the coefficients for the response of each algal group to N and P (the main effect of N or P plus the interaction coefficient for each algal group) and their associated confidence intervals (Ives, 2018). All algal group-specific coefficients for responses to N and P are reported from this second MLM.

Finally, to explore the relationship between community structure and primary production, we regressed day 33 ln-transformed GPP against the standardized cell count abundances within algal groups using a simple linear model (LM). We acknowledge that directly relating algal abundance to primary production would require biovolumes of taxa and that cell counts are an inadequate way to quantify biomass (Hillebrand, Durselen, Kirschtel, Pollingher, & Zohary, 1999; Lavoie, Campeau, Fallu, & Dillon, 2006). However, we were interested in how variation in community structure explained variation in primary production rather than directly predicting production from community data. Using z-scored cell counts as predictors results in model coefficients that can be interpreted in terms of standard deviation in the predictors, and therefore are comparable across taxa.

All statistical analyses were conducted in R version 3.4.3 (R Core Team, 2017), using the packages ‘lme4’ (Bates, Maechler, Bolker, & Walker, 2015) for LMMs and MLMs, ‘car’ (Fox et al., 2018) for F-tests, and ‘vegan’ (Oksanen et al., 2017) for the RDA.

RESULTS

Ambient conditions

At the routine sampling location in Mývatn, the mean water temperature was 12.8 ºC, mean water column pH was 9.21, mean TN was 0.328 mg L^{-1}, and mean TP was 0.223 mg L^{-1} during the time of the field experiment. The light attenuation was relatively low, with k_D values of 0.474 m^{-1} and 0.515 m^{-1} for day 10 and 33 incubations. Based on these attenuation coefficients, during the incubations, PAR on days 10 and 33 averaged 264 µmol m^{-2} s^{-1} and 229 µmol m^{-2} s^{-1}, which are above previously measured light saturating conditions for periphyton (Liboriussen & Jeppesen, 2006). Averaging the two k_D coefficients, the average PAR at 1.75 m between sunrise and sunset over the entire experiment was 156 µmol m^{-2} s^{-1}.

Nutrient release rates

Differences in P release rates between treatments remained relatively consistent through time: on days 4, 14 and 32, the release rates for the control treatment were 0.001, 0.003, and 0.003; for the medium treatment 0.007, 0.007 and 0.003; and for the high treatment 0.015, 0.017 and 0.054 µmol cm^{-2} h^{-1} (Table S1). On day 14, different methods were used to measure N and P concentrations from days 4 and 32. On days 4 and 14 of the nutrient release experiment, N (based on NH_4^+ or NH_3 measurements) release rates varied as expected across enrichment treatments, with minimal release from the controls (0.003 and 0.018 µmol cm^{-2} h^{-1}; Table S1) and greater release from the medium (0.31 and 0.13 µmol cm^{-2} h^{-1}) and high treatments (1.57 and 0.47 µmol cm^{-2} h^{-1}). However, on day 32, N release from the medium treatment was much reduced (0.040 µmol cm^{-2} h^{-1}) and no different from controls (0.039 µmol cm^{-2} h^{-1}), while the high treatment had a negative flux toward the sediment (0.39 µmol cm^{-2} h^{-1}). These results

suggest that either N release from the agar was complete by day 32 or that the elevated initial (mean: 319 mg L^{-1}) and final (296 mg L^{-1}) NH_4^+ concentrations in the water column of the high nutrient replicates reduced the diffusive flux from the sediment to overlying water. High concentrations of NH_4^+ had time to accumulate in the overlying water between day 14 and 32 measurements because the release rates were measured in closed microcosms. In contrast, experimental microcosms were open to exchange with the lake water, so these high NH_4^+ concentrations should not reflect experimental conditions. We designed our nutrient treatments with the goal of maintaining similar N:P ratios to those measured in the interstitial water at Mývatn (15.4:1, Gislason et al. 2004). Comparing the ratio of N and P fluxes suggests that the N:P ratios being released into the water column were higher than this target ratio early in the experiment but that they decreased through time. For instance, on day 4, the medium and high N:P flux ratios were 46 and 104, suggesting substantially more N release relative to P, but by day 14, the medium and high N:P flux ratios decreased to 18 and 28. However, these nutrient release measurements did not measure release of nutrients from the agar directly; rather they account for the release of nutrients from the agar and through the sediment layer. Because sediment conditions can influence release of nutrients into the overlying water column (e.g., oxic conditions can inhibit phosphate release; Carlton & Wetzel, 1988), these N:P flux ratios may not actually reflect the nutrient supply entering the sediment that was available to the epipelic community.

Estimated annual diffusive fluxes of PO_4^- and NH_4^+ in Mývatn (reported by Gislason et al., 2004) scale to 0.000037 and 0.001549 µmol cm^{-2} h^{-1} respectively, demonstrating that our microcosm treatments resulted in higher-than-natural flux rates. While we acknowledge our higher flux rates, our goal was to obtain responses to nutrient enrichments within the practical

time constraints of experimental manipulations. We acknowledge that results from the release rate study cannot be directly translated to the field, since they differed in other conditions, such as temperature which can increase N diffusion from agar (Rugenski, Marcarelli, Bechtold, & Inouye, 2008). Nonetheless, these results satisfy our objective in demonstrating that the control, medium, and high nutrient enrichment treatments were achieved over at least the first 14 days of the experiment.

Chlorophyll-a

In the field experiment, CHL was not affected by any interactions involving N, P, and time (Table 1). CHL declined through time, had a marginal but non-significant positive response to N enrichment, and had no response to P enrichment (Table 1; Table S2; Fig 1).

Ecosystem metabolism

Nitrogen enrichment reduced ecosystem metabolism in the field experiment, but these effects tended to change over the course of the experiment. GPP was significantly influenced by an N × time interaction (Table 1), whereby there was a negative effect of N on GPP on day 10 that was not observed on day 33 (Table S3; Fig 2). The results for NEP paralleled those for GPP, with a decline in the effect of N through time (Table 1; Fig S2). N had no effect on RESP (Table 1), and in the absence of non-significant interactions, RESP increased from day 10 to 33 (Table S4; Fig S2). P had no effects on GPP, NEP, or RESP (Table 1). Overall, these results indicate that N had a negative but transient effect on metabolism, while P had no effect.

We investigated the influence of CHL on GPP; there was a non-significant effect of CHL on GPP (Table S5) suggesting no relationship between whole-community biomass and primary production.

Community composition

Nutrients played a substantial role in shaping algal composition by the end of the field experiment. Partial Mantel tests indicated that N had a strong effect on composition ($p < 0.001$) and that P had a weaker but significant influence on composition ($p = 0.023$). Several patterns emerged when comparing algal group scores for RDA axis 1, which was the axis strongly influenced by N enrichment (Fig 3). All diatoms and cyanobacteria had negative RDA1 scores, which suggests a negative association with N enrichment, while all green algae had positive RDA1 scores, which suggests a positive association with N enrichment (Table 2). Fragilariaceae and *Scenedesmus* had the most negative and positive responses to N enrichment, respectively (Fig 3; Table 2). RDA2 had an inverse relationship with P enrichment (Fig 3); small diatoms, cyanobacteria, and Fragilariaceae had the most negative RDA2 scores, suggesting their positive response to P (Table 2).

In the MLMs incorporating the standardized cell count abundances of algal groups, the effects of nutrients on community composition are given by variation among taxa in their responses to N or P (i.e., the random slope for each algal group). In the model, N significantly affected community composition (LRT: $\chi^2(1) = 38.17$, $p < 0.001$), while the effect of P was marginally significant (LRT: $\chi^2(1) = 4.05$, $p = 0.044$). The coefficients from the second MLM treating each algal group as a categorical variable quantified the effects of N or P on the standardized cell count abundance of each algal group (i.e., a fixed slope for each algal group),

and generally reflected the patterns of the RDA scores (Table 2). Fragilariaceae had a strong negative response to N, while *Scenedesmus* had a strong positive response (Table 2; Figs 4, 5). Other green algae also had a positive response to N (Fig 4). Fragilariaceae, cyanobacteria, and small diatoms all appeared to have positive responses to P enrichment (Table 2; Figs 4, 5). Other green algae had a negative response to P, although the 95% confidence interval of the coefficient overlaps with zero (Fig 4). Other green algae and *Scenedesmus* also slightly declined in their relative abundance in response to P enrichment (Fig 5).

Linking composition and GPP

In order to relate algal composition to ecosystem function, we regressed ln-transformed GPP from day 33 against the standardized cell count abundances of each algal group. *Oocystis* standardized abundance showed the strongest relationship with GPP, which was negative (Fig 6; Table 3). However, this group was not strongly influenced by N or P (Table 2; Figs 3, 4, 6). The standardized abundances of *Scenedesmus* and Fragilariaceae, the two groups most influenced by nutrients, had positive relationships with GPP (Table 3). While these two groups had similar effects on GPP, their responses to N were in opposite directions (Fig 6).

DISCUSSION

Using a field experiment, we assessed how nutrient enrichment affected the biomass, production, and composition of sediment-associated algal communities. We found a negligible response of chlorophyll-*a* to nutrients, and primary production was insensitive to P and transiently inhibited by N. In contrast, N and P affected community composition by generating contrasting responses of different algal groups to nutrient enrichment. While there was an

association between community structure and primary production measured at the end of the experiment, it did not appear to be driven by nutrient enrichment, because groups with contrasting responses to nutrients had similar patterns between their standardized abundances and GPP, and the group whose standardized abundance had the strongest relationship with GPP did not respond to nutrient enrichment. These results highlight the substantial effects that nutrient enrichment can have on benthic algal community structure, even while having little effect on ecosystem processes such as metabolism.

We did not see evidence of community-wide nutrient limitation in biomass. A similar lack of biomass response to N or P enrichment was also reported in 42.6% of nutrient-diffusing substrate experiments in streams (Francoeur, 2001). The fact that our study began with intact epipelic communities, while a majority of nutrient-diffusing agar studies provide bare substrates for colonization, may have also influenced our lack of N or P stimulation of community biomass. In a meta-analysis, Hillebrand (2002) found that nutrient effects lessened as biomass increased; therefore, starting the experiment with an intact community may have lowered the ability to detect a response. The lack of community-wide biomass limitation may also be due to the large nutrient pool available to benthic algae in the sediment interstitial water, ultimately fueled by Mývatn's high nutrient loading, both external and internal (Gíslason et al., 2004). Studies in other systems have suggested that moderate to high background nutrient concentrations may explain a lack of benthic nutrient limitation (Hillebrand & Kahlert, 2001). Alternatively, production could have been more strongly limited by factors other than N and P. For example, other studies have suggested the potential for dissolved inorganic carbon to limit benthic algae in lentic systems (Niederhauser & Schanz, 1993; Turner et al., 1994). Silicon is often limiting for diatoms, which compose the majority of Mývatn's epipelic communities (both in our

experimental microcosms and *in situ*), and previous research has shown that silicon can become limiting to diatoms after N and P enrichment (Carrick & Lowe, 1988). While results from studies using nutrient-diffusing agar have shown mixed results regarding benthic algal nutrient limitation (Fairchild & Lowe, 1984; Fairchild et al., 1985; Hogan, McGowan, & Anderson, 2014; Lepori & Robin, 2014; Maberly, King, Dent, Jones, & Gibson, 2002; Steinman et al., 2016), few of these have assessed the limitation of sediment-associated epipelic communities. Meta-analysis results suggest that producer communities associated with marine sediments or soft-bottoms showed weaker responses to nutrient enrichment than benthic communities on hard substrates (Elser et al., 2007). Additionally, in several studies in which the water column was enriched with nutrients, epipelic community biomass showed no response to nutrients, but algae that grow on hard substrates demonstrated increased biomass under nutrient enrichment (Blumenshine, Vadeboncoeur, & Lodge, 1997; Nydick, Lafrancois, Baron, & Johnson, 2004; Vadeboncoeur et al., 2001). Together, these results and the results from our study suggest epipelic growth may in general not be limited by N or P due to their ability to acquire nutrients from the sediment (Blumenshine et al., 1997; Hansson, 1990).

The most apparent feature of nitrogen's effects on ecosystem metabolism was its temporal variability. In both the field and laboratory experiments, N had a negative effect on GPP and NEP early in the experiment, with this inhibitory effect lessening through time. While N or P inhibition is relatively uncommon, studies on benthic algae (Bernhardt & Likens, 2004; Lougheed et al., 2015; Reisinger et al., 2016; Ribot, von Schiller, Sabater, & Martí, 2015; Vizza et al., 2018) and phytoplankton (Steinman et al., 2016) have documented its occurrence. Furthermore, in a recent meta-analysis, 15% of included studies on primary producers from freshwater, marine, and terrestrial systems showed a negative response to nutrient addition

(Harpole et al., 2011). Bernhardt & Likens (2004) observed a negative effect of N on periphyton biomass and proposed that competition between heterotrophs (e.g., bacteria and fungi) and primary producers for inorganic nutrients contributed to this inhibitory effect. Similarly, the addition of dissolved organic carbon (DOC) to stream biofilms increased heterotroph demand for inorganic nutrients which consequentially reduced autotrophic biomass (Bechtold, Marcarelli, Baxter, & Inouye, 2012). We did not measure heterotroph abundance, but if N stimulated heterotrophic bacteria growth and consequentially suppressed algal growth through enhanced competition, one would expect a corresponding increase in ecosystem respiration with a decline in primary production. However, there was no effect of N on RESP in our field experiment, and N had a negative effect on RESP early in the lab experiment. Thus, we suggest that competition between algae and bacteria is not the likely explanation for our observations, which is consistent with previous research suggesting that competition between primary producers and heterotrophs will be less important in autochthonous (similar to Mývatn) than allochthonous systems (Rier & Stevenson, 2002).

Previous studies have suggested that toxic levels of nutrient enrichment may lead to inhibition of primary producers (Bernhardt & Likens, 2004; Harpole et al., 2011; Ribot et al., 2015). Because the experimental microcosms were open to exchange with the water column, we expect that the nutrient concentrations overlaying the sediment in the field experiment would be much lower than high concentrations measured in the release rate microcosms. However, we do not have nutrient concentration data from the field experiment, so we cannot rule out the possibility of N toxicity. Finally, as with any experimental manipulation in the field, our experimental design could have influenced the inferred temporal change in how N affected primary production. For instance, we measured production on day 33 of our field experiment, but

our nutrient release data suggest that the agar may have depleted its nutrient concentrations by this point. At the end of the 21-day lab experiment, medium-N enrichment had a positive effect on NEP which hints at the potential importance of duration of agar deployment (though differences in background nutrient concentrations in the water overlying the sediment from the two experiments may have also contributed to this effect). While other studies have deployed nutrient-diffusing agar for over 30 days (Hill & Knight, 1988; Lowe, Golladay, & Webster, 1986), these studies focused on biomass rather than metabolism (i.e., structural versus dynamic responses *sensu* Grimm & Fisher, 1986). Thus, we cannot rule out the possibility that complete nutrient release by the agar, rather than biologically meaningful changes in the community, is responsible for the absence of an N effect on production at the end of the field experiment.

The temporal variation in production in response to nutrient manipulation may have been influenced by nutrient-driven effects on other aspects of the algal community, such as physiological effects on the existing community or compositional shifts. We do not know the mechanism behind the decrease in GPP, NEP, and RESP with N enrichment early in our experiment, but briefly discuss here how potential mechanisms—physiological effects and compositional shifts—may explain our results. The initial negative effect of N on production could have been mediated through a negative physiological effect on the diatoms, which are dominant members of Mývatn's epipelic community. We describe below how NH_4^+ may not be the preferred form of N for diatoms, and they may have experienced consequent reduced photosynthetic abilities if their nutrient uptake was limited or if their growth was inhibited by NH_4^+ (Domingues, Barbosa, Sommer, & Galva, 2011). We acknowledge that data related to algal physiology (i.e., cellular N, P, and Si content), for diatoms in particular, could have been useful in addressing this potential explanation. However, the possibility of physiological effects does

not explain why the inhibitory effect of N was only transient. Alternatively, the inhibitory effect of N may reflect a compositional shift that occurred during the experiment. Because different algal groups had contrasting responses to nutrients but similar patterns with GPP, the relationship between GPP and community composition was not aligned with nutrient enrichment at the end of the experiment; however, it is possible that a transient shift in composition early in the experiment (and therefore not captured by our data) explains the early inhibitory effect of N. Fragilariaceae are the most abundant taxa in the benthos of Mývatn, and they showed a negative response to N. If declines in their abundance or inhibition of their photosynthetic activity occurred early in the experiment, this could have decreased production. The ratio of alive:dead Fragilariaceae cells at the end of the experiment, as determined by presence/absence of chloroplasts, was lowest in N-enriched microcosms, supporting this explanation (data not shown). It is possible that as other taxa became more abundant in the N-enriched microcosms, they compensated for the production lost by Fragilariaceae, resulting in comparable production to other treatments at the end of the experiment. This hypothesis does not directly link nutrients to production; rather, we suppose that compositional shifts causing increases and decreases in the standardized abundance of different algal groups may have created multiple compositions that achieved the same productivity. We did not test these mechanisms directly, and they are only possible explanations for our observations. Nonetheless, in mesocosms that were enriched with N to alter N:P ratios, Vrede et al. (2009) saw notable shifts in phytoplankton community composition that resulted in statistically similar production.

The strong effects of nutrient (especially N) enrichment on algal composition mirrored results in the literature. Carrick & Lowe (1988) report a decline in *Fragilaria* biovolume and an increase in green algae under enrichment of N and P. Other studies have shown increased cell

counts of green algae in benthic algal communities under nutrient enrichment (Guasch et al., 1995; Lepori & Robin, 2014) and a negative response of *Fragilaria* to nutrient enrichment (Carrick, Lowe, & Rotenberry, 1988). As recently reviewed by Glibert et al. (2016), varying preferences for inorganic N forms among algal taxa can ultimately affect community composition. Although NH_4^+ has often been considered the preferred form of inorganic N for primary producers (Dortch, 1990; Von Schiller, Martí, Riera, & Sabater, 2007), diatoms may respond negatively to enriched levels of NH_4^+ due to their preferred use of NO_3^- (Glibert et al., 2016). While this recent review focuses on N preferences of phytoplankton, the results likely extend to benthic taxa, as the preference for NO_3^- or NH_4^+ appears phylogenetically conserved and driven by the abundance of corresponding ion transporters on cell membranes (Glibert et al., 2016). NH_4^+ is the dominant form of N naturally available in the sediment, but the magnitude of our experimental enrichment may have provided a competitive advantage to green algae taxa such as *Scenedesmus*. Green algae have a preference for NH_4^+ over NO_3^- (Domingues et al., 2011; Litchman et al., 2007) and previous work has shown that *Scenedesmus* growth is stimulated under enhancement of reduced N forms (Donald, Bogard, Finlay, Bunting, & Leavitt, 2013).

While algal composition was associated with GPP, the effects of individual groups were more influential than compositional shifts from nutrient enrichment. For instance, *Oocystis* standardized abundance had a negative relationship with GPP but was unaffected by N or P enrichment. Additionally, Fragilariaceae and *Scenedesmus* standardized abundances showed similar positive relationships with GPP, but since these groups responded in opposite directions to N enrichment, there was no net effect of overall community composition on production, as would be characterized by our RDA. We are not sure why the standardized abundances of these

groups were related to production; however, previous studies have highlighted the potential for specific taxonomic groups to influence ecosystem processes. For instance, in phytoplankton communities some species may have relatively low contributions to primary production compared to their high relative abundance based on cell density, while other species can contribute substantially to primary production despite having minor relative abundance (Han & Furuya, 2000). Additionally, Ishida et al. (2008) did not see significant relationships between ecosystem function (denitrification) and overall periphyton community structure, but they found a significant relationship between denitrification rates and the biovolume of specific algal divisions, including diatoms. Similarly, the relationship between macroalgae productivity and the identity of individual species was much stronger than between productivity and overall community richness in marine subtidal environments (Bruno, Boyer, Duffy, Lee, & Kertesz, 2005). Together these results suggest that functional traits of individual taxa are likely more important for benthic ecosystem metabolism than overall community composition. This implies that individual taxa can have effects disproportionate to their relative abundance in their communities.

Overall, in our field experiment we did not observe epipelic biomass nutrient limitation and saw no lasting positive effect of N or P enrichment on algal production, which as described previously is potentially due to length of agar deployment. Nonetheless, nutrient enrichment had strong effects on community composition. The compositional shifts did not lead to changes in biomass or primary production because either (i) taxa had opposite responses to nutrient enrichment and therefore offset each other's effects on biomass and production, or (ii) taxa with the strongest effects on production were not affected by nutrient enrichment. Our results highlight the complexities arising from shifts in algal community composition, and they suggest

that future studies continue to investigate the relationship between benthic algal composition and ecosystem processes and how this relationship is affected by nutrients or other abiotic controls.

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CONFLICT OF INTEREST

The authors declare no conflict of interest pertaining to this study. The study results have not been submitted or published elsewhere.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES

Bartrons, M., Einarsson, Á., Nobre, R. L. G., Herren, C. M., Webert, K. C., Brucet, S., … Ives, A. R. (2015). Spatial patterns reveal strong abiotic and biotic drivers of zooplankton community composition in Lake Myvatn, Iceland. *Ecosphere*, *6*(June), 1–20.

Bates, D., Maechler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models

using lme4. *Journal of Statistical Software*, *67*(1), 1-48.

Bechtold, H. A., Marcarelli, A. M., Baxter, C. V., & Inouye, R. S. (2012). Effects of N, P, and organic carbon on stream biofilm nutrient limitation and uptake in a semi-arid watershed. *Limnology and Oceanography*, *57*(5), 1544–1554.

Bernhardt, E. S., & Likens, G. E. (2004). Controls on periphyton biomass in heterotrophic streams. *Freshwater Biology*, *49*(1), 14–27.

Blumenshine, S. C., Vadeboncoeurl, Y., & Lodge, D. M. (1997). Benthic-pelagic links:responses of benthos to water-column nutrient enrichment. *Journal of the North American Benthological Society*, *16*(3), 466–479.

Borchardt, M. A. (1996). Nutrients. In R. J. Stevenson, M. L. Bothwell, & R. L. Lowe (Eds.), *Algal Ecology: Freshwater Benthic Ecosystems* (pp. 183–227). San Diego: Academic Press.

Bothwell, M. L. (1989). Phosphorus-limited growth dynamics of lotic periphytic diatom communities: areal biomass and cellular growth rate responses. *Canadian Journal of Fisheries and Aquatic Sciences*, *46*, 1293–1301.

Bruno, J. F., Boyer, K. E., Duffy, J. E., Lee, S. C., & Kertesz, J. S. (2005). Effects of macroalgal species identity and richness on primary production in benthic marine communities. *Ecology Letters*, *8*(11), 1165–1174.

Cael, B. B., Heathcote, A. J., & Seekell, D. A. (2017). The volume and mean depth of Earth's lakes. *Geophysical Research Letters*, *44*(1), 209–218.

Carlton, R. G., & Wetzel, R. G. (1988). Phosphorus flux from lake sediments: Effect of epipelic algal oxygen production. *Limnology and Oceanography*, *33*(4), 562–570.

Carrick, H. J., & Lowe, R. L. (1988). Response of Lake Michigan benthic algae to in situ enrichment with Si, N, and P. *Canadian Journal of Fisheries and Aquatic Sciences*, *45*,

271–279.

Carrick, H. J., Lowe, R. L., & Rotenberry, J. T. (1988). Guilds of Benthic Algae along Nutrient Gradients: Relationships to Algal Community Diversity. *Journal of the North American Benthological Society*, *7*(2), 117.

Cole, B. E., Cloern, J. E., & Alpine, A. E. (1986). Biomass and productivity of three phytoplankton size classes in San Francisco Bay. *Estuaries*, *9*(2), 117–126.

Domingues, R. B., Barbosa, A. B., Sommer, U., & Galva, H. M. (2011). Ammonium , nitrate and phytoplankton interactions in a freshwater tidal estuarine zone: potential effects of cultural eutrophication. *Aquatic Sciences*, *73*, 331–343.

Donald, D. B., Bogard, M. J., Finlay, K., Bunting, L., & Leavitt, P. R. (2013). Phytoplankton-Specific Response to Enrichment of Phosphorus-Rich Surface Waters with Ammonium, Nitrate, and Urea. *PLoS ONE*, *8*(1).

Dortch, Q. (1990). The interaction between ammonium and nitrate uptake in phytoplankton. *Marine Ecology Progress Series*, *61*, 183–201.

Edwards, K. F., Thomas, M. K., Klausmeier, C. A., & Litchman, E. (2012). Allometric scaling and taxonomic variation in nutrient utilization traits and maximum growth rate of phytoplankton. *Limnology and Oceanography*, *57*(2), 554–566.

Edwards, K. F., Thomas, M. K., Klausmeier, C. A., & Litchman, E. (2015). Light and growth in marine phytoplankton: Allometric, taxonomic, and environmental variation. *Limnology and Oceanography*, *60*(2), 540–552.

Einarsson, Á., Gardarsson, A., Gislason, G. M., & Ives, A. R. (2002). Consumer – resource interactions and cyclic population dynamics of Tanytarsus gracilentus (Diptera: Chironomidae). *Journal of Animal Ecology*, *71*, 832–845.

Einarsson, Á., Hauptfleisch, U., Leavitt, P. R., & Ives, A. R. (2016). Identifying consumer-resource population dynamics using paleoecological data. *Ecology*, *97*(2), 361–371.

Einarsson, Á., Stefánsdóttir, G., Jóhannesson, H., Ólafsson, J. S., Gíslason, G. M., Wakana, I., … Gardarsson, A. (2004). The ecology of Lake Myvatn and the River Laxa: Variation in space and time. *Aquatic Ecology*, *38*(2), 317–348.

Elser, J. J., Bracken, M. E. S., Cleland, E. E., Gruner, D. S., Harpole, W. S., Hillebrand, H., … Smith, J. E. (2007). Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater , marine and terrestrial ecosystems. *Ecology Letters*, *10*(12), 1135-1142.

Fairchild, G. W., & Lowe, R. L. (1984). Artificial substrates which release nutrients: Effects on periphyton and invertebrate succession. *Hydrobiologia*, *114*(1), 29–37.

Fairchild, G. W., Lowe, R. L., & Richardson, W. B. (1985). Algal periphyton growth on nutrient-diffusing substrates: an in situ bioassay. *Ecology*, *66*(2), 465–472.

Fox, J., Weisberg, S., Price, B., Adler, D., Bates, D., Baud-Bovy, G., … Zeileis, A. (2018). car: Companion to Applied Regression. R package version 3.0-0. https://cran.r-project.org/web/packages/car/index.html

Francoeur, S. N. (2001). Meta-analysis of lotic nutrient amendment experiments: detecting and quantifying subtle responses. *Journal of the North American Benthological Society*, *20*(3), 358–368.

Freedman, D. A. (1983). A note on screening regression equations. *The American Statistician*, *37*(2), 152–155.

Gíslason, S. R., Eiríksdóttir, E. S., & Ólafsson, J. S. (2004). Chemical composition of interstitial water and diffusive fluxes within the diatomaceous sediment in Lake Myvatn, Iceland.

Aquatic Ecology, *38*(2), 163–175.

Glibert, P. M., Wilkerson, F. P., Dugdale, R. C., Raven, J. A., Dupont, C. L., Leavitt, P. R., … Kana, T. M. (2016). Pluses and minuses of ammonium and nitrate uptake and assimilation by phytoplankton and implications for productivity and community composition, with emphasis on nitrogen-enriched conditions. *Limnology and Oceanography*, *61*(1), 165–197.

Greenwood, J. L., & Rosemond, A. D. (2005). Periphyton response to long-term nutrient enrichment in a shaded headwater stream. *Canadian Journal of Fisheries and Aquatic Sciences*, *62*(9), 2033–2045.

Grimm, N. B., & Fisher, S. G. (1986). Nitrogen Limitation in a Sonoran Desert Stream. *Journal of the North American Benthological Society*, *5*(1), 2–15.

Guasch, H., Marti, E., & Sabater, S. (1995). Nutrient enrichment effects on biofilm metabolism in a Mediterranean stream. *Freshwater Biology*, *33*(3), 373–383.

Han, M., & Furuya, K. (2000). Size and species-specific primary productivity and community structure of phytoplankton in Tokyo Bay, *Journal of Plankton Research*, *22*(7), 1221–1235.

Hansson, L.-A. (1990). Quantifying the impact of periphytic algae on nutrient availability for phytoplankton. *Freshwater Biology*, *24*, 265–273.

Harpole, W. S., Ngai, J. T., Seabloom, E. W., Borer, E. T., Bracken, M. E. S., Elser, J. J., … Smith, J. E. (2011). Nutrient co-limitation of primary producer communities. *Ecology Letters*, *14*, 852–862.

Herren, C. M., Webert, K. C., Drake, M. D., Vander Zanden, M. J., Einarsson, A., Ives, A. R., & Gratton, C. (2017). Positive feedback between chironomids and algae creates net mutualism between benthic primary consumers and producers. *Ecology*, *98*(2), 447–455.

Hill, W. R., & Knight, A. W. (1988). Nutrient and Light Limitation of Algae in Two Northern

California Streams. *Journal of Phycology*, *24*, 125–132.

Hillebrand, H. (2002). Top-down versus bottom-up control of autotrophic biomass — a meta-analysis on experiments with periphyton. *Journal of the North American Benthological Society*, *21*(3), 349–369.

Hillebrand, H., Durselen, C. D., Kirschtel, D., Pollingher, U., & Zohary, T. (1999). Biovolume calculation for pelagic and benthic microalgae. *Journal of Phycology*, *35*, 403–424.

Hillebrand, H., & Kahlert, M. (2001). Effect of grazing and nutrient supply on periphyton biomass and nutrient stoichiometry in habitats of different productivity. *Limnology and Oceanography*, *46*(8), 1881–1898.

Hogan, E. J., McGowan, S., & Anderson, N. J. (2014). Nutrient limitation of periphyton growth in arctic lakes in south-west Greenland. *Polar Biology*, *37*, 1331–1342.

Ishida, C. K., Arnon, S., Peterson, C. G., Kelly, J. J., Gray, K. A., Kelly, J. J., & Gray, K. A. (2008). Influence of Algal Community Structure on Denitrification Rates in Periphyton Cultivated on Artificial Substrata. *Microbial Ecology*, *56*(1), 140–152.

Ives, A. R. (2013). LTREB Biological Limnology at Lake Mývatn 2012-current. LTER Network Information System Repository.

Ives, A. R. (2018). Mixed and Phylogenetic Models: A Conceptual Introduction to Correlated Data. Leanpub. Available from: https://leanpub.com/correlateddata

Ives, A. R., Einarsson, A., Jansen, V. A. A., & Gardarsson, A. (2008). High-amplitude fluctuations and alternative dynamical states of midges in Lake Myvatn. *Nature*, *452*(7183), 84–87.

Jackson, M. M., Turner, M. G., Pearson, S. M., & Ives, A. R. (2012). Seeing the forest and the trees: multilevel models reveal both species and community patterns. *Ecosphere*, *3*(9), 1–

16.

Jonasson, P. M. (1979). The Lake Myvatn ecosystem, Iceland. *Oikos*, *32*(1), 289–305.

Jónasson, P. M., & Adalsteinsson, H. (1979). Phytoplankton Production in Shallow Eutrophic Lake Mývatn, Iceland. *Oikos*, *32*(1), 113–138.

Karlsson, J., Byström, P., Ask, J., Ask, P., Persson, L., & Jansson, M. (2009). Light limitation of nutrient-poor lake ecosystems. *Nature*, *460*(7254), 506–509.

Lavoie, I., Campeau, S., Fallu, M.-. A., & Dillon, P. J. (2006). Diatoms and biomonitoring: should cell size be accounted for? *Hydrobiologia*, *573*, 1–16.

Lepori, F., & Robin, J. (2014). Nitrogen limitation of the phytobenthos in Alpine lakes: results from nutrient-diffusing substrata. *Freshwater Biology*, *59*, 1633–1645.

Liboriussen, L., & Jeppesen, E. (2006). Structure, biomass, production and depth distribution of periphyton on artificial substratum in shallow lakes with contrasting nutrient concentrations. *Freshwater Biology*, *51*(1), 95–109.

Lindegaard, C., & Jónasson, P. M. (1979). Abundance , population dynamics and production of zoobenthos in Lake Myvatn , Iceland. *Oikos*, *32*, 202–227.

Litchman, E., Klausmeier, C. A., Schofield, O. M., & Falkowski, P. G. (2007). The role of functional traits and trade-offs in structuring phytoplankton communities: Scaling from cellular to ecosystem level. *Ecology Letters*, *10*(12), 1170–1181.

Loeb, S. L., & Reuter, J. E. (1981). The epilithic periphyton community: a five-lake comparative study of community productivity, nitrogen metabolism and depth-distribution of standing crop. *Verhandlungen des Internationalen Verein Limnologie*, *21*, 346–352.

Lougheed, V. L., Hernandez, C., Andresen, C. G., Miller, N. A., Alexander, V., & Prentki, R. (2015). Contrasting responses of phytoplankton and benthic algae to recent nutrient

enrichment in Arctic tundra ponds. *Freshwater Biology*, *60*(10), 2169–2186.

Lowe, R. L., Golladay, S. W., & Webster, J. R. (1986). Periphyton Response to Nutrient Manipulation in Streams Draining Clearcut and Forested Watersheds. *Journal of the North American Benthological Society*, *5*(3), 221–229.

Maberly, S. C., King, L., Dent, M. M., Jones, R. I., & Gibson, C. E. (2002). Nutrient limitation of phytoplankton and periphyton growth in upland lakes. *Freshwater Biology*, *47*(11), 2136–2152.

Marcarelli, A. M., Bechtold, H. A., Rugenski, A. T., & Inouye, R. S. (2009). Nutrient limitation of biofilm biomass and metabolism in the Upper Snake River basin, southeast Idaho, USA. *Hydrobiologia*, *620*(1), 63–76.

Marcarelli, A. M., & Wurtsbaugh, W. A. (2006). Temperature and nutrient supply interact to control nitrogen fixation in oligotrophic streams: An experimental examination. *Limnology and Oceanography*, *51*(5), 2278–2289.

Marks, J. C., & Lowe, R. L. (1989). The independent and interactive effects of snail grazing and nutrient enrichment on structuring periphyton communities. *Hydrobiologia*, *185*(1), 9–17.

Marks, J. C., & Lowe, R. L. (1993). Interactive effects of nutrient availablility and light levels on the periphyton composition of a large oligotrophic lake. *Can.J.Fish.Aquat.Sci.*, *50*(1), 1270–1278.

Niederhauser, P., & Schanz, F. (1993). Effects of nutrient (N, P, C) enrichment upon the littoral diatom community of an oligotrophic high-mountain lake. *Hydrobiologia*, *269–270*(1), 453–462.

Nydick, K. R., Lafrancois, B. M., Baron, J. S., & Johnson, B. M. (2004). Nitrogen regulation of algal biomass, productivity, and composition in shallow mountain lakes, Snowy Range,

Wyoming, USA. *Canadian Journal of Fisheries and Aquatic Sciences*, *61*(7), 1256–1268.

O'Dell, J. W. (1993a). Method 350.1. Determination of ammonia nitrogen by semi-automated colorimetry. Environmental Monitoring Systems Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, OH, USA.

O'Dell, J. W. (1993b). Method 365.1. Determination of phosphorus by semi-automated colorimetry. Environmental Monitoring Systems Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, OH, USA.

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., … Wagner, H. (2017). vegan: Community Ecology Package. R package version 2.4-4. https://CRAN.R-project.org/package=vegan

Ólafsson, J. (1979). The chemistry of Lake Myvatn and River Laxa. *Oikos*, *32*(1), 82–112.

Ozersky, T., Volkova, E. A., Bondarenko, N. A., Timoshkin, O. A., Malnik, V. V., Domysheva, V. M., & Hampton, S. E. (2018). Nutrient limitation of benthic algae in Lake Baikal, Russia. *Freshwater Science*, *37*(3), 472–482.

Phillips, J. S., McCormick, A. R., Einarsson, A., Grover, S. N., & Ives, A. R. Spatiotemporal variation in the sign and magnitude of ecosystem engineer effects on lake ecosystem production. *Ecosphere* 10(6):e02760.

R Core Team. (2017). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Reisinger, A. J., Tank, J. L., & Dee, M. M. (2016). Regional and seasonal variation in nutrient limitation of river biofilms. *Freshwater Biology*, *35*(October 2015), 474–489.

Ribot, M., von Schiller, D., Sabater, F., & Martí, E. (2015). Biofilm growth and nitrogen uptake responses to increases in nitrate and ammonium availability. *Aquatic Sciences*, *77*(4), 695–

707.

Rier, S. T., & Stevenson, R. J. (2002). Effects of light, dissolved organic carbon, and inorganic nutrients on the relationship between algae and heterotrophic bacteria in stream periphyton. *Hydrobiologia*, *489*, 179–184.

Rosemond, A. D. (1993). Interactions among irradiance, nutrients, and herbivores constrain a stream algal community. *Oecologia*, *94*(4), 585–594.

Rosemond, A. D., & Brawley, S. H. (1996). Species-specific characteristics explain the persistence of Stigeoclonium tenue (Chlorophyta) in a woodland stream. *Journal of Phycology*, *32*, 54–63.

Rugenski, A. T., Marcarelli, A. M., Bechtold, H. A., & Inouye, R. S. (2008). Effects of temperature and concentration on nutrient release rates from nutrient diffusing substrates. *Journal of the North American Benthological Society*, *27*(1), 52–57.

Steinman, A., Abdimalik, M., Ogdahl, M. E., & Oudsema, M. (2016). Understanding planktonic vs. benthic algal response to manipulation of nutrients and light in a eutrophic lake. *Lake and Reservoir Management*, *32*(4), 402–409.

Tank, J. L, Bernot, M. J., & Rosi-Marshal, E. J. (2006). Nitrogen Limitation and Uptake. In F. R. Hauer, & G. A. Lamberti (Eds.), *Methods in Stream Ecology* (2nd ed.) (pp. 213-238). San Diego, CA: Academic Press.

Tank, J. L., & Dodds, W. K. (2003). Nutrient limitation of epilithic and epixylic biofilms in ten North American streams. *Freshwater Biology*, 1031–1049.

Taylor, J., & Tibshirani, R. J. (2015). Statistical learning and selective inference. *Proceedings of the National Academy of Sciences*, *112*(25), 7629–7634.

Thorbergsdóttir, I. M., & Gíslason, S. R. (2004). Internal loading of nutrients and certain metals

in the shallow eutrophic Lake Myvatn, Iceland. *Aquatic Ecology*, *38*(2), 191–207.

Turner, M. A., Howell, E. T., Robinson, G. G. C., Campbell, P., Hecky, R. E., & Schindler, E. U. (1994). Roles of nutrients in controlling growth of epilithon in oligotrophic lakes of low alkalinity. *Canadian Journal of Fisheries and Aquatic Sciences*, *51*, 2784–2793.

Underwood, G. J. C., Perkins, R. G., Consalvey, M. C., Hanlon, A. R. M., Oxborough, K., Baker, N. R., & Paterson, D. M. (2005). Patterns in microphytobenthic primary productivity: Species-specific variation in migratory rhythms and photosynthetic efficiency in mixed-species biofilms. *Limnology and Oceanography*, *50*(3), 755–767.

Vadeboncoeur, Y., Lodge, D. M., & Carpenter, S. R. (2001). Whole-Lake Fertilization Effects on Distribution of Primary Production between Benthic and Pelagic Habitats. *Ecology*, *82*(4), 1065–1077.

Vadeboncoeur, Y., Peterson, G., Vander Zanden, M. J., & Kalff, J. (2008). Benthic Algal Production Across Lake Size Gradients : Interactions Among Morphometry, Nutrients, and Light. *Ecology*, *89*(9), 2542–2552.

Vadeboncoeur, Y., Vander Zanden, M. J., & Lodge, D. M. (2002). Putting the Lake Back Together: Reintegrating Benthic Pathways into Lake Food Web Models. *BioScience*, *52*(1), 44.

Vizza, C., Pechal, J. L., Benbow, M. E., Lang, J. M., Chaloner, D. T., Jones, S. E., & Lamberti, G. A. (2018). Nitrate amendment reduces biofilm biomass and shifts microbial communities in remote, oligotrophic ponds. *Freshwater Science*, *37*(2), 251–263.

Von Schiller, D., Martí, E., Riera, J. L., & Sabater, F. (2007). Effects of nutrients and light on periphyton biomass and nitrogen uptake in Mediterranean streams with contrasting land uses. *Freshwater Biology*, *52*(5), 891–906.

Vrede, T., Ballantyne, A., Mille-Lindblom, C., Algesten, G., Gudasz, C., Lindahl, S., & Brunberg, A. K. (2009). Effects of N : P loading ratios on phytoplankton community composition, primary production and N fixation in a eutrophic lake. *Freshwater Biology*, *54*(2), 331–344.

Wacklin, P., Hoffmann, L., & Komarek, J. (2009). Nomenclatural validation of the genetically revised cyanobacterial genus Dolichospermum (Ralfs ex Bornet et Flahault) comb. nova. *Fottea*, *9*(1), 59–64.

Young, E. B., & Beardall, J. (2003). Photosynthetic function in Dunaliella tertiolecta (Chlorophyta) during a nitrogen starvation and recovery cycle. *Journal of Phycology*, *39*(5), 897–905.

Table 1. Type III F-tests from linear mixed models testing for the main and interactive effects of N, P, and time on ln-transformed chlorophyll-*a* (CHL), gross primary production (GPP), net ecosystem production (NEP), and respiration (RESP) in the field experiment. For statistically significant main effects, we indicate whether the main effect of a factor was positive (+) or negative (-) in the 'Effect' column. We denote statistically significant interaction effects with a (*) and explain the interaction in the main text when presenting the results.

	Factor	Effect	F	df_{num}	$df_{residual}$	P-value
CHL	*N*		3.09	2	50.37	0.054
	P		0.56	2	49.61	0.573
	Time	(-)	6.05	1	29.24	0.020
	N × *P*		0.84	4	23.81	0.512
	N × *Time*		1.09	2	29.58	0.350
	P × *Time*		1.41	2	29.58	0.260
GPP	*N*	(-)	9.91	2	46.90	< 0.001
	P		1.21	2	45.93	0.309
	Time		2.17	1	29.32	0.152
	N × *P*		0.74	4	23.79	0.577
	N × *Time*	(*)	5.46	2	29.68	0.010
	P × *Time*		1.23	2	29.68	0.307
NEP	*N*		19.92	2	46.89	< 0.001
	P		0.29	2	45.95	0.751
	Time	(+)	7.04	1	29.31	0.013
	N × *P*		2.39	4	23.72	0.079
	N × *Time*	(*)	8.84	2	29.67	0.001
	P × *Time*		1.83	2	29.67	0.177
RESP	*N*		2.34	2	46.89	0.107
	P		2.85	2	45.95	0.068
	Time		0.04	1	29.31	0.836
	N × *P*		0.13	4	23.72	0.971
	N × *Time*		1.89	2	29.67	0.168
	P × *Time*		2.22	2	29.67	0.126

Table 2. Algal groups differed in their responses to additions of nitrogen (N) and phosphorus (P). Scores for each algal group from the redundancy analysis (RDA) for axes RDA1 and RDA2 and coefficients from the multilevel model (MLM) for group-specific responses to N and P, in which each algal group's response to nutrients is modeled as a fixed effect, are shown. MLM coefficients are the main effects for N or P plus the interaction term for each algal group. The coefficients shown are the means obtained by parametric bootstrapping over 2000 simulations.

	RDA axes scores		MLM coefficients	
Algal Group	RDA1	RDA2	N	P
Fragilariaceae	-1.08	-0.15	-0.74	0.36
Navicula	-0.27	0.04	-0.20	0.04
Epithemia	-0.04	0.23	-0.08	-0.15
Large Diatoms	-0.07	-0.14	-0.02	0.12
Cyanobacteria	-0.07	-0.52	0.07	0.39
Small Diatoms	-0.04	-0.58	0.09	0.42
Oocystis	0.03	-0.08	0.05	0.05
Pediastrum	0.20	-0.12	0.17	0.03
Other Green	0.89	0.06	0.63	-0.25
Scenedesmus	1.14	-0.20	0.87	-0.13

Table 3. Results from a linear regression of ln-transformed gross primary production (GPP) from day 33 in the field experiment against the standardized abundance of algal groups based on cell counts. Coefficients ± SE for each algal group are shown. Asterisks (*) indicate p-values < 0.05.

Algal group	Coefficient	$F_{1,22}$	P-value
Oocystis	-0.217 ± 0.039	30.17	< 0.001*
Other Green	-0.078 ± 0.051	2.35	0.140
Pediastrum	-0.038 ± 0.040	0.92	0.347
Large Diatoms	0.015 ± 0.038	0.16	0.695
Small Diatoms	0.027 ± 0.048	0.31	0.582
Cyanobacteria	0.034 ± 0.039	0.74	0.400
Epithemia	0.050 ± 0.037	1.82	0.191
Navicula	0.080 ± 0.042	3.70	0.067
Fragilariaceae	0.128 ± 0.054	5.55	0.028*
Scenedesmus	0.196 ± 0.067	8.49	0.008*

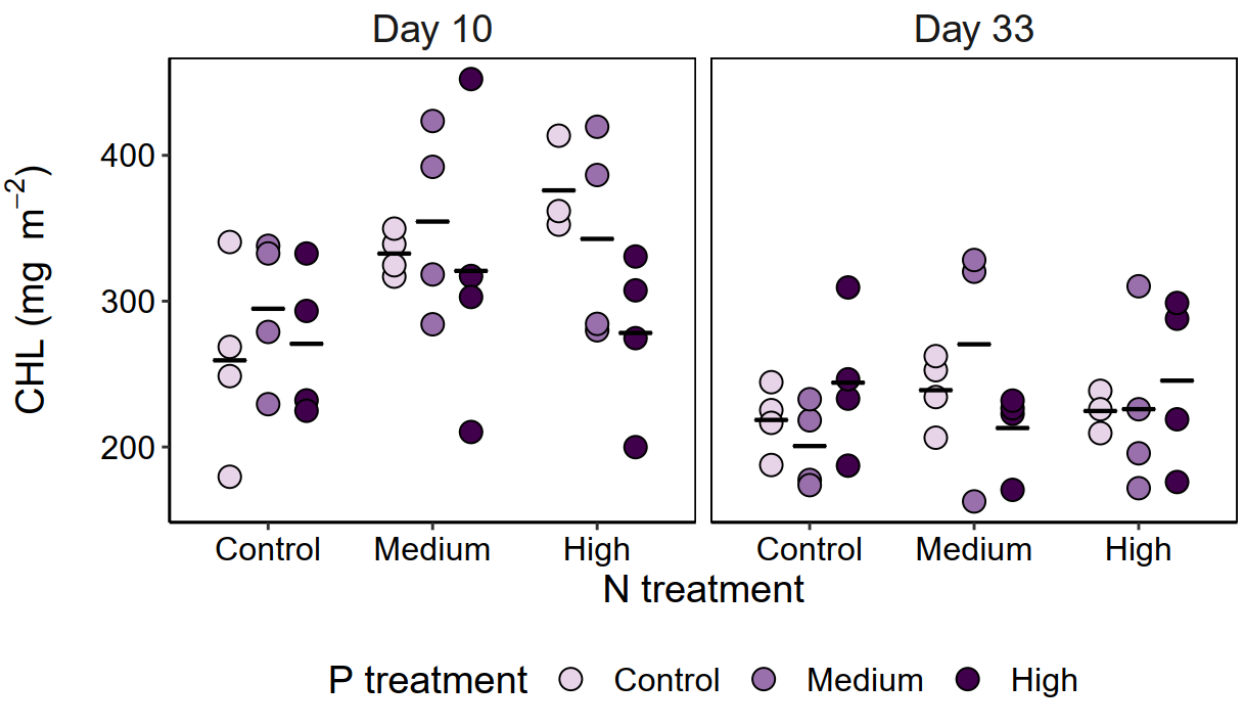

Figure 1. Benthic chlorophyll-*a* (CHL) concentrations measured from the field experiment on day 10 and day 33, across 3 levels of nitrogen (N) enrichment and 3 levels of phosphorus (P) enrichment. Each point is a measurement from an individual microcosm. Horizontal lines represent the mean for each N × P treatment combination.

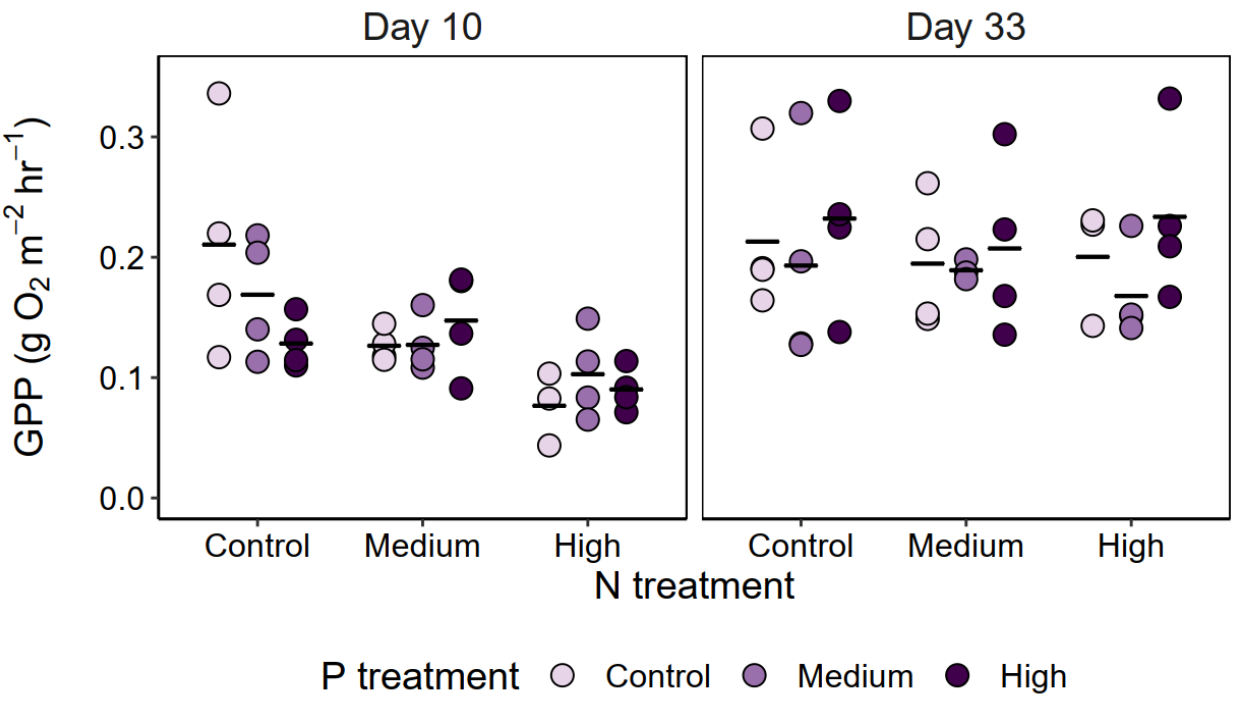

Figure 2. Gross primary production (GPP) measured from the field experiment on day 10 and day 33, across 3 levels of nitrogen (N) enrichment and 3 levels of phosphorus (P) enrichment. Each point is a measurement from an individual microcosm. Horizontal lines represent the mean for each N × P treatment combination.

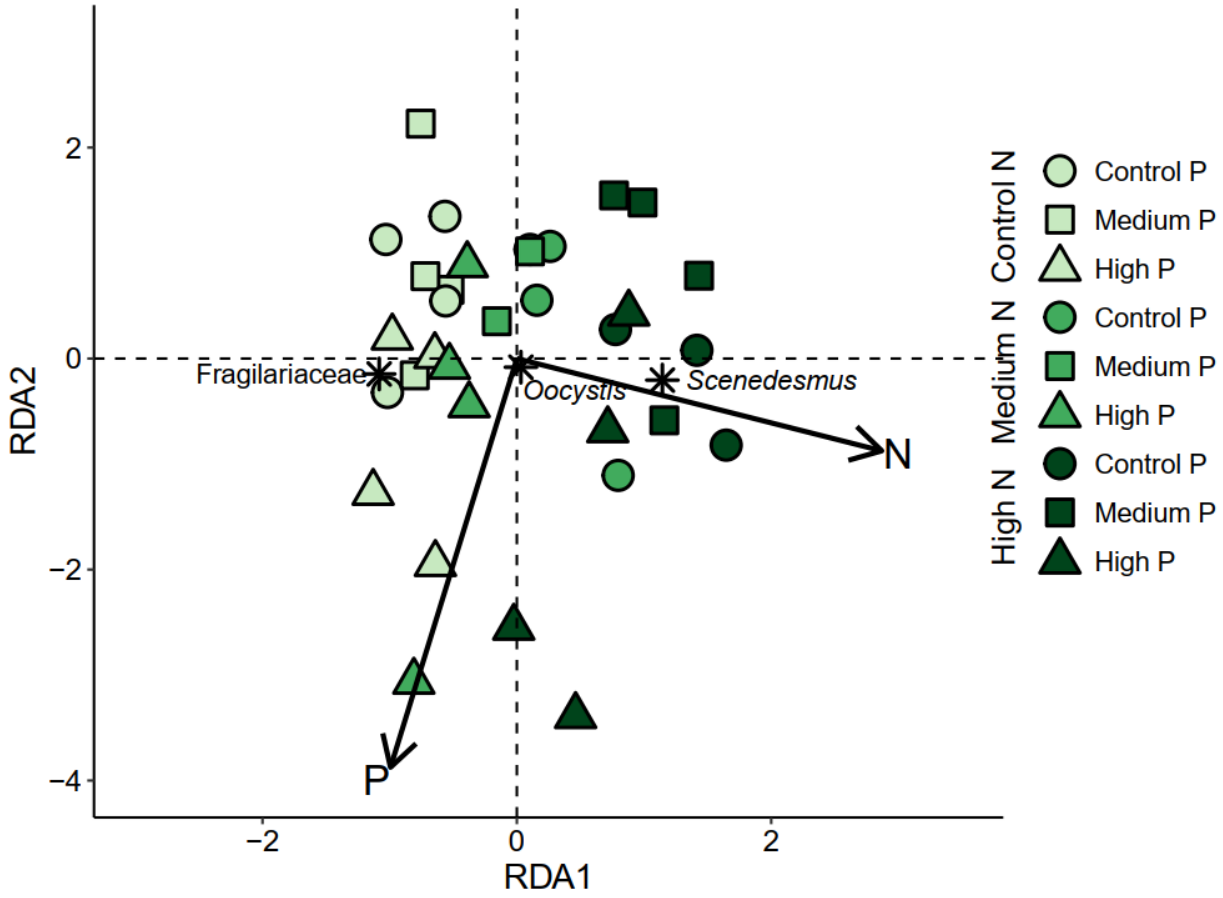

Figure 3. Ordination from redundancy analysis (RDA) comparing algal composition across the nitrogen (N) and phosphorus (P) treatments. Coordinates of RDA taxa scores for axes 1 and 2 were used to plot Fragilariaceae, *Scenedesmus*, and *Oocystis* (shown as asterisks); these taxa are labeled because they were the groups with the most negative response to N, most positive response to N, and strongest effect on GPP, respectively. Vectors show the biplot scores for constraining variables N and P.

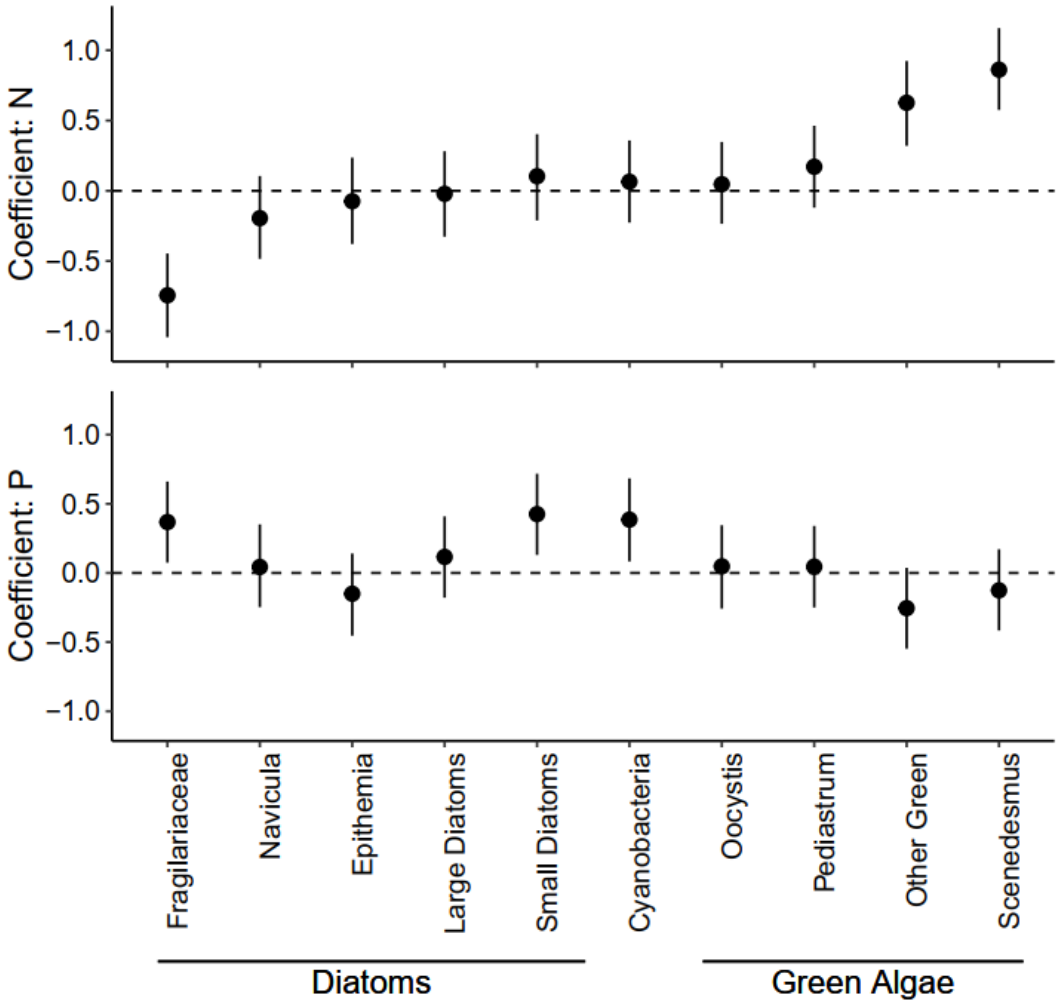

Figure 4. Coefficients showing the specific responses of each algal group to nitrogen (N) and phosphorus (P) treatments. Coefficients for each algal group (the main effect for N or P plus the interaction term for each algal group) are the mean coefficients obtained by parametric bootstrapping over 2000 simulations. Error bars show the 95% confidence interval of each coefficient.

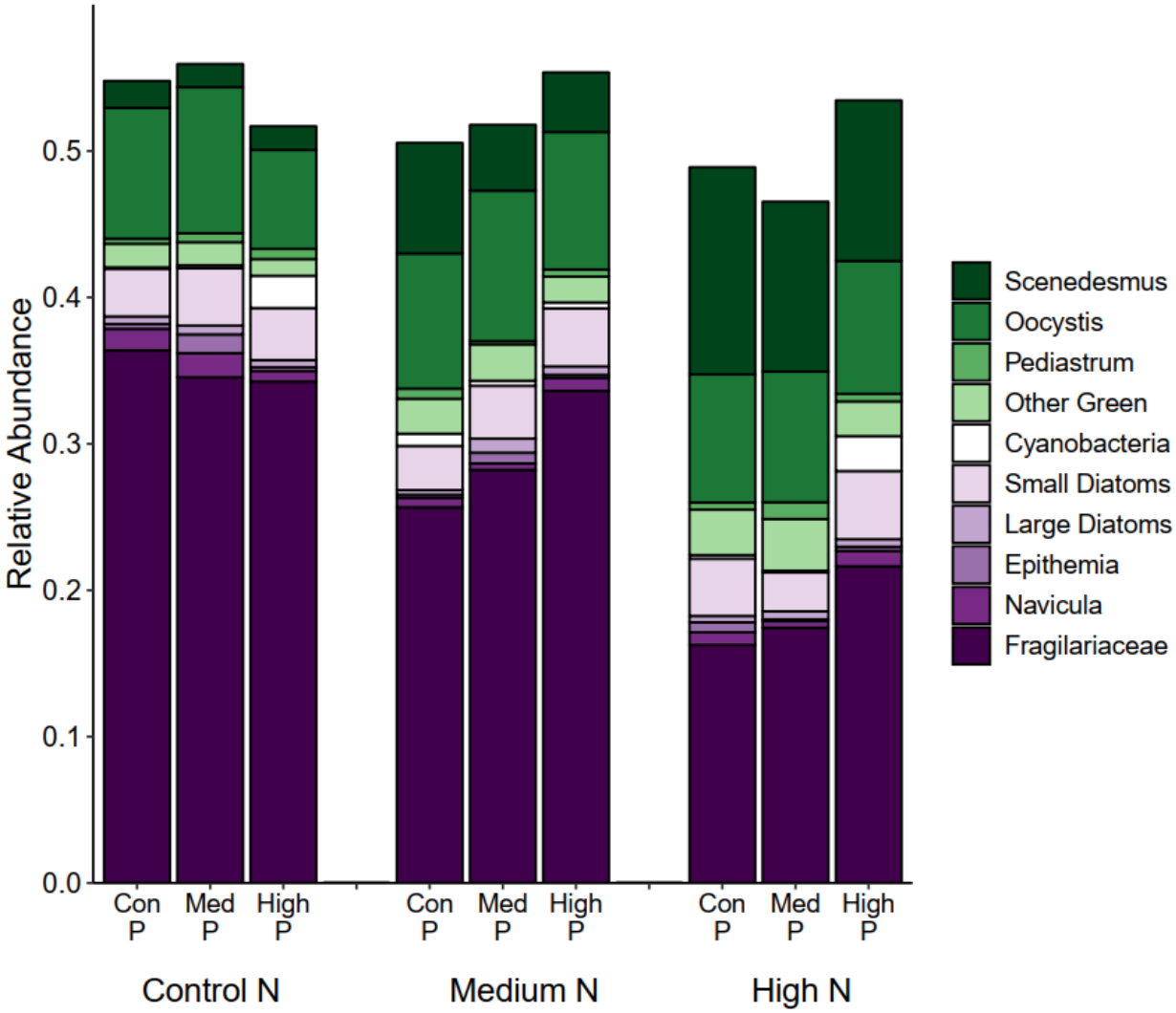

Figure 5. Mean relative abundance of the algal groups across nitrogen (N) and phosphorus (P) treatments. The sums of bars do not reach 1.0 because some of the counted cells were dead and only living cells are shown.

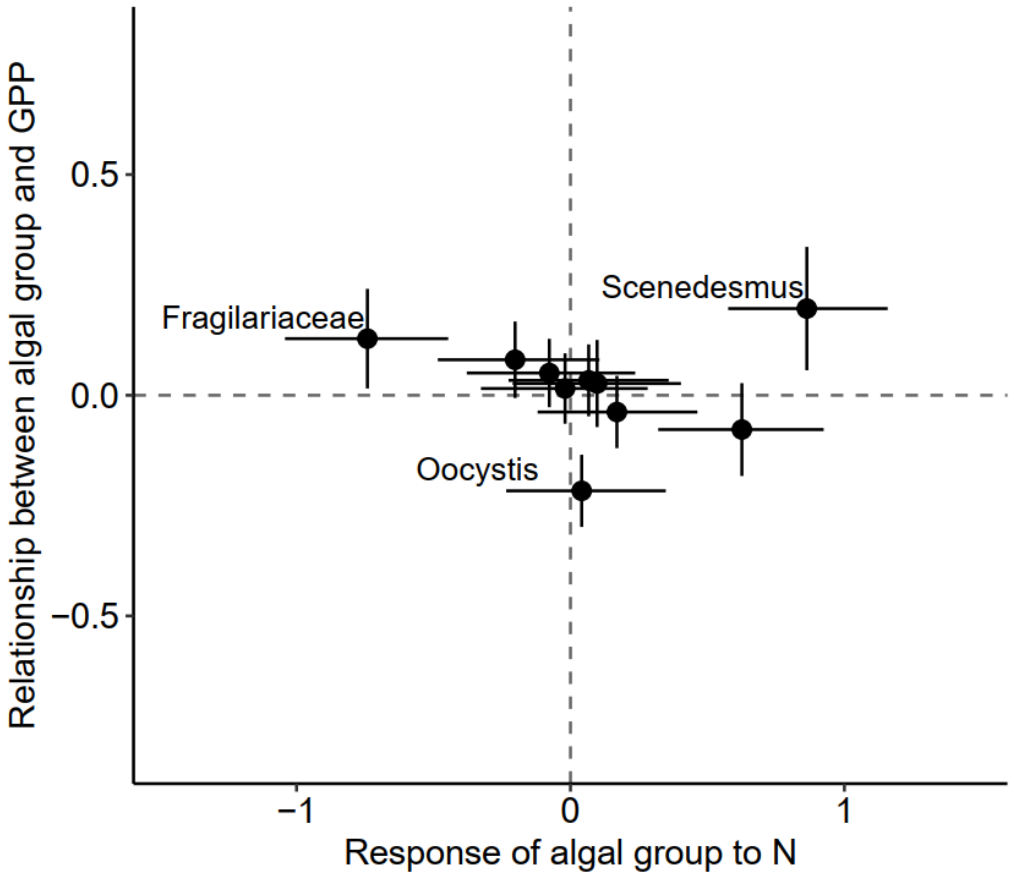

Figure 6. The response of each algal group to nitrogen (N) enrichment is plotted against the coefficient of each algal group from the regression of ln-transformed gross primary production (GPP) from day 33 against the standardized abundances of the different algal groups based on cell counts. Error bars show the 95% confidence intervals of each algal group-specific coefficient. Fragilariaceae, *Scenedesmus*, and *Oocystis* are labeled because they were the groups with the most negative response to N, most positive response to N, and strongest relationship with GPP, respectively.

Appendix A: Supplementary methods and results for Chapter 1

Supplemental Information: METHODS

Laboratory Experiment

The setup for the accompanying laboratory experiment was similar to that of the field experiment. We followed the same protocols for sediment collection (conducted 23 July 2016), sediment preparation, and nutrient treatments as described for the field experiment (see METHODS: *Experimental Design and Sampling*). The experiment was started on 26 July 2016 (day 0). After receiving nutrient-enriched agar and sieved sediment, lab microcosms were filled with water from a well located 0.5 km from the lakeshore and known to be nutrient poor (0.16 µM PO4, 1.07 µM NO3, 0.214 µM NH_4). This water source was used largely for convenience, but we acknowledge that the difference in background nutrient concentrations in the field and lab experiments may have influenced our results. Microcosms were placed into polystyrene coolers and kept outside the Mývatn Research Station during the experiment. We originally attempted to manipulate light conditions in the field (which later proved infeasible due to methodological constraints), which is why we manipulated light availability in the lab experiment with shade cloth (4 layers and 0 layers), such that microcosms were in either 'shaded' or 'unshaded' coolers. HOBO Pendant data loggers (Onset Computer Corporation, Bourne, Massachusetts, USA) were placed on the top of each cooler to track light and temperature throughout the experiment and indicated a 40% reduction of light between the two light conditions. The shaded treatment resulted in similar average light conditions (185 µmol m^{-2} s^{-1}) to estimated light levels in Mývatn at 1.75 m depth (151 µmol m^{-2} s^{-1}), while the average light levels of the unshaded treatment were higher (312 µmol m^{-2} s^{-1}) than estimated *in situ* 1.75 m depth light levels. The average air temperature during the lab experiment (12.6 ºC) was similar to the average lake water

temperature (11.6 ºC) during this time period. Each nutrient treatment had three replicates for each light condition. On days 4 and 21 we measured CHL, NEP, RESP, and GPP using the same methods as the field experiment.

Statistical Analysis

Like the field experiment, we used linear mixed models (LMMs) to examine effects of N and P enrichment, as well as shading treatment, on ln-transformed CHL, GPP, NEP, and RESP. For each response variable, we included fixed effects of N, P, and shading treatments, time, and all two-way interactions and random effects of cooler and microcosm identity. Methods for model fitting and p-value reporting are identical to the field experiment (METHODS: *Statistical Analysis*).

In an effort to investigate the linkage between biomass and production, we used additional LMMs to examine the effects of CHL on GPP. We used ln-transformed GPP as the response variable, ln-transformed CHL, time, and their interaction as fixed effects, and rack and microcosm identity as random effects. Separate LMMs were performed for the field and lab experiments.

Supplemental Information: RESULTS

Lab Experiment

In the lab experiment, we saw no effect of nutrients or shading on CHL (Table S6). When non-significant interactions were removed, CHL increased through time in the lab experiment (Table S6; Fig S3). The results indicate a limited response of CHL to nutrient enrichment.

GPP was influenced by a significant N × time interaction in the lab experiment (Table S6). Like the field experiment, GPP was lower when N was added compared to control N

treatments on day 4, but this inhibitory effect did not persist to the end of the experiment; on day 21, GPP was highest in the medium N treatments, while the control and high N treatments were similar (Fig S4). There was also a significant N × P interaction (Table S6); GPP was lower in microcosms with N amendment relative to controls, but in microcosms with high N enrichment, the presence of P lessened the negative effects of N (Fig S4). However, the high-N, high-P treatment did not result in higher GPP relative to the control-N, control-P treatment. NEP generally followed these trends with a marginally significant N × P interaction (Table S6). NEP significantly increased during the lab experiment (Table S6) and was highest in the medium N treatments at the end of the experiment (Fig S4). There was no effect of the shading treatment on GPP or NEP (Table S6). RESP in the lab experiment largely paralleled the GPP results with significant N × time and N × P interactions (Table S6; Fig S4). On day 4, there was decreased RESP in N-enriched microcosms, but by day 21 RESP was highest in the medium N treatments. Additionally, the negative effect of N was less apparent in the presence of P (Table S6; Figure S4). RESP was also influenced by a shading × time interaction (Table S6); there was higher RESP in the shaded microcosms early in the experiment, but this effect did not persist to day 21.

Relationship between biomass and production

There was no significant effect of CHL on GPP in the field or lab experiment (Table S5). Results from this analysis for the lab experiment mirrored the GPP models with the experimental treatments as fixed effects and demonstrated an increase in GPP through time (Table S5).

Table S1. Release rates of nitrogen (N) and phosphorus (P) were measured from a set of microcosms (separate from the field and lab experiments) with three nutrient treatments: control (no N, no P), medium (0.05 M NH_4Cl, 0.0065 M KH_2PO_4), high (0.5 M NH_4Cl, 0.033 M KH_2PO_4), n=2 for each. Note that a separate method was used to quantify nutrient concentrations on day 14 than days 4 and 32, preventing temporal comparisons of N and P release rates.

		Flux (µmol cm^{-2} h^{-1})	
Day	Treatment	N	P
4	Control	0.0027	0.0013
	Medium	0.3067	0.0067
	High	1.5734	0.0151
14	Control	0.0178	0.0025
	Medium	0.1319	0.0072
	High	0.4691	0.0167
32	Control	0.0386	0.0028
	Medium	0.0402	0.0032
	High	-0.3903	0.0538

Table S2. Mean ± SD chlorophyll-a concentrations (mg m^{-2}) from day 10 and 33 measurements in the field experiment for all nitrogen (N) and phosphorus (P) treatment combinations. n=4 for all treatment combinations except for the high-N, control-P combination (n=3) for days 10 and 33 and the medium-N, medium-P combination (n=3) for day 33.

		Control N	Medium N	High N
Day 10	Control P	259 ± 66.2	333 ± 14.7	376 ± 32.9
	Medium P	295 ± 51.1	355 ± 64.4	343 ± 71.1
	High P	271 ± 51.5	321 ± 99.7	278 ± 57.0
Day 33	Control P	219 ± 23.6	239 ± 24.6	225 ± 14.6
	Medium P	201 ± 29.4	270 ± 93.3	226 ± 60.4
	High P	244 ± 50.4	213 ± 28.5	245 ± 58.2

Table S3. Mean ± SD gross primary production (GPP) measurements (g O_2 m^{-2} h^{-1}) from day 10 and 33 measurements in the field experiment for all nitrogen (N) and phosphorus (P) treatment combinations. n=4 for all treatment combinations except for the high-N, control-P combination (n=3) for days 10 and 33 and the medium-N, medium-P combination (n=3) for day 33.

		Control N	Medium N	High N
Day 10	Control P	0.210 ± 0.094	0.126 ± 0.014	0.077 ± 0.030
	Medium P	0.169 ± 0.050	0.127 ± 0.023	0.103 ± 0.037
	High P	0.128 ± 0.021	0.147 ± 0.043	0.090 ± 0.018
Day 33	Control P	0.213 ± 0.064	0.195 ± 0.054	0.200 ± 0.050
	Medium P	0.193 ± 0.091	0.189 ± 0.008	0.168 ± 0.039
	High P	0.232 ± 0.079	0.207 ± 0.073	0.234 ± 0.070

Table S4. Type II F-tests from linear mixed models testing for the main effects of N, P, and time on ln-transformed chlorophyll-*a* (CHL), gross primary production (GPP), net ecosystem production (NEP), and respiration (RESP) in the field experiment. Statistical outputs for interaction terms from the Type II F-tests are omitted because they are identical to those from the Type III F-tests, which are shown in Table 1 of the main text.

	Factor	F	df_{num}	$df_{residual}$	P-value
CHL	*N*	2.89	2	23.79	0.075
	P	0.29	2	24.17	0.751
	Time	48.24	1	29.52	< 0.001
	N × *P*				
	N × *Time*				
	P × *Time*				
GPP	*N*	6.62	2	23.76	0.005
	P	0.22	2	25.36	0.805
	Time	41.32	1	29.63	< 0.001
	N × *P*				
	N × *Time*				
	P × *Time*				
NEP	*N*	14.07	2	23.70	< 0.001
	P	1.16	2	23.73	0.330
	Time	48.93	1	29.62	< 0.001
	N × *P*				
	N × *Time*				
	P × *Time*				
RESP	*N*	1.03	2	23.70	0.374
	P	0.50	2	23.74	0.616
	Time	20.96	1	29.62	< 0.001
	N × *P*				
	N × *Time*				
	P × *Time*				

Table S5. Type III F-tests from linear mixed models testing for the main and interactive effects of ln-transformed chlorophyll-a (CHL) and time on ln-transformed gross primary production (GPP) in the field and laboratory experiments.

Field Experiment	F	df_{num}	$df._{residual}$	P-value
ln-CHL	0.13	1	35.97	0.716
time	0.04	1	41.44	0.843
ln-CHL × *time*	0.39	1	42.63	0.534
Lab Experiment				
ln-CHL	0.65	1	99.70	0.423
time	6.41	1	98.86	0.013
ln-CHL × *time*	0.43	1	98.45	0.514

Table S6. Type II and type III F-tests from linear mixed models testing for the main and interactive effects of N, P, light, and time on ln-transformed chlorophyll-*a* (CHL), gross primary production (GPP), net ecosystem production (NEP), and respiration (RESP) in the laboratory experiment. Statistical outputs for interaction terms from the Type II F-tests are omitted because they are identical to those from the Type III F-tests.

		Type II				**Type III**			
	Factor	F	df_{num}	$df_{residual}$	P-value	F	df_{num}	$df_{residual}$	P-value
CHL	*N*	0.91	2	38.30	0.411	0.23	2	80.47	0.794
	P	2.46	2	38.29	0.099	1.39	2	80.53	0.254
	Light	1.99	1	1.68	0.315	0.01	1	62.40	0.930
	Time	7.88	1	48.00	0.007	0.09	1	48.00	0.768
	N × *P*					0.28	4	38.30	0.887
	N × *Light*					0.72	2	38.31	0.492
	N × *Time*					0.30	2	48.00	0.739
	P × *Light*					0.38	2	38.31	0.685
	P × *Time*					0.89	2	48.00	0.418
	Light × *Time*					0.06	1	48.00	0.812
GPP	*N*	11.12	2	37.74	< 0.001	17.05	2	78.54	< 0.001
	P	0.65	2	37.89	0.530	0.13	2	79.25	0.877
	Light	1.66	1	1.69	0.346	1.55	1	56.00	0.218
	Time	242.87	1	47.25	< 0.001	26.40	1	47.47	< 0.001
	N × *P*					4.29	4	37.94	0.006
	N × *Light*					0.05	2	37.85	0.955
	N × *Time*					8.17	2	47.36	0.001
	P × *Light*					0.12	2	38.06	0.886
	P × *Time*					0.64	2	47.36	0.534
	Light × *Time*					1.43	1	47.39	0.237
NEP	*N*	3.41	2	37.98	0.043	4.26	2	81.23	0.017
	P	0.62	2	38.11	0.544	0.20	2	81.91	0.819
	Light	0.42	1	1.35	0.611	2.81	1	68.51	0.098
	Time	288.10	1	47.17	< 0.001	30.58	1	47.39	< 0.001
	N × *P*					2.68	4	38.16	0.046
	N × *Light*					0.08	2	38.07	0.927
	N × *Time*					1.23	2	47.28	0.303
	P × *Light*					0.24	2	38.29	0.784

	P × *Time*					0.14	2	47.28	0.867
	Light × *Time*					3.62	1	47.32	0.063
RESP	*N*	18.63	2	37.54	< 0.001	19.25	2	78.50	< 0.001
	P	0.25	2	37.67	0.779	0.34	2	78.97	0.713
	Light	2.21	1	1.97	0.277	17.49	1	12.28	0.001
	Time	101.07	1	47.23	< 0.001	12.97	1	47.44	0.001
	N × *P*					3.35	4	37.72	0.019
	N × *Light*					0.08	2	37.65	0.924
	N × *Time*					12.66	2	47.33	< 0.001
	P × *Light*					0.09	2	37.81	0.913
	P × *Time*					0.58	2	47.33	0.564
	Light × *Time*					27.18	1	47.36	< 0.001

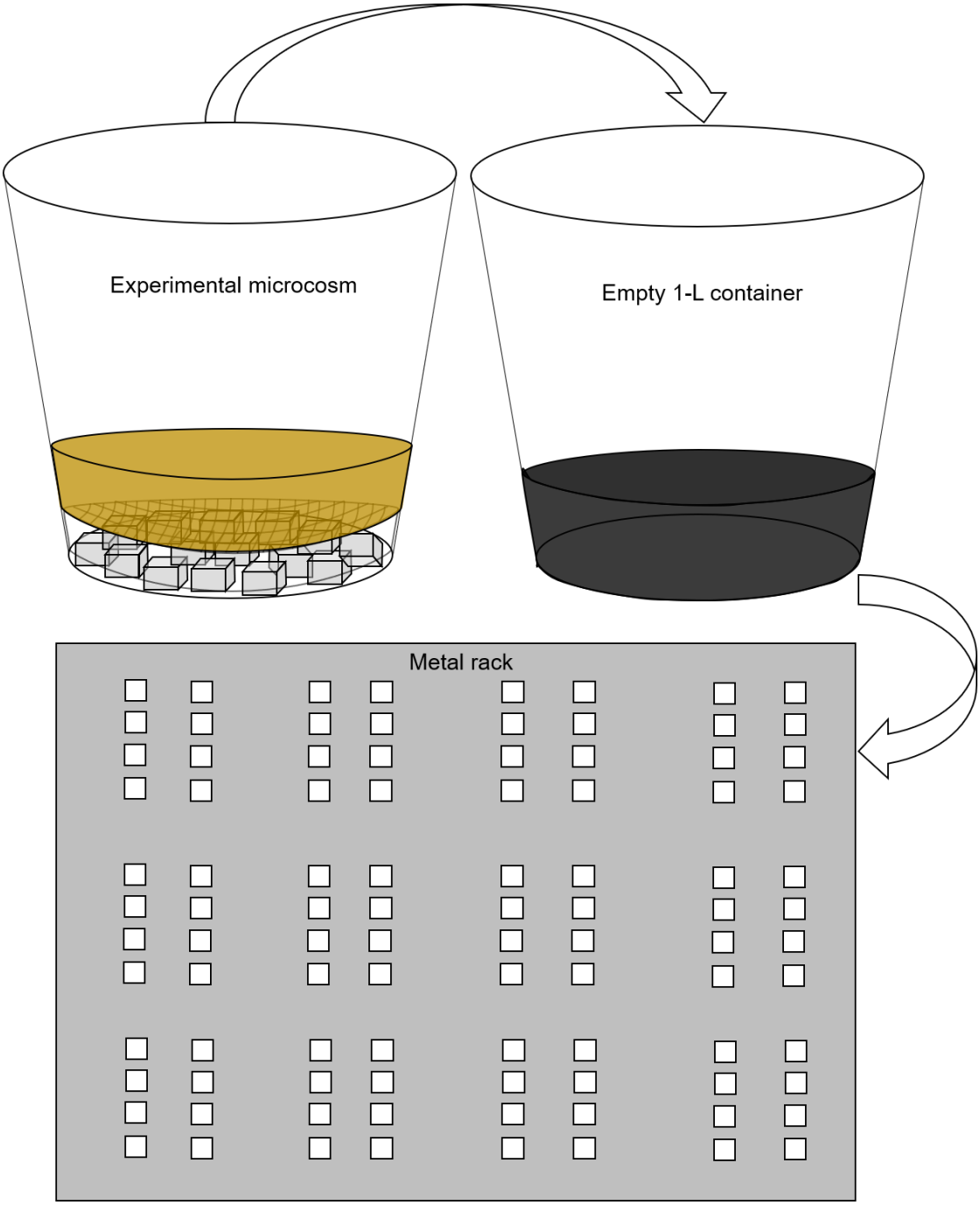

Fig S1. Diagram representing the setup for the in-lake experiment (not shown to scale). Experimental microcosms consisted of clear 1-L containers. A mesh-covered, plastic ring that fit snugly against the inner walls of the microcosms was placed over the agar cubes. Sediment overlaid the agar and mesh-covered ring. Separate empty 1-L plastic containers were securely zip-tied to holes in the metal rack (12 per rack), and the bottoms of these empty containers were covered with layers of black tape to shade the bottoms of the experimental microcosms. The experimental microcosms were placed into the empty 1-L containers and the metal racks were deployed to the bottom of the lake at 1.75 m depth for the duration of the experiment.

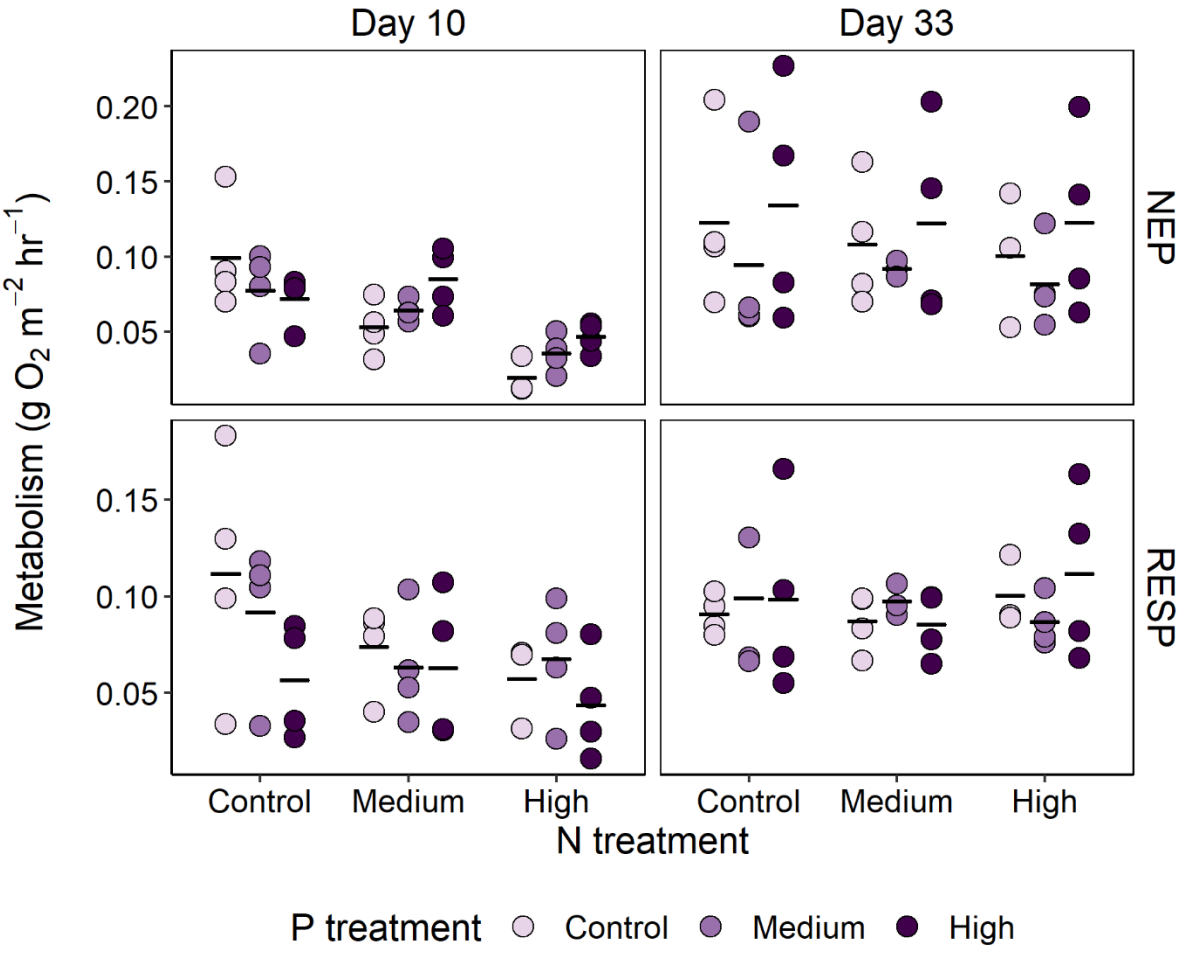

Fig S2. Net ecosystem production (NEP) and community respiration (RESP) measured from the field experiment on day 10 and day 33 across 3 levels of nitrogen (N) enrichment and 3 levels of phosphorus (P) enrichment. Each point is a measurement from an individual microcosm. Horizontal lines represent the mean for each N × P treatment combination.

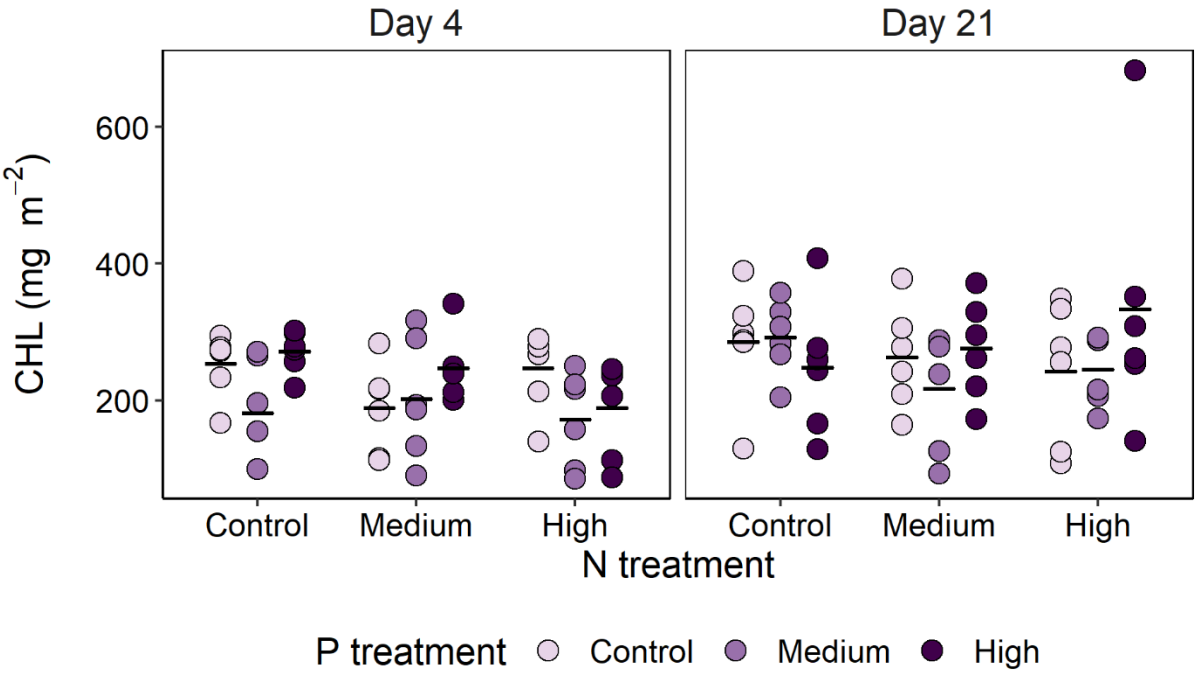

Figure S3. Benthic chlorophyll-*a* (CHL) concentrations measured from the laboratory experiment on day 4 and day 21 across 3 levels of nitrogen (N) enrichment and 3 levels of phosphorus (P) enrichment. Each point is a measurement from an individual microcosm. Horizontal lines represent the mean for each N × P treatment combination.

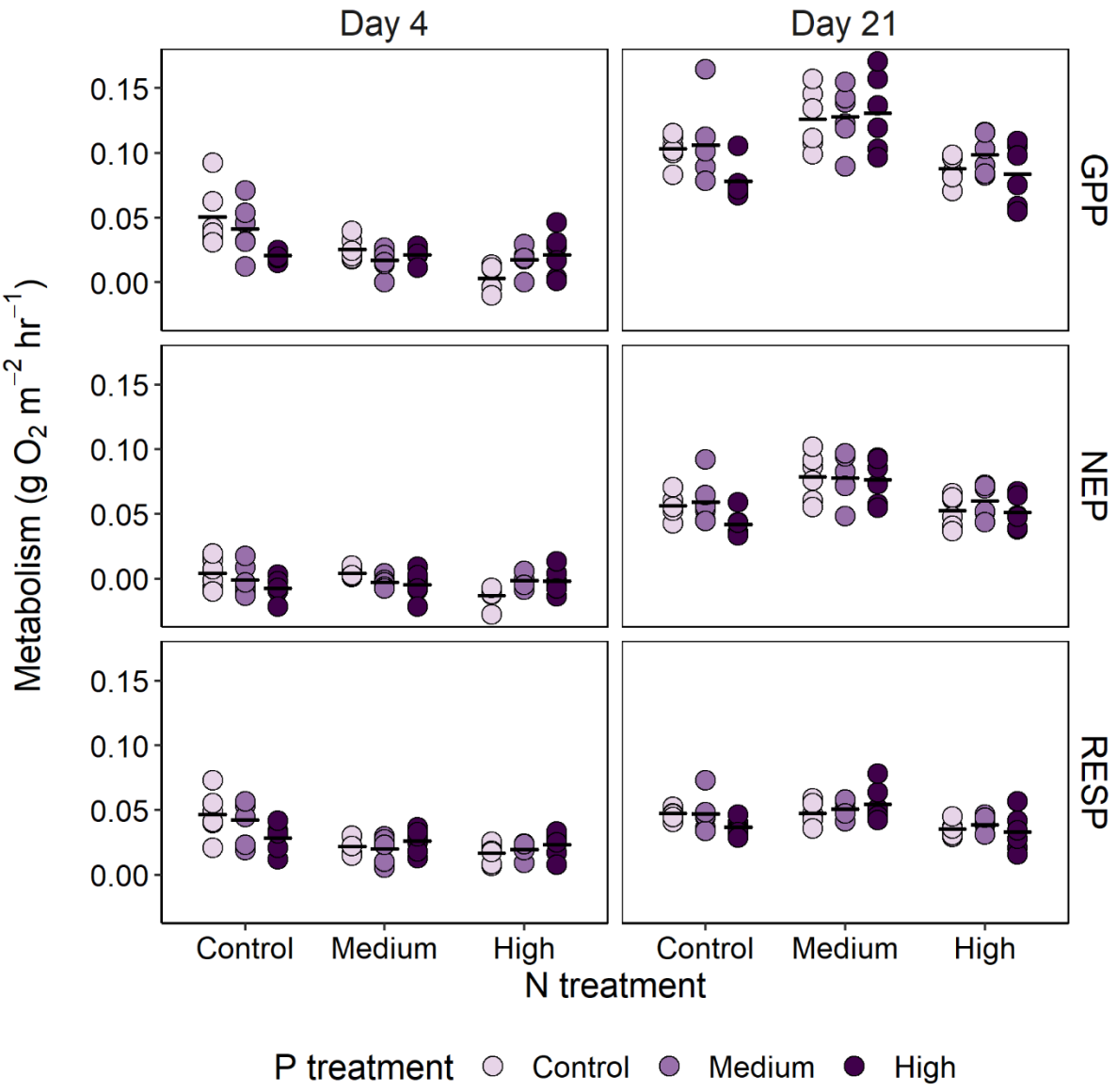

Figure S4. Gross primary production, net ecosystem production (NEP) and community respiration (RESP) measured from the laboratory experiment on day 4 and day 21 across 3 levels of nitrogen (N) enrichment and 3 levels of phosphorus (P) enrichment. Each point is a measurement from an individual microcosm. Horizontal lines represent the mean for each N × P treatment combination.

CHAPTER 2

Intra- and interannual shifts in the partitioning of benthic and pelagic primary production in a shallow lake

Status: in preparation for *Limnology and Oceanography*

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Running head: shallow lake production partitioning

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ABSTRACT

The relative contributions of benthic and pelagic primary production affect ecosystem processes, but studies documenting natural variation in the partitioning of production (i.e., autotrophic structure) are relatively rare. This study partitions primary production into benthic and pelagic components in shallow Lake Mývatn for seven summers (2012-2018). Using biweekly benthic gross primary production (GPP) measurements for a site in the center of the lake, we estimated the irradiance at which benthic production is half its maximum rate, and accounting for ambient light conditions, we determined maximum productivity rates (P_{max}) for each sample date. In 2018, we measured pelagic photosynthesis-irradiance relationships to determine a pelagic half-saturation light level and to quantify the relationship between pelagic P_{max} and chl-a, which we applied to biweekly chl-a data to estimate associated pelagic P_{max} rates. With these parameters and corresponding incident light and water clarity data, we estimated in situ benthic and pelagic GPP. By increasing light attenuation, phytoplankton contributed to declines in estimated benthic GPP. These effects were strongest in three years with dense cyanobacteria blooms, in which the benthic fraction of estimated total production declined from >95% to <22%. Benthic P_{max} rates varied through time, but we found no biotic or abiotic factors that explained this variation. Nonetheless, our results suggest that benthic P_{max} rates influence how a shift toward pelagic-dominated autotrophic structure affects total production. Overall, Mývatn exhibited substantial variation in autotrophic structure, which may affect energy flow and highlights the value of ecological monitoring for understanding temporal dynamics in the balance between benthic and pelagic production.

INTRODUCTION

Benthic and pelagic primary producers jointly constitute whole-lake primary production, and their relative contributions to total production influence ecosystem function. Interactions among lake bathymetry, morphology, water clarity, and nutrient concentrations influence the partitioning of whole-system primary production into benthic and pelagic components (i.e., "autotrophic structure," sensu Higgins et al. 2014) (Sand-Jensen and Borum 1991; Vadeboncoeur et al. 2008). Some static lake characteristics (e.g., basin shape) can create general patterns in the partitioning of primary production; for example, deep, steep-sided lakes with little illuminated benthic habitat are commonly pelagic-dominated, while shallow lakes with expansive, flat littoral surfaces are more likely to support high benthic production (Wetzel 2001; Vadeboncoeur et al. 2008; Cremona et al. 2016). However, a lake's autotrophic structure is not necessarily fixed through time, because variation in physicochemical factors (e.g., water clarity, nutrient concentrations) can alter the relative contribution of benthic and pelagic primary producers to total production, with these shifts potentially affecting ecosystem function. For example, long-term (i.e., decadal) and short-term (i.e., seasonal) shifts in the partitioning of primary production can impact aquatic food webs by altering consumer reliance on either benthic or pelagic derived carbon (Chandra et al. 2005; Turschak et al. 2014; Stewart et al. 2017). In particular, shallow lakes may experience dramatic shifts in autotrophic structure alternating between regimes characterized as 'clear' or 'turbid' states, dominated by benthic or pelagic production, respectively (Scheffer et al. 1993; Scheffer and van Nes 2007; Genkai-Kato et al. 2012).

The competitive relationship between pelagic (phytoplankton) and benthic (periphyton, epipelon, macrophytes) primary producers largely mediates the partitioning of production and

temporal variation in autotrophic structure (Jäger and Diehl 2014). Specifically, epipelic benthic algae living on the sediment surface can limit phytoplankton production by reducing nutrient release rates from the sediment to the water column (Carlton and Wetzel 1988; Hansson 1988; Dodds 2003), and phytoplankton negatively affects benthic algal production by reducing their access to light via shading (Sand-Jensen and Borum 1991; Hansson 1992). Thus, stimulation of pelagic primary producers may consequently decrease benthic primary production and the contribution of benthic algae to whole-lake production (Vadeboncoeur et al. 2003, 2008). These interactions may influence future patterns of autotrophic structure in many lakes, given the global concern of eutrophication and associated increases in the frequency of cyanobacteria blooms (Taranu et al. 2015).

In addition to in situ light availability, parameters governing the relationship between photosynthesis and irradiance (i.e., the P-I curve) affect realized rates of benthic primary production. Photosynthetic rates increase linearly at low light levels and plateau at a maximum rate of production (P_{max}) at saturating light intensities. P_{max} can be thought of as a synoptic metric representing the "functional abundance" of primary producers that depends on cell density, taxonomic composition, maximum photosynthetic rates of constituent species, cellular investment in photosynthetic pigments, and other factors that affect primary production. Variation in P_{max} can potentially affect lake autotrophic structure. For instance, when modeling autotrophic structure across a eutrophication gradient, Vadeboncoeur et al. (2008) found that benthic production was <50% of total production when benthic P_{max} was low, while benthic production typically dominated when P_{max} was intermediate or high, even in mesotrophic conditions. P_{max} may particularly influence the benthic contribution to total production in

shallow lakes, in which saturated light levels occur over a larger benthic area than in deeper lakes.

While the direct reduction in benthic light availability is a well-documented negative effect of phytoplankton on in situ benthic primary production (Hansson 1992; Vadeboncoeur et al. 2001, 2003), it is less clear how phytoplankton shading affects P_{max}. An effect of phytoplankton on benthic P_{max} would imply that processes determined by phytoplankton (e.g., extended periods of shading) alter the functional abundance of benthic primary producers. Several studies describe associations between ambient light conditions in lakes and benthic P-I parameters (Vadeboncoeur and Lodge 2000; Liboriussen and Jeppesen 2003; Vadeboncoeur et al. 2014; Brothers et al. 2016; Devlin et al. 2016). For example, epipelic and epilithic P_{max} rates may decline with increasing depths (Jónsson 1992; Vadeboncoeur and Lodge 2000), and high periphyton P_{max} rates are associated with clear-water systems (Brothers et al. 2016). The link between benthic P_{max} rates and the ambient light environment may be partly mediated by light's effect on algal biomass; previous studies have shown that experimental shading can reduce chl-a concentrations in epipelic (Hansson 1988) and lotic periphyton (Steinman et al. 1990) communities, and other studies have found positive relationships between benthic P_{max} rates and primary producer biomass (Boston and Hill 1991; Hill and Boston 1991; Dodds et al. 1999). Together, these results suggest that if shifts in the benthic light environment have the potential to decrease algal biomass, they may thereby affect P_{max} rates.

In addition to P_{max}, phytoplankton may affect other parameters of the P-I curve. Algae can increase intracellular chl-a content in response to low light (Falkowski and LaRoche 1991; Lowe and Pillsbury 1995), thereby increasing efficiency of benthic algae without necessarily changing biomass. Thus, while P_{max} may markedly affect the relative contribution of benthic

algae to total production at saturating light levels, the photosynthetic efficiency of benthic algae at low light levels may influence their contribution in periods of strong light-limitation (e.g., during dense phytoplankton blooms).

Overall, a shift to a pelagic-dominated autotrophic structure could affect the relative contribution of benthic algae to total production by influencing their P-I curve parameters in addition to directly reducing benthic light availability. However, characterizations of benthic photosynthesis in lakes are lacking (Vadeboncoeur et al. 2008; Brothers et al 2016), especially measurements that capture temporal variability in the photosynthetic response of benthic algae to light (but see Jónsson 1992; Liboriussen and Jeppesen 2003; Malkin et al. 2010; Daniels et al. 2015; Vesterinen et al. 2016). Thus, the effect of phytoplankton on benthic algal primary production potential (i.e., their photosynthetic capacity based on P-I parameters) remains an understudied dimension of benthic-pelagic coupling.

While lake autotrophic structure may vary temporally, capturing these dynamics is often challenging. Whole-lake and mesocosm experiments (Björk-Ramberg and Ånell 1985; Vadeboncoeur et al. 2001; Vasconcelos et al. 2016), observational studies (Liboriussen and Jeppesen 2003; Althouse et al. 2014), and models (Vadeboncoeur et al. 2008; Genkai-Kato et al. 2012; Higgins et al. 2014) are useful for investigating factors and mechanisms that shift the partitioning of benthic and pelagic production. However, temporal, comparative measurements of benthic and pelagic primary production under natural conditions are relatively rare (Liboriussen and Jeppesen 2003; Althouse et al. 2014; Cremona et al. 2016), which limits understanding which types of lakes are likely to experience temporally dynamic autotrophic structure and under what conditions these shifts are likely to occur. Furthermore, ongoing consequences of global change to lake ecosystems (e.g., eutrophication, increased dissolved

organic carbon (DOC) loading, invasive species establishment) (Smith and Schindler 2009; Havel et al. 2015; Solomon et al. 2015; Taranu et al. 2015) will likely affect the partitioning of benthic and pelagic primary production (Vadeboncoeur et al. 2001; Karlsson et al. 2009; Higgins et al. 2014). Knowing the natural variation in autotrophic structure could provide insight into how these ongoing stressors will affect lakes in the future (Althouse et al. 2014).

Lake Mývatn is well-suited for examining temporal variability in the partitioning of benthic and pelagic production and the ecological consequences of shifting autotrophic structure. Mývatn's shallow depth supports high epipelic primary production on the surface of its nutrient-rich sediments, with benthic algae generally contributing a majority of total production (Ólafsson 1979a, Einarsson et al. 2004). However, Mývatn is also naturally eutrophic, with high external loading from nutrient-rich groundwater springs (Ólafsson 1979a) and potentially high internal loading of nutrients from the sediment (Gíslason et al. 2004). The resulting high nutrient availability in the pelagic habitat implies the potential for phytoplankton dominance (Jäger and Diehl 2014). Cyanobacteria blooms in Mývatn occur with variable timing and intensity, such that blooms spread throughout much of the lake in some years, while the lake maintains a fairly clear-water state in other years (Einarsson et al. 2004). Additionally, Phillips (2020) modeled whole-ecosystem metabolism (integrating both pelagic and benthic habitats) over multiple years in Mývatn and showed that cyanobacterial blooms were strongly linked to the photosynthetic potential of the ecosystem. Because benthic pathways dominate energy flow in Mývatn's food web (Jónasson 1979), shifts in autotrophic structure may have important ecosystem-wide consequences.

In this study we partition primary productivity into benthic and pelagic components, which complements recent results on whole-ecosystem metabolism from the same time period

and study site in Mývatn (Phillips 2020). We analyze monitoring data associated with benthic and pelagic primary producers in Mývatn, including regular measurements of benthic gross primary production (GPP) and phytoplankton biomass (chl-a), for seven summers (2012-2018). Using these long-term data, as well as supplemental measurements to characterize pelagic primary production, we examine temporal trends in benthic and pelagic maximum primary production rates (P_{max}). We also estimate in situ benthic and pelagic GPP for the seven summers based on these P_{max} rates and corresponding incident irradiance data and light attenuation coefficients. Our primary objectives were to 1) determine which biotic and abiotic factors contribute to variation in benthic P_{max} rates, 2) examine competitive effects of phytoplankton on benthic production, and 3) investigate how variation in benthic P_{max} rates may influence the overall effect that a shift in autotrophic structure has on total (i.e., summed benthic and pelagic) GPP.

METHODS

Study system and monitoring site

Lake Mývatn, located in northeast Iceland (65°35’ N, 17°00’ W), is naturally eutrophic and shallow. The main basin, which includes our study site, has a mean depth of 2.3 m and maximum depth of 4.2 m (Jónasson 1979). During the ice-free periods, there is no stratification in the main basin, with wind action during the summer helping to maintain mixing (Ólafsson 1979b). The lake’s water renewal time is 27 d (Ólafsson 1979b), and the River Laxá forms its major outlet. Inputs to the lake include nutrient-rich springs along the eastern shore and the Grænilækur River draining the spring-fed Lake Grænavatn; together these annually contribute 1.5, 1.4, and 340 g m^{-2} y^{-1} for N, P, and Si, respectively, to Mývatn (Ólafsson 1979a). Water

column nutrient availability varies temporally and spatially; though representative concentrations near the center of the lake are 91 mg m^{-3}, 16 mg m^{-3} and 381 mg m^{-3} for total dissolved nitrogen, total dissolved phosphorus and silicon, respectively (Ólafsson 1979a). Internal loading also contributes to nutrient availability, with estimated diffusive nutrient fluxes from the sediment of 0.13 g P m^{-2} y^{-1} and 1.89 g N m^{-2} y^{-1} for PO_4^{3-} and NH_4^+, respectively (Gíslason et al. 2004). However, actual nutrient flux rates into the overlying water are lower than the diffusive rates within the sediment, with a net flux from overlying water toward the sediment in much of the summer, likely due to uptake by epipelic algal communities (Thorbergsdóttir and Gíslason 2004).

Benthic primary production substantially contributes to Mývatn's whole lake production. Much of the lake has a soft-bottomed substrate, and epipelic diatoms (especially Fragilariceae) are major contributors to benthic primary production. Mats of filamentous green algae (*Cladophora glomerata* and *Aegagropila linnaei*) can also cover substantial portions of the main basin, though their spatial extent is highly variable on a decadal time scale (Einarsson et al. 2004). While the lake's shallow depth allows for high benthic light availability, intermittent phytoplankton blooms and wind-driven sediment resuspension reduce water column transmissivity and create a variable light environment for benthic algae (Jónasson and Adalsteinsson 1979; Phillips et al. 2019). Cyanobacteria blooms are a natural occurrence in Mývatn, and the water column's fairly low N:P ratios and high phosphorus concentrations are favorable for nitrogen fixing taxa such as *Dolichospermum* (Ólafsson 1979a). Annual *Dolichospermum* spp. blooms typically develop in Mývatn's smaller northern basin (8.2 km^2 compared to 29.1 km^2 in the main basin), which is connected to the main basin via a narrow passage. However, the spatial extent and intensity with which the blooms spread throughout the

main basin are highly variable across years (Einarsson et al. 2004). Thus, in some years, cyanobacteria blooms are unnoticeable in Mývatn's main basin, while in other years, dense blooms cover much of the lake (Einarsson et al. 2004). In addition to cyanobacteria, other phytoplankton include chlorophytes (*Oocystis*, *Sphaerocystis*, *Pediastrum*), diatoms (Fragilariacea), and chrysophytes (*Uroglena*, *Dinobryon*), with the abundances of these taxa often varying spatially across the lake (Jónasson and Adalsteinsson 1979; Dickman et al. 1993; Bartrons et al. 2015).

Our study incorporates data collected from a site located near the center of Mývatn's main basin (depth 3.3 m) that has been routinely monitored since 2012 (Ives 2013). Approximately every two weeks from late May to late August during 2012-2018, physicochemical and biological data were collected. Water column profiles of dissolved oxygen (DO) concentrations and temperature (ProODO Probe, YSI, Yellow Springs, Ohio, USA) and photosynthetically active radiation (PAR; µmol photons m^{-2} s^{-1}) (Li-192 Quantum Underwater Sensor, Li-COR, Lincoln, Nebraska, USA) were recorded at 0.5 m intervals. For each sampling day, we determined a light attenuation coefficient (k_D) by regressing ln-transformed PAR against water column depth. Hourly irradiance data were recorded by a local weather station, and from 2013 onward, illuminance data above the lake's surface were recorded every 15-30 min by a light logger (HOBO Pendant data logger, Onset Computer Corporation, Bourne, Massachusetts, USA) affixed to the top of a buoy at the study site. Using the routinely collected PAR measurements from water column profiles and corresponding incident light data, we estimated PAR levels just below the water's surface ($PAR_{water\ surface}$) based on weather station irradiance ($PAR_{water\ surface}$ = 1.76225 × downwelling irradiance, df = 46, t = 19.89, R^2 = 0.89, $p < 0.001$) and light logger illuminance ($PAR_{water\ surface}$ = 0.0084519 × lux, df = 53, t = 14.07, R^2 = 0.78, p

< 0.001). A sonde (Hydrolab DS5X, Hach, Loveland, Colorado, USA) at the monitoring site also recorded phycocyanin levels (a cyanobacteria pigment) and other physicochemical data.

On each sample date, water (3-5 analytical replicates) was collected from a single homogenized, integrated water column sample and filtered onto Whatman glass fiber filters (GF/F) for subsequent analysis of pelagic chl-a. We also collected five replicate sediment cores and used sediment from the surface of each core to determine benthic chl-a concentrations and water content. There were minor modifications during the study regarding the depth of sediment that was collected for these analyses; in 2014, 2015, and the first three sampling dates of 2016, the top 1.5-cm layer of sediment was used, but the top 0.75-cm layer was used otherwise. The filtered water and sediment samples were frozen, and chlorophyll-a was then extracted in 100% methanol for 24 h in the dark and read on a fluorometer (AquaFluor, Turner Designs, San Jose, California, USA), using acidification to correct for phaeophytin concentrations. We accounted for water content of the sediment by dividing benthic chl-a concentrations by the proportion dry weight of the sediment sample. Benthic chl-a and pelagic chl-a data were aggregated by date for subsequent analysis.

Benthic primary production

A central objective of this study was to estimate in situ benthic and pelagic primary production, and this required information about the parameters underlying P-I curves for benthic and pelagic primary producers. Benthic GPP was routinely measured during the study, with these measures spanning a range of ambient light levels driven by the conditions on each sampling day. Below, we describe how we used these routine GPP measurements that occurred across a range of light levels to accomplish two goals: 1) determine a light level corresponding the half-

saturation point of benthic primary production and 2) estimate the P_{max} associated with each GPP measurement based on this parameter.

On routine sample dates, we measured benthic GPP, which we refer to as the 'observed' GPP (GPP_{obs}). Ten intact sediment cores (separate from those for benthic chl-a) were collected in clear, acrylic tubes (height: 50 cm, inner diameter: 5 cm) and incubated either without any shading (n=6 but n=5 in 2012) or in complete darkness (n=4 but n=5 in 2012), achieved by applying opaque, black PVC coverings to the acrylic tubes. During incubations, acrylic tubes were sealed airtight with opaque rubber stoppers, which prevented gas exchange with the atmosphere and the surrounding lake water (sensu Phillips et al. 2019). Tubes were suspended from a floating rack, such that tubes were incubated 0.5 m below the water's surface. Incubations were generally conducted in a nearshore bay rather than at the routine monitoring site, but water temperatures at the monitoring site and incubation location are similar. We measured DO concentrations (mg O_2 L^{-1}) from the water in the tubes at the start and end of the incubation period with ProODO probes. Before taking the initial and final DO readings, we gently stirred the water in the tubes with the DO probe. We calculated net ecosystem production (NEP) and ecosystem respiration (ER) as the hourly change in O_2 concentrations (mg O_2 L^{-1} h^{-1}) in the light and dark tubes, respectively. Under the assumption that ER is equal in the light and dark, we calculated GPP_{obs} for each sample date as the summed magnitudes of the mean NEP and mean ER rates. We converted GPP_{obs} to areal units by multiplying the volumetric metabolic rates by the water column heights in the tubes. This approach ignores any hypothetical production by phytoplankton in the ~20 cm of water overlying the sediment, but phytoplankton are not observable in this water layer. Temperature affects respiration and primary production rates (Davison 1991; Phillips 2020), so it should also affect NEP; however, because this method for

measuring metabolism does not allow us to quantify GPP directly, it precludes us from fully correcting for temperature's effects on NEP. In other words, while we can correct ER for temperature because it is measured directly, it is difficult to fully understand how temperature is affecting our NEP measurements, as we cannot directly relate ambient temperature to GPP.

GPP_{obs} measurements were conducted on days spanning a range of ambient light conditions. We fit a Michaelis-Menten type equation $GPP = \frac{P_{max} \cdot I}{K + I}$ (eq 1) (sensu Turner et al. 1983; Jónsson 1992; Daniels et al. 2015) to this set of data, in which I is the in situ light level, P_{max} is the maximum rate of primary production, and K is the light level at which GPP = ½ P_{max}. We fit eq (1) to GPP_{obs} and the mean PAR level that was recorded at 0.5 m during each incubation (Fig 1) using nonlinear least squares with the nls() function in R. This produced a K parameter for benthic primary production ($K_{benthic}$ = 110.86 µmol m^{-2} s^{-1}), which we assume to be fixed through time. Using the fixed $K_{benthic}$ parameter, we then estimated a P_{max} value for each sample date based on the measured GPP_{obs} and light conditions during the incubation, such that P_{max} was allowed to vary through the study's duration. For each GPP_{obs}, we calculated P_{max} given the light during the incubation ($I_{incubation}$) by algebraically rearranging eq (1): $P_{max} = GPP_{obs} \cdot \frac{I_{incubation} + K_{benthic}}{I_{incubation}}$.

Despite the utility of our approach, it also has certain limitations. Namely, we assume $K_{benthic}$ is a fixed value, such that benthic primary producers reach half their photosynthetic maximum at the same light level across time. In our case, it is not possible to determine temporal variation in this parameter, or other parameters influencing the sub-saturated portion of the P-I curve (e.g., the initial slope in the low-light region of the P-I curve using a hyperbolic-tangent function), as we did not conduct incubations across a gradient of light for each sample date. A number of functions characterize the relationship between photosynthesis and light (Jassby and

Platt 1976); we chose the Michaelis-Menten curve in part because it eased the algebraic calculation of P_{max} from GPP_{obs} and incubation light levels. As the incubations were performed only 0.5 m below the water's surface, many GPP_{obs} values should well-approximate maximum benthic photosynthetic rates under light saturating conditions. However, incubations sometimes occurred on days which likely did not provide adequate light for the onset of light-saturation (e.g., overcast conditions) (Fig 1), and our approach allowed us to estimate benthic P_{max} rates and subsequently estimate in situ benthic GPP across the study duration, thereby taking advantage of the full temporal extent of the data.

Pelagic primary production

As for benthic primary production, estimating in situ pelagic primary production across years was a central objective of the study. To accomplish this, we measured pelagic primary production across a range of light levels and fit Michaelis-Menten P-I curves to these data (see below). This approach allowed us to 1) estimate a half-saturation irradiance level for pelagic primary producers (i.e., the light level at which photosynthetic rates are half their maximum value and analogous to our approach for benthic primary production) and 2) determine an empirical relationship between pelagic chl-a concentrations and P_{max}. The latter objective allowed us to translate our routinely measured chl-a measurements into corresponding pelagic P_{max} rates.

In the summer of 2018, we performed pelagic DO incubations across a range of light levels. The incubations were conducted at three sites (our study site in the main basin and two sites in the north basin), with three incubations per site, in June, July, and August. While these incubations include samples from outside our study site, these measurements are relevant

because phytoplankton blooms in the north basin can later enter the main basin. We measured pelagic metabolism in clear, acrylic tubes (33-cm tall and 5-cm diameter) filled with homogenized, integrated water column samples. We manipulated light availability in individual tubes by either completely darkening them (using PVC shades as in the benthic incubations), partially excluding light by wrapping them with different layers of mosquito netting, or allowing them to receive full ambient light (i.e., leaving the tubes as they were). Each light treatment had 3 replicates (except for full light, where n =5), though samples were occasionally lost in the field. Additionally, for one north basin site, there were only full light and full dark treatments for all three incubations. To determine the extent of light reduction imposed by the shade treatments, we measured PAR outside and inside the acrylic tubes with their shading treatments and stoppers. During the incubations, we estimated PAR availability within the tubes at their 0.5 m depth based on the average incoming light levels recorded by an irradiance logger, the light attenuation coefficient (k_D) from light profiles recorded during the incubation, and the light reduction capacity of each shade treatment. Pelagic incubation methods were otherwise analogous to those for the benthic incubations described above. We measured chl-a concentrations from water in the full light tubes and used the mean concentration for each site-date combination for subsequent P-I curve fitting (below).

We fit a single P-I model to pelagic production data from all sites and sample dates. We included the mean chl-a concentration for each site-date combination as a covariate, such that chl-a concentrations were assumed to drive variation in P_{max} across incubations. Assuming that GPP is equal to the summed magnitudes of NEP and ER, we fit a modified eq (1): $NEP = \frac{P_{max} \cdot chl \cdot I}{I + K_{pelagic}} - ER$, to our data using the nls() function in R (Fig 2). This produced a fixed K parameter ($K_{pelagic}$ = 46.09 µmol m^{-2} s^{-1}) and allowed P_{max} rates to vary as driven by chl-a

concentrations. The fitted model described the relationship between chl-a (μg L^{-1}) and P_{max} (g O_2 m^{-2} h^{-1}) (estimate ± SE = 0.0032 ± 0.0003; t = 10.06, df = 120, $p < 0.001$), which we subsequently used to estimate P_{max} rates corresponding to our pelagic chl-a concentrations from 2012-2018. We investigated the potential for photoinhibition by fitting our data to the model presented in Platt et al. (1980) and found little suggestion of photoinhibition.

We acknowledge several limitations associated with our approach in handing the pelagic primary production measurements. Specifically, we assume $K_{pelagic}$ and the relationship between pelagic P_{max} and chl-a are fixed across multiple years, even though these characteristics were based on a limited number of incubations within a single summer. Temporal variation in taxonomic composition and abiotic conditions (e.g., nutrients, light) may affect the accuracy of extending the estimated $K_{pelagic}$ parameter, as these factors can influence photosynthetic potential and efficiency (Richardson et al. 1983; Litchman and Klausmeier 2008; Edwards et al. 2015). While it would be possible to allow $K_{pelagic}$ to vary across our date-site combinations from our supplemental incubations in 2018, we opted for fixing this value to mirror our approach as for the benthic data. Furthermore, we could not account for how $K_{pelagic}$ might vary through 2012-2018 based on the routine chl-a concentrations. Our approach relies on chl-a concentrations to explain pelagic P_{max} rates, but if pigment composition varies taxonomically, this may affect interpretation of the relationship between chl-a concentrations and P_{max} rates. Nonetheless, previous studies have approximated pelagic P_{max} rates from phytoplankton biomass (Guildford et al. 1994; Vadeboncoeur et al. 2008). As *Dolichospermum* was the dominant phytoplankton group when we conducted our supplemental measurements in 2018, our estimates of pelagic primary production may be particularly affected when other algal divisions (i.e., chlorophytes) were dominant.

Estimating in-situ benthic and pelagic production

We estimated in situ benthic and/or pelagic primary production for dates with corresponding benthic GPP_{obs} or pelagic chl-a data, which provided us with respective benthic and pelagic P_{max} rates as described above. We calculated in situ light (I_z) at a given depth, z, based on the measured light attenuation coefficient (k_D) and light at the water's surface (I_0) as $I_z = I_0 e^{-k_D \cdot z}$. For 2012-2016 and May 2017 we used weather station irradiance data to estimate I_0 (i.e., $PAR_{water\ surface}$), and for the remainder of 2017 and all of 2018, during which the irradiance data were unavailable, we used the illuminance data from the logger affixed to a buoy at the sampling site (see "Methods: Study system and routine monitoring site"). To factor out day-to-day differences in irradiance, we calculated hourly surface water PAR levels across a five-day window (including the sample date, the two preceding days, and the two following days). We assumed constant $K_{benthic}$ and $K_{pelagic}$ values across all years of the study. For benthic production, we estimated in situ hourly GPP (across a 24-hr period comprised of the mean hourly light levels for the corresponding five-day window) based on eq (1): $GPP = \frac{benthic\ P_{max} \cdot I_{3.3}}{K_{benthic} + I_{3.3}}$, where $I_{3.3}$ is the estimated irradiance at 3.3 m. To estimate hourly in situ pelagic GPP, we integrated eq (1) through the maximum depth at which phytoplankton were assumed to occur (z_{chl}; see below): $\int_0^{z_{chl}} \frac{P_{max} \cdot I_0 \cdot e^{-k_D \cdot z}}{K_{pelagic} + I_0 \cdot e^{-k_D \cdot z}}\ dz$. We then summed hourly benthic and pelagic GPP to estimate daily in situ GPP for each habitat. Because photosynthetic parameters for benthic and pelagic algal communities likely vary spatially across Mývatn [e.g., due to differences in depth (Devlin et al. 2016) or heterogeneous phytoplankton distribution (Bartrons et al. 2015)], we present in situ GPP rates for a hypothetical column of the lake extending above our 3.3-m deep sampling site rather than extrapolating our estimates across varying depths of the lake. We

present our P_{max} and estimated in situ GPP rates in units of carbon by assuming a photosynthetic quotient of 1, corresponding to a 1:1 molar ratio for O_2 and C (Thorbergsdóttir and Gíslason 2004). To present the partitioning of primary production into benthic and pelagic components, we calculated the benthic fraction of total primary production (BF) at our study site by dividing daily benthic GPP by the sum of daily benthic and pelagic (i.e., total) GPP (Vadeboncoeur et al. 2008; Higgins et al. 2014).

As routine pelagic chl-a measurements were obtained from integrated water column samples, we lack quantitative information about the vertical distribution of phytoplankton. While phytoplankton distribution through the water column may be heterogeneous due variation in the physical environment (e.g., vertical gradients in temperature, nutrients, light), the distribution of chl-a into distinct layers is unlikely for well-mixed lakes (Klausmeier and Litchman 2001; Longhi and Beisner 2009). We assume a uniform distribution of chl-a throughout Mývatn's water column, such that z_{chl} = 3.3 m (see above). Pelagic production is therefore assumed to decrease with depth due to light attenuation through the water column (as our P-I equation does not incorporate photoinhibition). However, previous measurements of pelagic production at Mývatn have shown that in certain conditions (i.e., sunny days) photosynthetic rates can increase to the depth that provides optimal light conditions (Jónasson and Adalsteinsson 1979). During thick blooms at Mývatn, cyanobacteria can concentrate within the upper layer of the water column and subsequently affect the vertical limit for phytoplankton production (Jónasson and Adalsteinsson 1979). In the appendix, we present temporal comparisons of benthic and pelagic P_{max} and estimated in situ benthic and pelagic GPP under the assumption that phytoplankton chl-a is evenly distributed through the euphotic zone (Fig S1, S2), whose lower boundary occurs where the in situ light level is 1% of surface PAR (Reynolds 2006). The euphotic zone typically

exceeded our study site depth of 3.3 m, but 2014 and 2015 had minimum euphotic zone depths of 1.92 m and 2.02 m, respectively (Fig S3).

Error for estimated hourly in situ benthic GPP was propagated based on error in the GPP_{obs} measurement from the field incubations and error in the estimation of $K_{benthic}$. For hourly in situ pelagic GPP, error was propagated based on error in estimating $K_{pelagic}$ and the coefficient describing the relationship between P_{max} and chl-a, as well as the fact that variance in the estimates of these parameters were correlated (i.e., they were obtained from the same model fit to our data shown in Fig 2). Errors associated with hourly benthic and pelagic GPP were propagated when obtaining errors for the estimated daily GPP rates for each respective habitat. Lastly, the errors associated with daily benthic and pelagic GPP rates were propagated when calculating the error in the benthic fraction of total production.

In addition to investigating temporal trends in benthic and pelagic production, we also visually explored how variation in pelagic biomass and benthic P_{max} may influence autotrophic structure (sensu Vadeboncoeur et al. 2008). To accomplish this, we focused on five benthic P_{max} values (minimum, first quartile, median, third quartile, maximum) to summarize the variation in our data. This subset of P_{max} values encompasses variation in benthic P_{max} described by other studies (Liboriussen and Jeppesen 2003; Devlin et al. 2016); thus, our exploration might be relevant for understanding the role of benthic P_{max} in influencing autotrophic structure in other systems. We then qualitatively compared how partitioned GPP (i.e., either benthic or pelagic GPP), the combined benthic and pelagic (i.e., total) GPP, and the benthic fraction of total GPP vary across our observed range of pelagic chl-a concentrations (0.48 to 64.42 µg L^{-1}) for each of these five benthic P_{max} values. In our dataset, light attenuation coefficients were strongly related to pelagic chl-a concentrations; we empirically modeled this relationship so that k_D (m^{-1}) could

be expressed as a function of pelagic chl-a (µg L^{-1}) ($k_D = 0.92 \cdot \ln(pelagic\ chl + 9.31) - 1.73$; Fig S4), which allowed us to estimate light attenuation coefficients across the hypothetical combinations of pelagic chl-a and benthic P_{max}. We standardized the incident light conditions for this visualization using the average diel irradiance patterns (Fig S5), which consisted of mean hourly incoming PAR values across the five-day windows corresponding to our sample dates.

Time series analysis

We investigated biotic and abiotic factors contributing to variation in our measured benthic P_{max} rates. In our analysis, we included benthic P_{max} as the response variable and three predictor variables: benthic algal biomass (benthic chl-a standardized to percent sediment dry weight), temperature during the incubation in which we measured GPP_{obs}, and the cumulative benthic light availability preceding the routine incubation. We calculated cumulative benthic light availability by summing hourly benthic light levels for the sampling date and the preceding two days, such that this metric quantifies the recently experienced light environment by benthic algae. This metric incorporates variation in incident light levels over the three-day period, although we assumed the same k_D value as we measured on the routine sampling date for determining benthic light levels for the two days preceding the incubation. We included benthic chl-a in our analysis based on the hypothesis that benthic algal abundance would have a positive effect on maximum rates of primary production. We included temperature as this can affect rates of P_{max} (Davison 1991). Lastly, we included the three-day cumulative light availability under the rationale that the light environment experienced by benthic algae could potentially affect their maximum photosynthetic ability. In addition, to describe the effect of pelagic primary producers

on realized in situ benthic production, we conducted a similar analysis to the one above in which estimated daily benthic GPP was the response variable and pelagic chl-a was the predictor.

To statistically describe the effect of a specific predictor variable on our dependent variable of interest, we compared models with and without the given predictor variable using a likelihood-ratio test (LRT). Models accounted for temporal autocorrelation within years and were fit as linear models using generalized least squares with the gls() function within the 'nlme' package (Pinheiro et al. 2019) in R. Before the analyses, we first ln-transformed benthic P_{max} rates and benthic chl-a, as they had skewed distributions, and then z-transformed all variables across years by subtracting the respective variable's mean from each observation and dividing by that variable's standard deviation. All analyses were performed in R version 3.6.1 (R Core Team 2019).

RESULTS

Ambient conditions across years

Across all sampling dates, the average water column temperature at 1-m depth was 12.1 °C. The water column light environment was variable, with observed light attenuation coefficients ranging from 0.18 to 2.39 m^{-1}. Years generally began with relatively low light attenuation coefficients (mean k_D was 0.45 m^{-1} across sample dates in May and June), but k_D increased in the latter parts of some summers (Fig 3). These periods of low water clarity are associated with increased pelagic chl-a concentrations, which also showed intra and inter-annual variation (Fig 3). Based on water column phycocyanin data and visual observations, the increases in pelagic chl-a concentrations during 2014, 2015, and 2018 can be attributed to cyanobacteria blooms of *Dolichospermum* spp. (Fig 3).

Benthic and pelagic P_{max} rates

The pelagic P_{max} rates estimated from pelagic chl-a data were generally much lower than benthic P_{max} rates measured using ecosystem metabolism incubations (Fig 4, S1), suggesting that the capacity for benthic primary producers to contribute to total primary production typically exceeds that of phytoplankton. The magnitude of pelagic P_{max} was often only 1-10% of the corresponding benthic P_{max}. However, pelagic P_{max} approached or exceeded benthic P_{max} rates during the cyanobacteria blooms in 2014 and 2015 (Fig 4).

Benthic P_{max} varied within years, and we tested whether certain abiotic and biotic factors contributed to these trends. We did not find a significant influence of benthic chl-a (LRT: χ^2 = 0.26, df = 1, p = 0.609), the temperature at which the metabolism incubation was performed (LRT: χ^2 = 0.05, df = 1, p = 0.823), or the cumulative light levels for the time period preceding the incubation (LRT: χ^2 = 0.59, df = 1, p = 0.443) on benthic P_{max} (Fig S6).

Partitioning of benthic and pelagic primary production

We estimated in situ benthic and pelagic GPP using the values of P_{max} from each sample date, incident light levels, and the measured light attenuation coefficients. Estimated benthic GPP at times exceeded estimated pelagic GPP by an order of magnitude, but during periods of increased phytoplankton abundance, estimated pelagic GPP exceeded benthic GPP and dominated primary production (Fig 5, S2). There was a negative effect of phytoplankton on benthic primary production, in which estimated in situ benthic GPP was negatively associated with pelagic chl-a concentrations (LRT: χ^2 = 18.72, df = 1, p < 0.001). This result parallels the relationship between light attenuation coefficients and phytoplankton biomass (Fig S4).

The benthic fraction of total estimated production (BF) at our study site varied among and within years. In all years, benthic primary production comprised a majority of total production in early summer (late May through June), where BF ranged from 60% to 98% (Fig 6). In 2014, 2015, and 2018, there were marked declines in BF, which reached < 1% in 2014 and 2015. In contrast, BF was never < 43% in the four years without dense cyanobacteria blooms (2012, 2013, 2016, 2017) (Fig 6). Mirroring the effects of phytoplankton on in situ benthic GPP, increased water column light attenuation likely influenced the temporal patterns for BF.

We visualized how variation in benthic P_{max} and pelagic biomass (chl-a) influence total production and the partitioning of primary production (Fig 7). This visualization shows two pathways through which increasing phytoplankton abundance may alter autotrophic structure. First, as phytoplankton biomass increases, in situ benthic GPP is expected to decrease due to lower water clarity (Fig 7a). However, the increase in pelagic GPP associated with increased phytoplankton biomass at least partially compensates for the declines in benthic GPP (Fig 7b). Both the declines in benthic light availability and the increase in pelagic GPP reduce the relative contribution of benthic algae to total production (Fig 7c).

This visualization also suggests that benthic P_{max} influences autotrophic structure by affecting the point at which pelagic GPP may compensate for the declines in benthic GPP. For instance, pelagic GPP surpasses benthic GPP at a lower phytoplankton biomass when maximum benthic photosynthetic rates are low compared to when they are high (Fig 7a). Thus, benthic P_{max} may affect whether increasing phytoplankton biomass leads to a decline, maintenance, or increase in total GPP (Fig 7b). For low benthic P_{max} values, increased phytoplankton biomass resulted in maintenance or increase of total GPP (Fig 7b), although at high benthic P_{max} values (e.g., median, Q3, max), total GPP declined at low to intermediate phytoplankton biomass,

indicating that the initial increase in pelagic GPP was not fully compensating for declines in benthic GPP. Additionally, assuming our highest observed benthic P_{max} value, phytoplankton biomass would not fully compensate for the declines in benthic GPP over our observed range of pelagic chl-a concentrations (Fig 7b). Similarly, if we assume a low benthic P_{max} value (i.e., our minimum observed value), BF is expected to decline below 50% at a lower phytoplankton abundance (and correspondingly higher water clarity) than if P_{max} is assumed to be high (i.e., our maximum observed rate) (Fig 7c). In other words, benthic P_{max} in part affects the point at which autotrophic structure shifts from benthic-dominated to pelagic-dominated.

DISCUSSION

Our results demonstrate the potential for large temporal shifts in autotrophic structure within and among years in Lake Mývatn. Benthic algae substantially contributed to total GPP during relatively clear-water periods, but during cyanobacterial blooms, pelagic GPP dominated total estimated production. Similarly, a recent study that examined ecosystem metabolism (integrated across pelagic and benthic habitats) from 2012-2018 at the same study site in Mývatn showed that cyanobacteria blooms contributed to the temporal variation in whole-ecosystem production (Phillips 2020). When dense cyanobacteria blooms spread through much of the lake in 2014, 2015, and 2018, our estimated benthic fraction of total production rapidly declined. In mid-2012, the contribution of benthic algae to total production fell slightly below 50% in conjunction with an uptick in estimated pelagic GPP, which may have been caused by high densities of *Oocystis* at this time (Bartrons et al. 2015). The declines in estimated benthic GPP and its contribution to total production (i.e., BF) were strongly linked to reductions in light availability associated with increased phytoplankton abundance. Thus, while shallow Lake

Mývatn's autotrophic structure is often benthic dominated, strong competitive effects of phytoplankton on benthic primary producers can shift the partitioning of total production.

Values of benthic P_{max} ranged from 29 to 288 mg C m^{-2} hr^{-1}, representing large variation in the functional abundance of benthic primary producers. We investigated whether biotic and abiotic factors contribute to the observed variation in benthic P_{max}. We examined the importance of benthic chl-a and the recently experienced light environment because of the potential links between benthic light availability, algal biomass, and maximum photosynthetic potential. Hansson (1988) showed that when light was reduced, epipelic biomass on lake sediments declined, and in a light exclusion experiment, Steinman et al. (1990) attributed declines in periphyton carbon fixation to a reduction in algal biomass and physiological stress associated with persistently dark conditions. However, in our study both benthic chl-a and the recently experienced light environment had no significant effects on benthic P_{max}. While cyanobacteria blooms in Mývatn can block virtually all incident light from reaching the benthos, the blooms tend to be ephemeral. Thus, it is possible that water clarity may improve such that the duration or severity of shading experienced by benthic algae in Mývatn during blooms is not strong enough to elicit responses in P_{max}. Alternatively, the absence of an association between benthic chl-a and P_{max} might indicate that chl-a concentration is not a reliable surrogate for benthic algal biomass, as suggested by discrepancies between benthic chl-a and algal biovolume (Baulch et al. 2009). Furthermore, our sampling of the top 0.75-cm or 1.5-cm (2014, 2015, and early 2016) layer of sediment likely included non-photosynthetically active chlorophyll (Cyr 1998; Liboriussen and Jeppesen 2003), which may further complicate using benthic chl-a as a surrogate for the biomass of photosynthetically active algae. Thus, the absence of an association between benthic chl-a and

benthic P_{max} does not necessarily imply that P_{max} is unaffected by the biomass of photosynthetically active algae.

While we did not observe an effect of the ambient light environment on benthic P_{max}, light availability may nonetheless affect photosynthetic potential of benthic algae in other ways. For example, through photoacclimation, changes to the light environment affect cellular investment in photosynthetic pigments (Richardson et al. 1983; Falkowski and LaRoche 1991), such that per-capita investment in photosynthetic pigments may increase during light limited conditions (Devlin et al. 2016). Along these lines, Jasper and Bothwell (1986) interpreted a strong correlation between chl-a standardized P_{max} and recent ambient light conditions as due to light-induced changes in cellular chl-a content. These observations suggest that changes in the benthic light environment may affect other P-I parameters more strongly than P_{max}, and previous studies have found strong negative relationships between ambient light and the initial slope of the P-I curve (i.e., photosynthetic efficiency at non-saturating light intensities) (Hill et al. 2001; Hill and Dimick 2002; Phillips 2020). In our study, we lacked the necessary data to explore how ambient benthic light conditions could affect photosynthetic efficiency or photoacclimation, though understanding this relationship could more fully illustrate phytoplankton's effects on benthic primary producers. For instance, increased photosynthetic efficiency partially (though not fully) compensated for declines in periphyton primary production that resulted from shading in streams (Hill et al. 2001; Hill and Dimick 2002). Monitoring benthic P-I parameters (i.e., maximum photosynthetic rates and photosynthetic efficiency) both before and after (sensu Daniels et al. 2015) a shift to a pelagic-dominated autotrophic structure would help fully illustrate the different ways (i.e., changing photosynthetic parameters vs. the direct reduction in light availability) by which phytoplankton shading affects benthic primary producers.

Changes in autotrophic structure may result in compensatory production shifts such that a decrease in primary production in one habitat may be compensated for by increased production in the other, thereby maintaining constant rates of whole-system productivity (Vadeboncoeur et al. 2001; Brothers et al. 2016; Genzoli and Hall 2016). Conversely, shifts in autotrophic structure may cause declines in total production if the increased primary production in one habitat does not equal the declines in primary production in the other (Karlsson et al. 2009; Brothers et al. 2013; Higgins et al. 2014). Similar to previous studies (Genkai-Kato et al. 2012; Brothers et al. 2016), our visualization across hypothetical combinations of benthic P_{max} and pelagic algal biomass illustrates the role of benthic P_{max} in shaping the overall effect that a shift in autotrophic structure has on total GPP. We found that phytoplankton are more likely to compensate for shading-induced declines in benthic GPP if benthic P_{max} is low. In contrast, even the highest pelagic chl-a concentrations that we observed are unlikely to offset declines in benthic GPP associated with high benthic P_{max}. This conclusion, however, must be made with caution, as our maximum observed benthic P_{max} (288 mg C m^{-2} h^{-1}) was atypical for Mývatn. Nonetheless, similarly high values have been reported in other shallow lakes (Liboriussen and Jeppesen 2003). Furthermore, if phytoplankton biomass exceeded our maximum observation, pelagic production may compensate for declining benthic GPP, even if benthic P_{max} is high. Our maximum observed pelagic chl-a value (64 µg L^{-1}) corresponds to near-maximum phycocyanin reading from the seven years of monitoring (Fig 3). Thus, we likely explored compensatory effects between benthic and pelagic algae over the range of phytoplankton biomass that is representative of Mývatn, but other lakes may support higher phytoplankton biomass (e.g., Søndergaard et al. 2016). Additionally, the strong relationship between light attenuation coefficients and pelagic chl-a at Mývatn (Fig S4) directly linked benthic shading with enhanced pelagic production

(because of increased chl-a concentrations) in our visualization. However, high background light attenuation found in other systems (e.g., due to high DOC levels, turbidity, or suspended solids) would reduce overall production in benthic and pelagic habitats, and the manifested effect of background light attenuation on the partitioning of production would likely depend on factors such as lake basin shape, which influences the availability of illuminated benthic substrates (Vadeboncoeur et al. 2008). The above caveats underscore that the compensation point between benthic and pelagic production depends on the relative potential for production under the given light conditions in each habitat. Finally, we acknowledge that our observed relationship between pelagic P_{max} and phytoplankton biomass influences the point at which pelagic production compensates for declines in benthic production. Specifically, changes to the scaling between chl-a and P_{max} (i.e., due to abiotic conditions, taxonomic composition of phytoplankton) may affect how strongly phytoplankton biomass relates to pelagic P_{max} and, consequently, in situ pelagic production.

Benthic-pelagic coupling theory predicts that shallow lakes with large phosphorus pools in the sediment should be able to shift between regimes dominated by phytoplankton and periphyton (Genkai-Kato et al. 2012). Movement from one state to the other is facilitated by positive feedback loops, in which amassing phytoplankton biomass continually shades benthic algae, thereby enhancing nutrient release from the sediment and further increasing pelagic production (Liboriussen and Jeppesen 2003; Genkai-Kato et al. 2012; Jäger and Diehl 2014). Despite cyanobacteria being a natural feature of Mývatn (Einarsson et al. 2004) and the lake's relatively high P loading rates of 4.1 mg P m^{-2} d^{-1} (Ólafsson 1979a), there are no records of a multi-year shift to a turbid state caused by cyanobacteria dominance. Therefore, Mývatn does not seem prone to regime shifts between benthic and pelagic GPP domination (Scheffer et al. 1997,

Carpenter 2003). Similar year-to-year variability in the dominance of cyanobacteria has been reported in other shallow lakes (Søndergaard et al. 2016).

Several aspects of Mývatn's ecology and morphology may reduce the likelihood of regime shifts. First, Mývatn supports high densities of chironomid larvae, which may mitigate the release of nutrients to the water column by stabilizing and oxygenating the sediment through their tube-building and bioturbation behaviors (Ólafsson and Paterson 2004; Zhang et al. 2010; Holker et al. 2015). Oxygenation is particularly important for mitigating phosphate mobilization from sediments because phosphate forms precipitates with iron and other compounds in aerobic conditions (Wetzel 2001). Second, the sediment interface at Mývatn may remain oxic regardless of benthic photosynthesis because the lake is shallow and well-mixed. Therefore, even when there is shading from phytoplankton and hence little oxygen production from benthic photosynthesis, release of phosphate from the sediment may be minimal. Third, the lake's short residence time (~27 days) may also contribute to the ephemeral nature of cyanobacteria blooms. Previous studies have proposed short residence times as a factor limiting the dominance of phytoplankton in estuaries and coastal areas (Valiela et al. 1997; Cebrian et al. 2014). If *Dolichospermum* growth rates slow as the bloom progresses (e.g., due to nutrient limitation), the lake's high flushing rates may remove colonies more quickly than they grow. In summary, these biological and physical characteristics of Mývatn may decouple the positive feedback cycle underlying regime shifts between periphyton and phytoplankton dominated states, thereby preventing a long-term shift to a turbid state.

This study highlights the temporal dynamics in the partitioning of primary production between benthic and pelagic habitats in a shallow lake. Mývatn supports both high benthic and pelagic production at different times. Benthic P_{max} values spanned almost an order of magnitude,

with this variation in part influencing in situ benthic GPP and, consequently, total estimated GPP and autotrophic structure. While our visualization of hypothetical combinations of benthic P_{max} and phytoplankton biomass suggests that pelagic production may compensate for declines in benthic GPP when benthic P_{max} values are low to moderate, pelagic GPP may not compensate for the high values of benthic GPP when P_{max} values are high. Even if phytoplankton compensate for declines in benthic GPP, a shift in autotrophic structure may nonetheless be consequential in Mývatn, where benthic energy pathways dominate the food web, as well as other systems in which consumers heavily rely on benthic-derived resources (Hampton et al. 2011; Vander Zanden et al. 2011). While monitoring temporal variation in lake autotrophic structure under ambient conditions is not commonly undertaken (but see Liboriussen and Jeppesen 2003; Althouse et al. 2014), Mývatn shows how dynamic autotrophic structure can be, and that it would be misleading to characterize its autotrophic structure without accounting for temporal variation. Many factors associated with increasing anthropogenic stressors and global change (i.e., eutrophication, invasive species, DOC loading, shoreline development) may alter the partitioning of benthic and pelagic primary production, with potential consequences on energy flow, trophic interactions, and nutrient fluxes in aquatic ecosystems (Karlsson et al. 2009; Smith and Schindler 2009; Hampton et al. 2011; Althouse et al. 2014; Higgins et al. 2014; Solomon et al. 2015; Taranu et al. 2015). Thus, a baseline understanding of the temporal dynamics of autotrophic structure may assist in predicting the response of aquatic ecosystems to future change.

REFERENCES

Althouse, B., S. Higgins, and M. J. Vander Zanden. 2014. Benthic and planktonic primary production along a nutrient gradient in Green Bay, Lake Michigan, USA. Freshw. Sci. **33**:

487–498.

Bartrons, M., Á. Einarsson, R. L. G. Nobre, C. M. Herren, K. C. Webert, S. Brucet, S. R. Ólafsdóttir, and A. R. Ives. 2015. Spatial patterns reveal strong abiotic and biotic drivers of zooplankton community composition in Lake Myvatn, Iceland. Ecosphere **6**: 1–20.

Baulch, H. M., M. A. Turner, D. L. Findlay, R. D. Vinebrooke, and W. F. Donahue. 2009. Benthic algal biomass — measurement and errors. **66**: 1989–2001.

Björk-Ramberg, S., and C. Ånell. 1985. Production and chlorophyll concentration of epipelic and epilithic algae in fertilized and nonfertilized subarctic lakes. Hydrobiologia **126**: 213–219.

Boston, H. L., and W. R. Hill. 1991. Photosynthesis-light relations of stream periphyton communities. Limnol. Oceanogr. **36**: 644–656.

Brothers, S. M., S. Hilt, S. Meyer, and J. Kohler. 2013. Plant community structure determines primary productivity in shallow, eutrophic lakes. Freshw. Biol. **58**: 2264–2276.

Brothers, S., Y. Vadeboncoeur, and P. Sibley. 2016. Benthic algae compensate for phytoplankton losses in large aquatic ecosystems. Glob. Chang. Biol. **22**: 3865–3873.

Carlton, R. G., and R. G. Wetzel. 1988. Phosphorus flux from lake sediments: Effect of epipelic algal oxygen production. Limnol. Oceanogr. **33**: 562–570.

Carpenter, S. R. 2003. Regime shifts in lake ecosystems: patterns and variation. International Ecology Institute, Oldendorf/Luhe, Germany.

Cebrian, J., D. Corcoran, and J. Lartigue. 2014. Eutrophication-driven shifts in primary producers in shallow coastal systems: Implications for system functional change. **37**: 180–197.

Chandra, S., M. J. Vander Zanden, A. C. Heyvaert, B. C. Richards, B. C. Allen, and C. R. Goldman. 2005. The effects of cultural eutrophication on the coupling between pelagic

primary producers and benthic consumers. Limnol. Oceanogr. **50**: 1368–1376.

Cremona, F., A. Laas, L. Arvola, D. Pierson, P. Noges, and T. Noges. 2016. Numerical Exploration of the Planktonic to Benthic Primary Production Ratios in Lakes of the Baltic Sea Catchment. Ecosystems **19**: 1386–1400.

Cyr, H. 1998. How does the vertical distribution of chlorophyll vary in littoral sediments of small lakes? Freshw. Biol. **39**: 25–40.

Daniels, W. C., G. W. Kling, and A. E. Giblin. 2015. Benthic community metabolism in deep and shallow Arctic lakes during 13 years of whole-lake fertilization. Limnol. Oceanogr. **60**: 1604–1618.

Davison, I. R. 1991. Environmental effects on algal photosynthesis: temperature. J. Phycol. **27**: 2–8.

Devlin, S. P., M. J. Vander Zanden, and Y. Vadeboncoeur. 2016. Littoral-benthic primary production estimates: Sensitivity to simplifications with respect to periphyton productivity and basin morphometry. Limnol. Oceanogr. Methods **14**: 138–149.

Dickman, M., K. Stewart, and M. Servant-Vildary. 1993. Spatial heterogeneity of summer phytoplankton and water chemistry in a large volcanic spring-fed lake in northern Iceland. Arct. Alp. Res. **25**: 228–239.

Dodds, W. K. 2003. The role of periphyton in phosphorus retention in shallow freshwater aquatic systems. J. Phycol. **39**: 840–849.

Dodds, W. K., B. J. F. Biggs, and R. L. Lowe. 1999. Photosynthesis-irradiance patterns in benthic microalgae: variations as a function of assemblage thickness and community structure. J. Phycol. **35**: 42–53.

Edwards, K. F., M. K. Thomas, C. A. Klausmeier, and E. Litchman. 2015. Light and growth in

marine phytoplankton: Allometric, taxonomic, and environmental variation. Limnol. Oceanogr. **60**: 540–552.

Einarsson, Á., G. Stefánsdóttir, H. Jóhannesson, J. S. Ólafsson, G. M. Gíslason, I. Wakana, G. Gudbergsson, and A. Gardarsson. 2004. The ecology of Lake Myvatn and the River Laxa: Variation in space and time. Aquat. Ecol. **38**: 317–348.

Falkowski, P. G., and J. LaRoche. 1991. Acclimation to spectral irradiance in algae. J. Phycol. **27**: 8–14.

Genkai-Kato, M., Y. Vadeboncoeur, L. Liboriussen, and E. Jeppesen. 2012. Benthic-planktonic coupling , regime shifts, and whole - lake primary production in shallow lakes. Ecology **93**: 619–631.

Genzoli, L., and R. O. Hall. 2016. Shifts in Klamath River metabolism following a reservoir cyanobacterial bloom. Freshw. Biol. **35**: 795–809.

Gíslason, S. R., E. S. Eiríksdóttir, and J. S. Ólafsson. 2004. Chemical composition of interstitial water and diffusive fluxes within the diatomaceous sediment in Lake Myvatn, Iceland. Aquat. Ecol. **38**: 163–175.

Guildford, S. J., L. L. Hendzel, H. Kling, and E. Fee. 1994. Effects of Lake Size on Phytoplankton Nutrient Status. Can. J. Fish. Aquat. Sci. **51**: 2769–2783.

Hampton, S. E., S. C. Fradkin, P. R. Leavitt, and E. E. Rosenberger. 2011. Disproportionate importance of nearshore habitat for the food web of a deep oligotrophic lake. Mar. Freshw. Res. **62**: 350–358.

Hansson, L.-A. 1988. Effects of competitive interactions on the biomass development of planktonic and periphytic algae in lakes. Limnol. Oceanogr. **33**: 121–128.

Hansson, L.-A. 1992. Factors regulating periphytic algal biomass. Limnol. Ocean. **37**: 322–328.

Havel, J. E., K. E. Kovalenko, S. M. Thomaz, S. Amalfitano, and L. B. Kats. 2015. Aquatic invasive species: challenges for the future. Hydrobiologia **750**: 147–170.

Higgins, S. N., B. A. Althouse, S. P. Devlin, Y. Vadeboncoeur, and M. J. Vander Zanden. 2014. Potential for large-bodied zooplankton and dreissenids to alter the productivity and autotrophic structure of lakes. Ecology **95**: 2257–2267.

Hill, W. R., and H. L. Boston. 1991. Community development alters photosynthesis-irradiance relations in stream periphyton. Limnol. Oceanogr. **36**: 1375–1389.

Hill, W. R., and S. M. Dimick. 2002. Effects of riparian leaf dynamics on periphyton photosynthesis and light utilisation efficiency. Freshw. Biol. **47**: 1245–1256.

Hill, W. R., P. J. Mulholland, and E. R. Marzolf. 2001. Stream ecosystem responses to forest leaf emergence in spring. Ecology **82**: 2306–2319.

Holker, F., M. J. Vanni, J. J. Kuiper, and others. 2015. Tube-dwelling invertebrates: tiny ecosystem engineers have large effects in lake ecosystems. Ecol. Monogr. **85**: 333–351.

Ives, A. R. 2013. LTREB Biological Limnology at Lake Mývatn 2012-current. LTER Network Information System Repository.

Jäger, C. G., and S. Diehl. 2014. Resource competition across habitat boundaries: Asymmetric interactions between benthic and pelagic producers. Ecol. Monogr. **84**: 287–302.

Jasper, S., and M. L. Bothwell. 1986. Photosynthetic Characteristics of Lotic Periphyton. Can J Fish Aquat Sci **43**: 1960–1969.

Jassby, A. D., and T. Platt. 1976. Mathematical formulation of the relationship photosynthesis and light for phytoplankton. Limnol. Oceanogr. **21**: 540–547.

Jónasson, P. M. 1979. The Lake Mývatn ecosystem, Iceland. Oikos **32**: 289–305.

Jónasson, P. M., and H. Adalsteinsson. 1979. Phytoplankton Production in Shallow Eutrophic

Lake Mývatn, Iceland. Oikos **32**: 113–138.

Jónsson, S. G. 1992. Photosynthesis and production of epilithic algal communities in Thingvallavatn. Oikos **64**: 222–240.

Karlsson, J., P. Byström, J. Ask, P. Ask, L. Persson, and M. Jansson. 2009. Light limitation of nutrient-poor lake ecosystems. Nature **460**: 506–509.

Klausmeier, C. A., and E. Litchman. 2001. Algal games: The vertical distribution of phytoplankton in poorly mixed water columns. Limnol. Oceanogr. **46**: 1998–2007.

Liboriussen, L., and E. Jeppesen. 2003. Temporal dynamics in epipelic, pelagic and epiphytic algal production in a clear and a turbid shallow lake. Freshw. Biol. **48**: 418–431.

Litchman, E., and C. A. Klausmeier. 2008. Trait-Based Community Ecology of Phytoplankton. Annu. Rev. Ecol. Evol. Syst. **39**: 615–639.

Longhi, M. L., and B. E. Beisner. 2009. Environmental factors controlling the vertical distribution of phytoplankton in lakes. J. Plankton Res. **31**: 1195–1207.

Lowe, R. L., and R. W. Pillsbury. 1995. Shifts in benthic algal community structure and function following the appearance of zebra mussels (Dreissena polymorpha) in Saginaw Bay, Lake Huron. J. Great Lakes Res. **21**: 558–566.

Malkin, S. Y., S. A. Bocaniov, R. E. Smith, S. J. Guildford, and R. E. Hecky. 2010. In situ measurements confirm the seasonal dominance of benthic algae over phytoplankton in nearshore primary production of a large lake. Freshw. Biol. **55**: 2468–2483.

Ólafsson, J. 1979a. The chemistry of Lake Mývatn and River Laxa. Oikos **32**: 82–112.

Ólafsson, J. 1979b. Physical Characteristics of Lake Mývatn and River Laxá. Oikos **32**: 38–66.

Ólafsson, J. S., and D. M. Paterson. 2004. Alteration of biogenic structure and physical properties by tube-building chironomid larvae in cohesive sediments. Aquat. Ecol. **38**: 219–

Phillips, J. S. 2020. Time-varying responses of lake metabolism to light and temperature. Limnol. Oceanogr. **65**: 652–666.

Phillips, J. S., A. R. McCormick, Á. Einarsson, S. N. Grover, and A. R. Ives. 2019. Spatiotemporal variation in the sign and magnitude of ecosystem engineer effects on lake ecosystem production. Ecosphere **10**: e02760.

Pinheiro J, Bates D, DebRoy S, Sarkar D, R Core Team (2019). _nlme: Linear and Nonlinear Mixed Effects Models_. R package version 3.1-140, https://CRAN.R-project.org/package=nlme

Platt, T., C. L. Gallegos, and W. G. Harrison. 1980. Photoinhibition of photosynthesis in natural assemblages of marine phytoplankton. J. Mar. Res. **38**: 103-.

R Core Team 2019. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Reynolds, C. S. 2006. The ecology of phytoplankton. Cambridge University Press.

Richardson, K., J. Beardall, and J. A. Raven. 1983. Adaptation of unicellular algae to irradiance: an analysis of strategies. New Phytol. **93**: 157–191.

Sand-Jensen, K., and J. Borum. 1991. Interactions among phytoplankton, periphyton, and macrophytes in temperate freshwaters and estuaries. Aquat. Bot. **41**: 137–175.

Scheffer, M., S. H. Hosper, M. L. Meijer, B. Moss, and E. Jeppesen. 1993. Alternative equilibria in shallow lakes. Trends Ecol. Evol. **8**: 275–279.

Scheffer, M., and E. H. van Nes. 2007. Shallow lakes theory revisited : various alternative regimes driven by climate , nutrients , depth and lake size. 455–466.

Scheffer, M., S. Rinaldi, A. Gragnani, L. R. Mur, and E. H. van Ness. 1997. On the dominance of filamentous cyanobacteria in shallow, turbid lakes. Ecology **78**: 272–282.

Smith, V. H., and D. W. Schindler. 2009. Eutrophication science : where do we go from here ? Trends Ecol. Evol. **24**: 201–207.

Solomon, C. T., S. E. Jones, B. C. Weidel, and others. 2015. Ecosystem Consequences of Changing Inputs of Terrestrial Dissolved Organic Matter to Lakes : Current Knowledge and Future Challenges. 376–389.

Søndergaard, M., S. E. Larsen, L. S. Johansson, T. L. Lauridsen, and E. Jeppesen. 2016. Ecological classification of lakes: Uncertainty and the influence of year-to-year variability. Ecol. Indic. **61**: 248–257.

Steinman, A. D., P. J. Mulholland, A. V Palumbo, T. F. Flum, J. W. Elwood, and D. L. DeAngelis. 1990. Resistance of lotic ecosystems to a light elimination disturbance: a laboratory stream study. Oikos **58**: 80–90.

Stewart, S. D., D. P. Hamilton, W. T. Baisden, M. Dedual, P. Verburg, I. C. Duggan, B. J. Hicks, and B. S. Graham. 2017. Variable littoral-pelagic coupling as a food-web response to seasonal changes in pelagic primary production. Freshw. Biol. **62**: 2008–2025.

Taranu, Z. E., I. Gregory-Eaves, P. R. Leavitt, and others. 2015. Acceleration of cyanobacterial dominance in north temperate- subarctic lakes during the Anthropocene. Ecol. Lett.

Thorbergsdóttir, I. M., and S. R. Gíslason. 2004. Internal loading of nutrients and certain metals in the shallow eutrophic Lake Myvatn, Iceland. Aquat. Ecol. **38**: 191–208.

Turner, M. A., D. W. Schindler, R. W. Graham. 1983. Photosynthesis-irradiance relationships of epilithic algae measured in the laboratory and in situ. In Periphyton of Freshwater Ecosystems pp. 73-87 (Editor: R. G. Wetzel). Springer, Dordrecht.

Turschak, B. A., D. Bunnell, S. Czesny, T. O. Hook, J. Janssen, D. Warner, and H. A. Bootsma. 2014. Nearshore energy subsidies support Lake Michigan fishes and invertebrates following

major changes in food web structure. Ecology **95**: 1243–1252.

Vadeboncoeur, Y., S. P. Devlin, P. B. Mcintyre, and M. J. Vander Zanden. 2014. Is there light after depth? Distribution of periphyton chlorophyll and productivity in lake littoral zones. Freshw. Sci. **33**: 524–536.

Vadeboncoeur, Y., E. Jeppesen, M. J. Vander Zanden, H.-H. Schierup, K. Christoffersen, and D. M. Lodge. 2003. From Greenland to green lakes: Cultural eutrophication and the loss of benthic pathways in lakes. Limnol. Oceanogr. **48**: 1408–1418.

Vadeboncoeur, Y., and D. M. Lodge. 2000. Periphyton production on wood and sediment: substratum-specific response to laboratory and whole-lake nutrient manipulations. J. North Am. Benthol. Soc. **19**: 68–81.

Vadeboncoeur, Y., D. M. Lodge, and S. R. Carpenter. 2001. Whole-Lake Fertilization Effects on Distribution of Primary Production between Benthic and Pelagic Habitats. Ecology **82**: 1065–1077.

Vadeboncoeur, Y., G. Peterson, M. J. Vander Zanden, and J. Kalff. 2008. Benthic Algal Production Across Lake Size Gradients : Interactions Among Morphometry , Nutrients , and Light. Ecology **89**: 2542–2552.

Valiela, I., J. McClelland, J. Hauxwell, P. J. Behr, D. Hersh, and K. Foreman. 1997. Macroalgal blooms in shallow estuaries: Controls and ecophysiological and ecosystem consequences. Limnol. Oceanogr. **42**: 1105–1118.

Vasconcelos, F. R., S. Diehl, P. Rodriguez, P. Hedstrom, J. Karlsson, and P. Bystrom. 2016. Asymmetrical competition between aquatic primary producers in a warmer and browner world. Ecology **97**: 2580–2592.

Vesterinen, J., S. P. Devlin, J. Syvaranta, and R. I. Jones. 2016. Accounting for littoral primary

production by periphyton shifts a highly humic boreal lake towards net autotrophy. Freshw. Biol. **61**: 265–276.

Vander Zanden, M. J., Y. Vadeboncoeur, and S. Chandra. 2011. Fish Reliance on Littoral – Benthic Resources and the Distribution of Primary Production in Lakes. 894–903.

Wetzel, R. G. 2001. Limnology: Lake and River Ecosystems. (Third Edition). Academic Press, San Diego, CA.

Zhang, L., X. Gu, C. Fan, J. Shang, Q. Shen, Z. Wang, and J. Shen. 2010. Impact of different benthic animals on phosphorus dynamics across the sediment-water interface. J. Environ. Sci. **22**: 1674–1682.

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AUTHOR CONTRIBUTIONS

ARI developed the long-term monitoring and sampling efforts. All authors participated in data collection. ARM led the analysis of the data with input from other authors. ARM wrote the first draft of the manuscript with input from other authors on subsequent drafts.

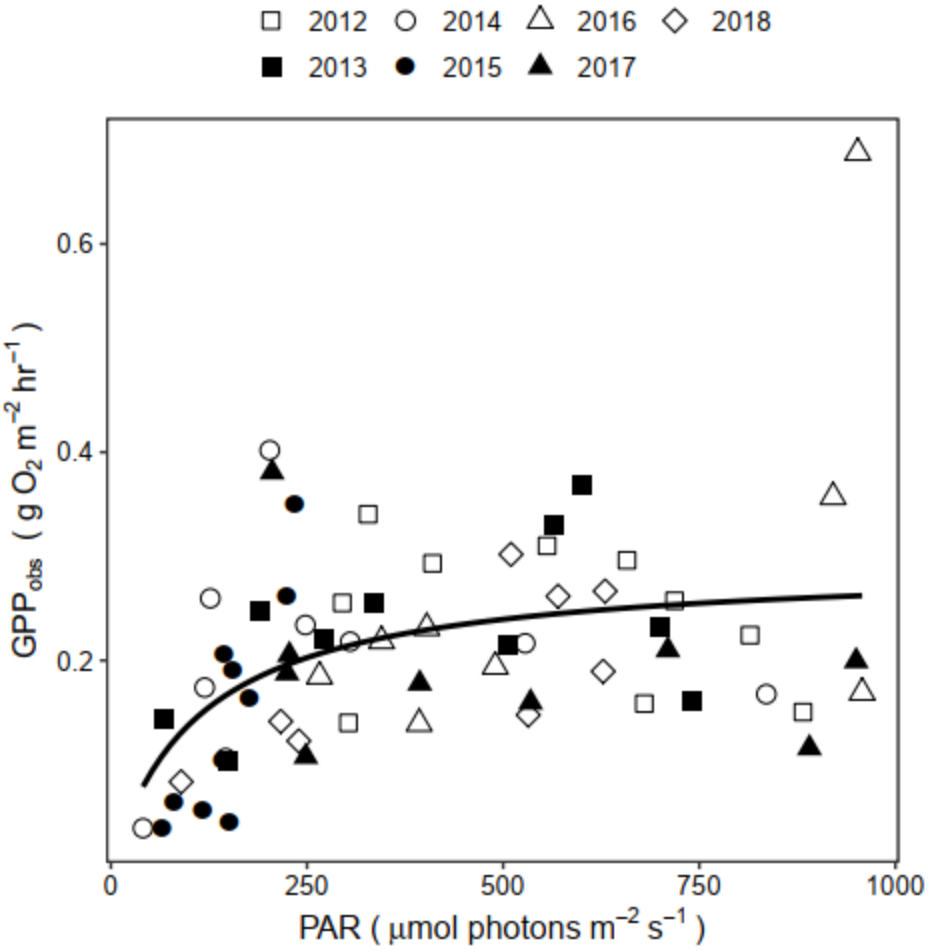

Figure 1. Observed benthic gross primary production (GPP_{obs}) measured approximately every two weeks from late May to late August for seven years of monitoring at our study site. Each point represents a single sample date, with the in situ irradiance level during the incubation. The line shows the fit of a Michaelis-Menten type P-I curve to these data.

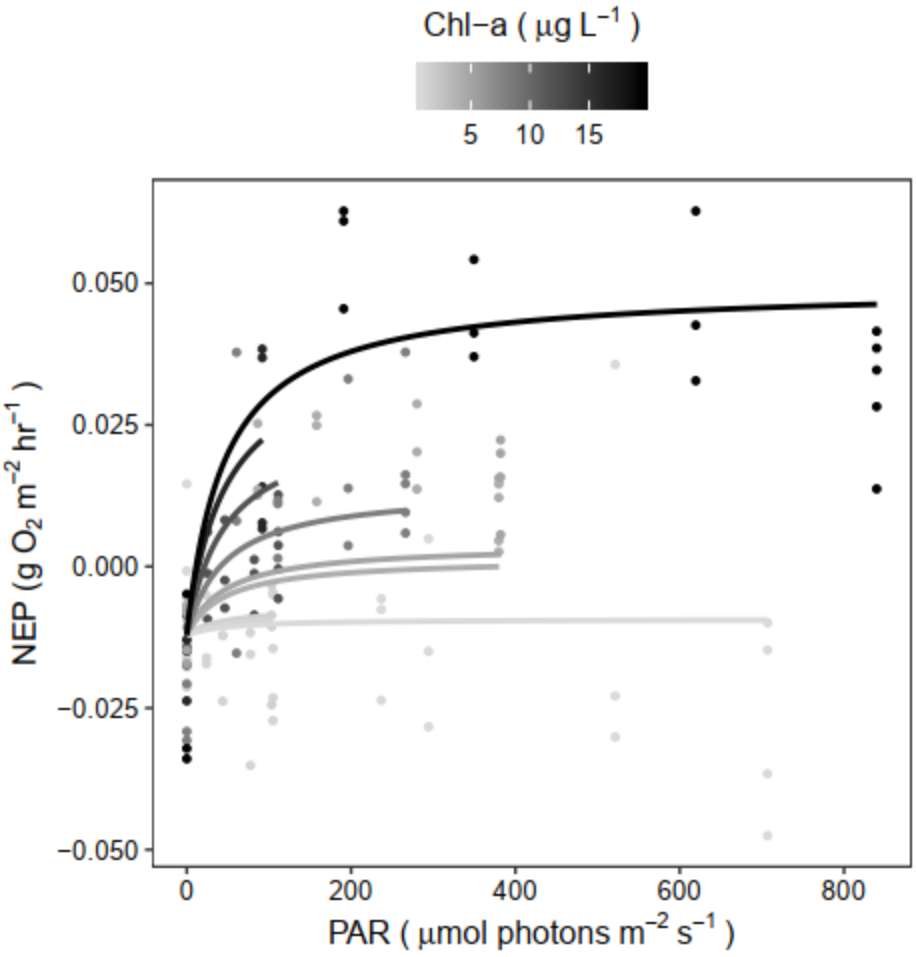

Figure 2. The relationship between pelagic primary production and irradiance was characterized at three sites, with three incubations per site in 2018. The line shows a Michaelis-Menten P-I curve fit to the production data, which included pelagic chl-a as a covariate influencing variation in the maximum productivity rate for each incubation.

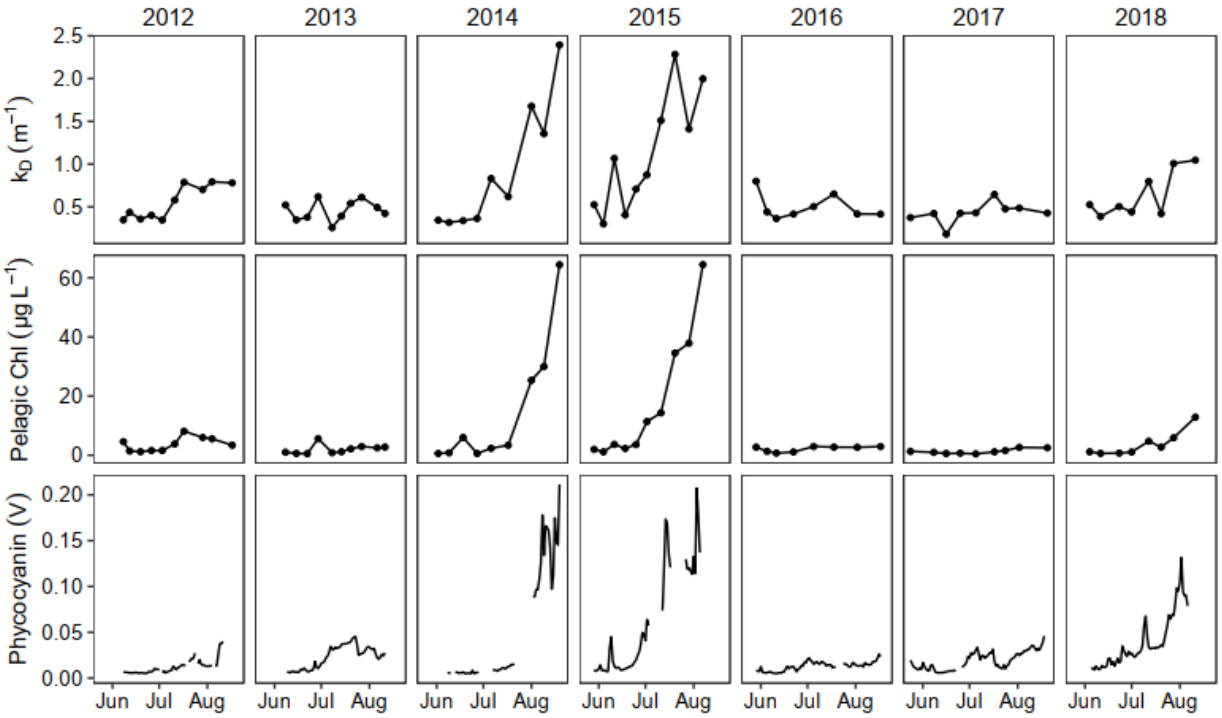

Figure 3. Temporal variation in water column clarity and phytoplankton abundance at our study site. Light attenuation coefficients (k_D) and pelagic chl-a were routinely sampled approximately every two weeks. Phycocyanin data are shown as daily averages. Gaps in the phycocyanin are periods during which the sonde was inactive.

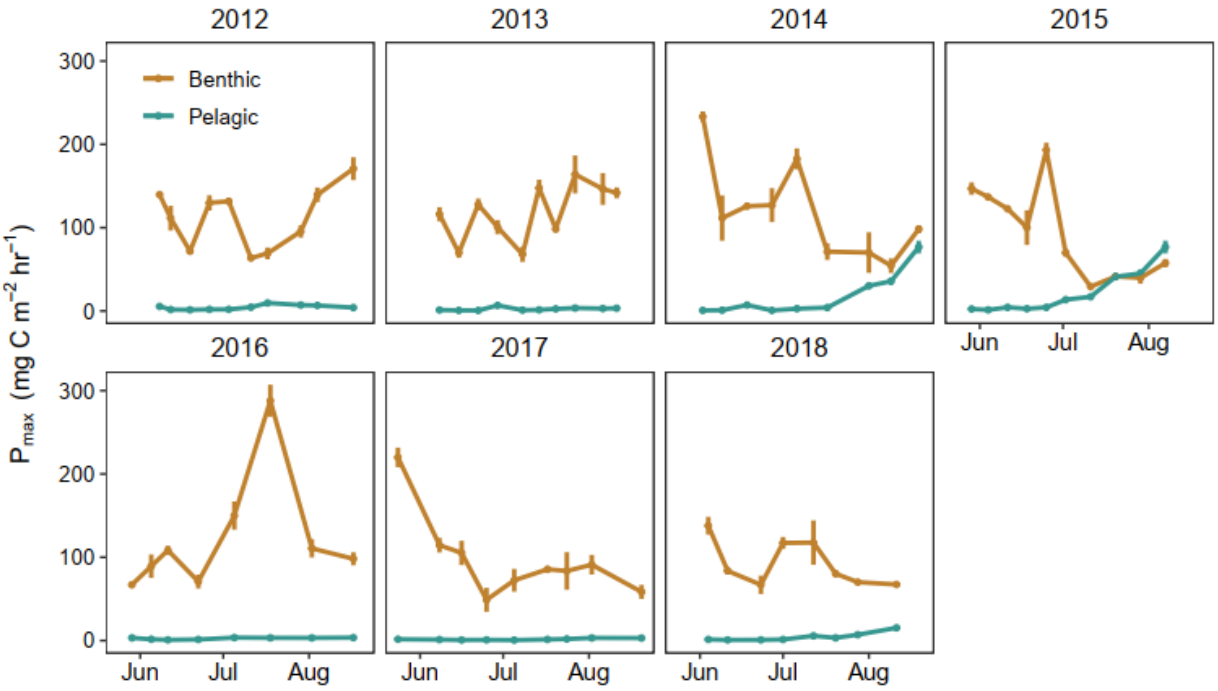

Figure 4. Temporal variation in benthic and pelagic maximum rates of primary production (P_{max}) at the study site. Standard errors are shown for each sample date and are based on propagation of standard errors from the dark and light replicate cores (benthic) or standard errors associated with the parameter relating P_{max} to chl-a (pelagic).

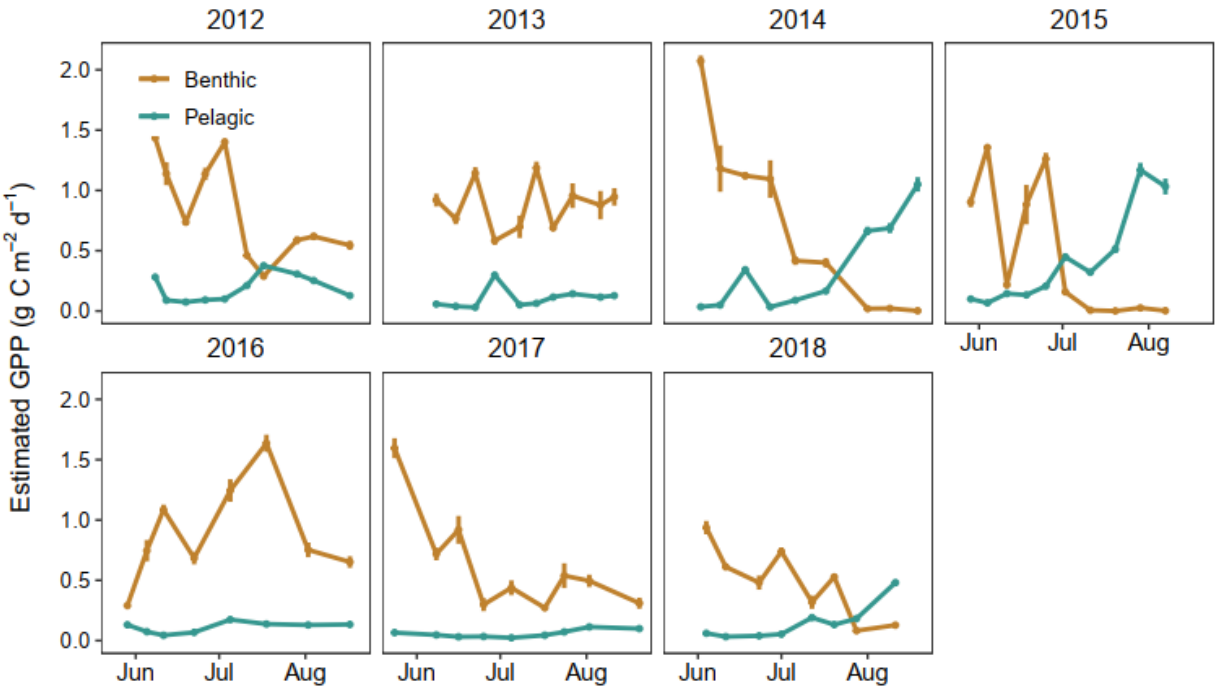

Figure 5. Temporal variation in estimated benthic and pelagic daily gross primary production (GPP) at the study site. GPP was estimated from using P_{max} rates respective to either benthic or pelagic producers, light attenuation coefficients, and incident light levels corresponding to each sample date.

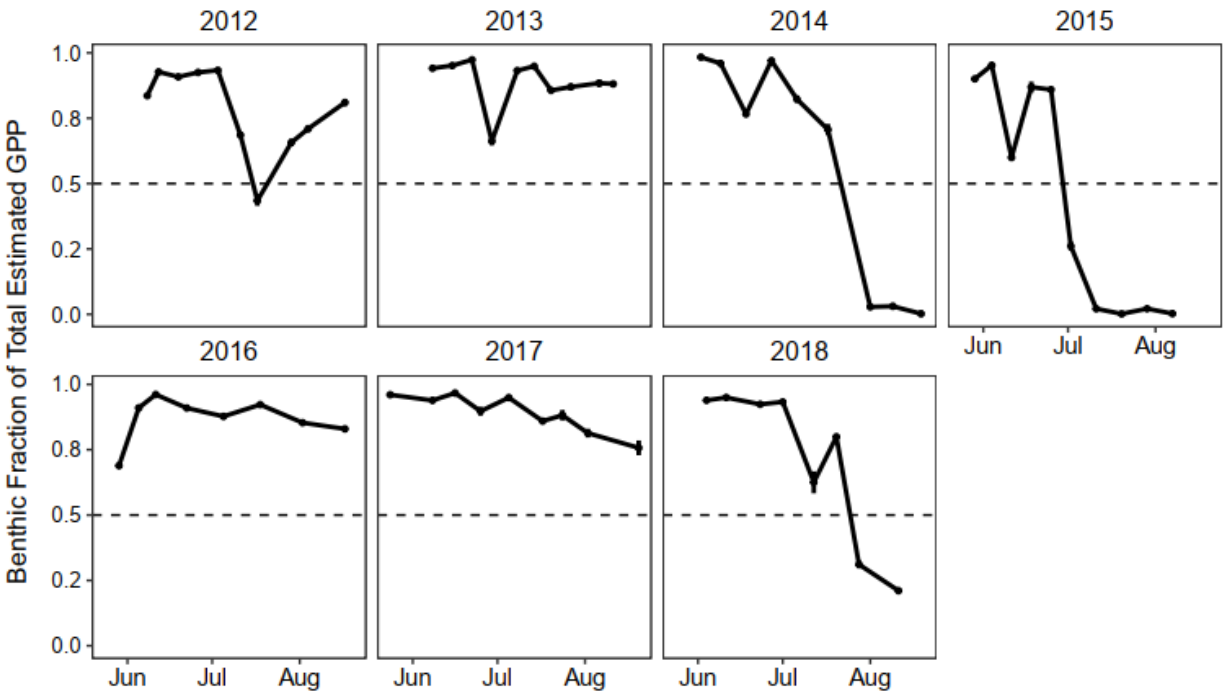

Figure 6. The benthic fraction of total estimated production (daily benthic GPP divided by the sum of benthic and pelagic daily GPP rates) for the study site.

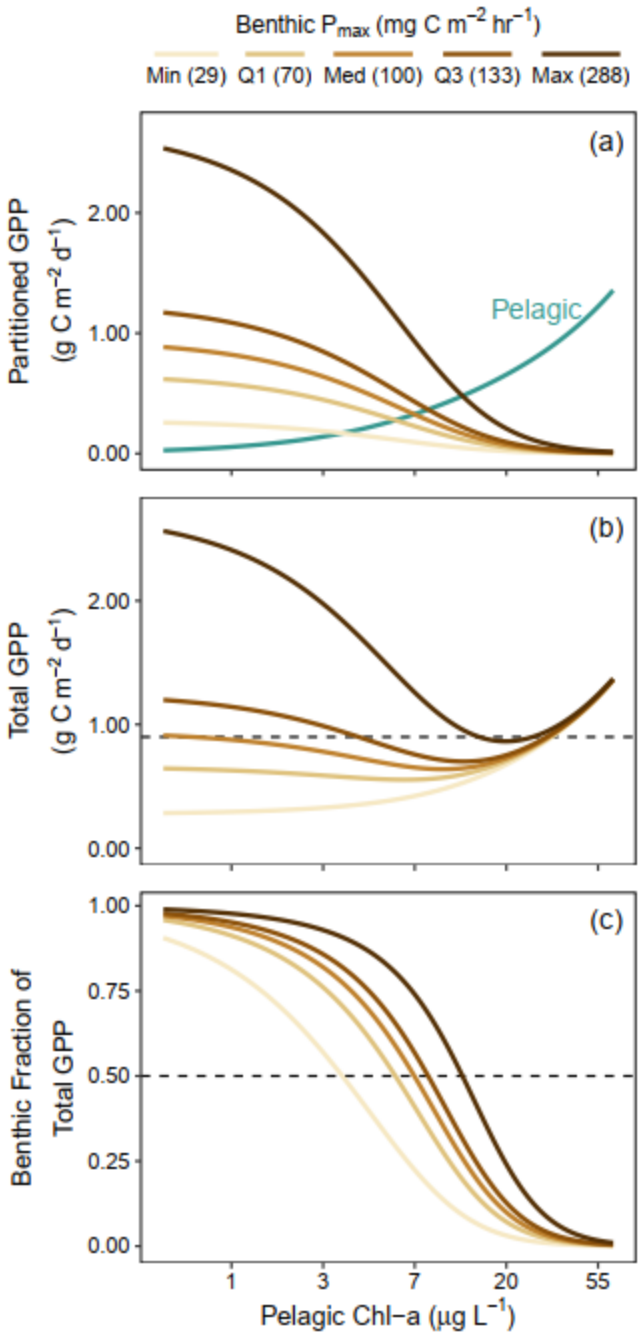

Figure 7. The partitioned gross primary production (GPP) for (a) the benthic or pelagic habitat separately, (b) total GPP, and (c) the benthic fraction of total GPP were estimated for a subset of benthic P_{max} values encompassing the variation we observed in our measurements (the minimum, first quartile, median, third quartile, and maximum values which are respectively equivalent to

29, 70, 100, 133, and 288 mg C m^{-2} h^{-1}). For each benthic P_{max} value (depicted as brown lines), we estimated these metrics across the range of pelagic chl-a observed in our study. Pelagic chl-a concentrations were translated into light attenuation coefficients based on an empirical model fit to our data from 2012-2018. We used average diel incident light levels to standardize external light conditions across the depicted scenarios. In panel (a), a single line depicts pelagic GPP because it is unaffected by benthic P_{max}. The horizontal line in panel (b) represents the mean daily total GPP estimated across all our sampling dates. The horizontal line at 50% in panel (c) represents the point at which benthic and pelagic algae equally contribute to total GPP. Pelagic chl-a concentrations are shown on a natural-log scale.

Appendix B: Supplemental figures for Chapter 2

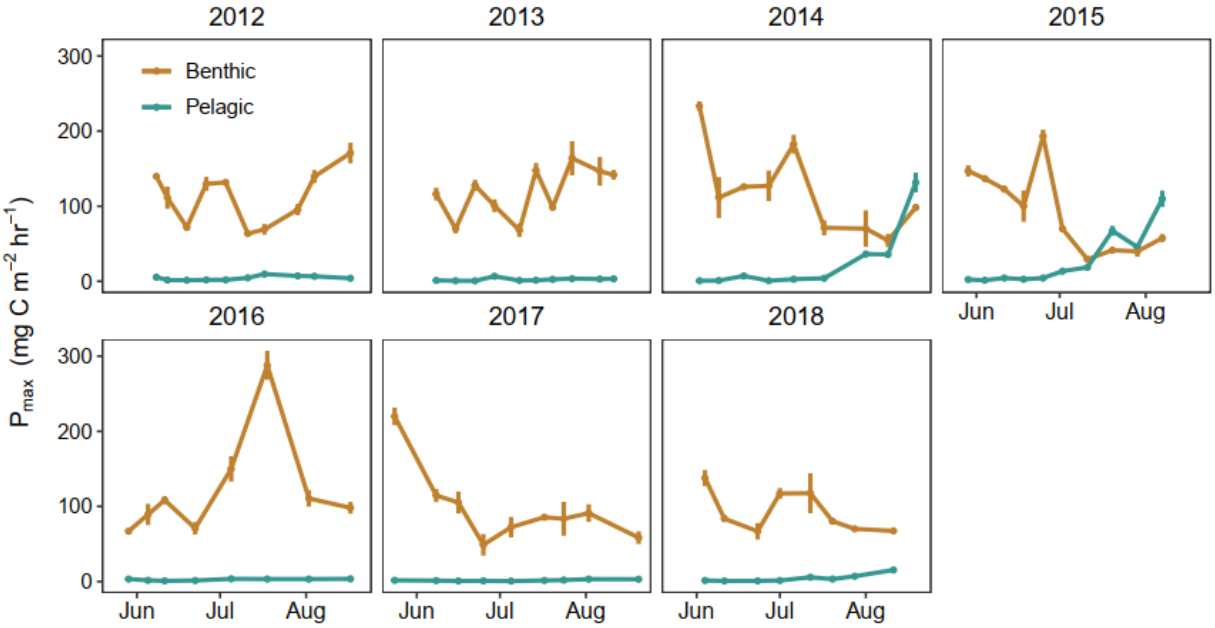

Figure S1. Interannual variation in benthic and pelagic maximum rates of primary production (P_{max}) at the 3.3 m deep study site, under the assumption that phytoplankton biomass is evenly distributed through the euphotic zone. This figure is analogous to Figure 4, except the version in the main text assumes that pelagic chl-a is evenly distributed throughout the entire water column.

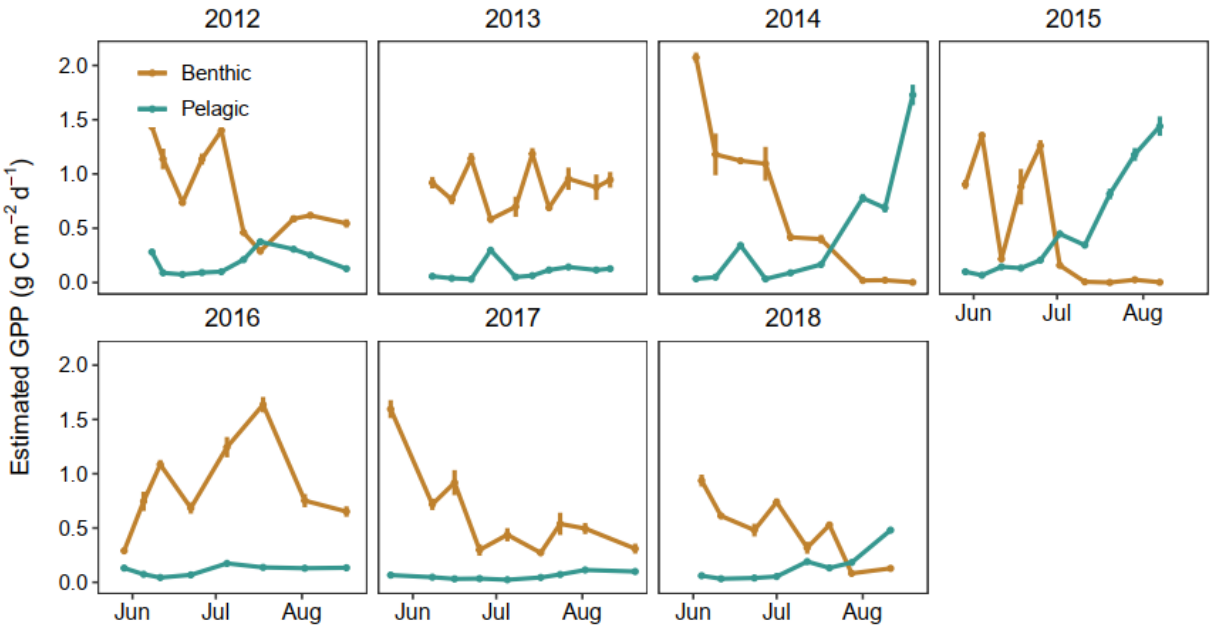

Figure S2. Interannual variation in estimated benthic and pelagic daily gross primary production (GPP) at the 3.3 m deep study site, under the assumption that phytoplankton biomass is evenly distributed through the euphotic zone. This figure is analogous to Figure 5, except the version in the main text assumes that pelagic chl-a is evenly distributed throughout the entire water column.

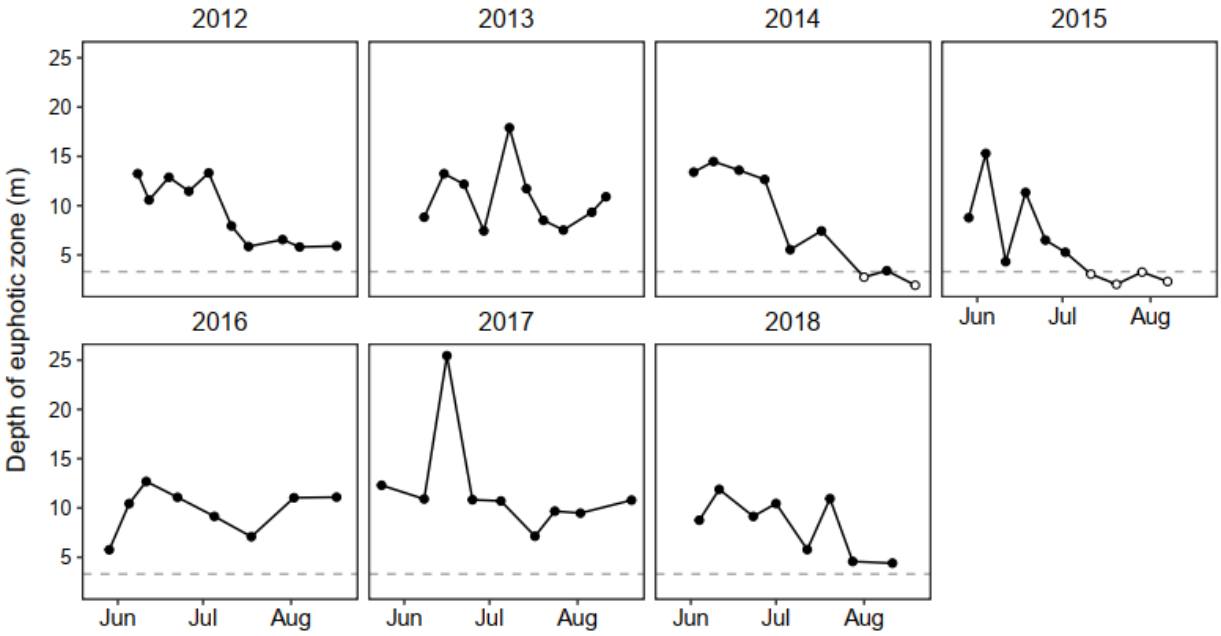

Figure S3. Depth of the euphotic zone, where 1% of surface light reaches. The horizontal line depicts the depth of the study site (3.3 m). Open symbols illustrate points during which the euphotic zone was less than the site depth.

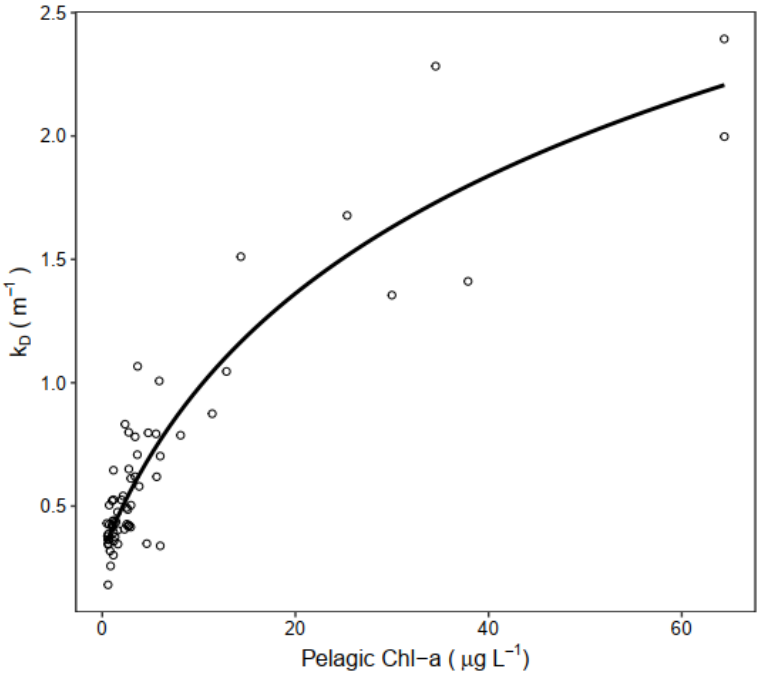

Figure S4. Pelagic chl-a concentrations affected measured light attenuation coefficients (k_D).

The curve shows the function fit to the data: $k_D = 0.92 \cdot \log(pelagic\ chl + 9.31) - 1.73$

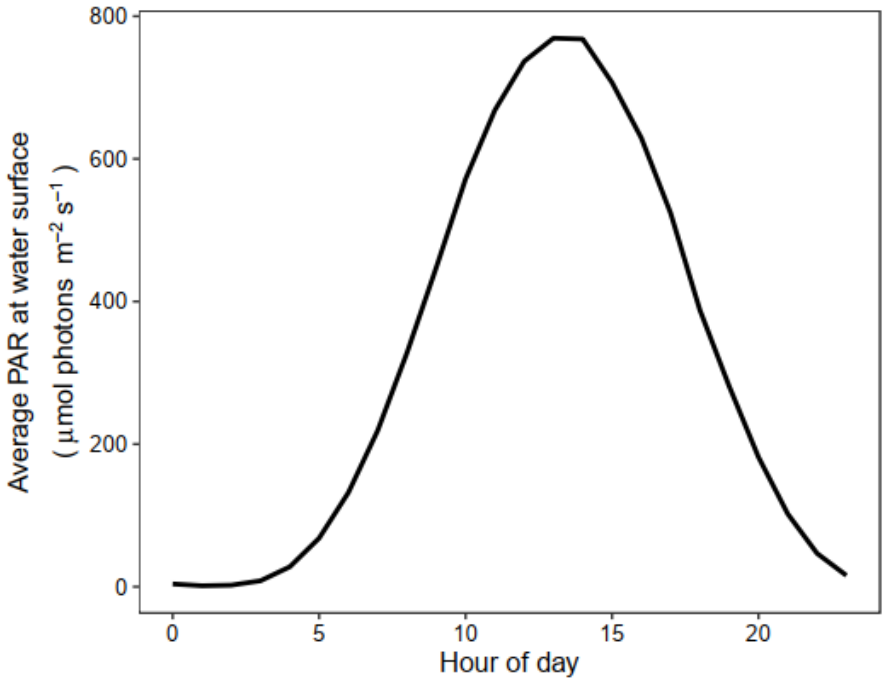

Figure S5. Mean photosynthetically active radiation (PAR) at the water's surface. Mean hourly light levels were calculated from all the aggregated five-day windows around our sample dates. These diel light levels were used as the incident light for the visualizations presented in the main text Figure 7.

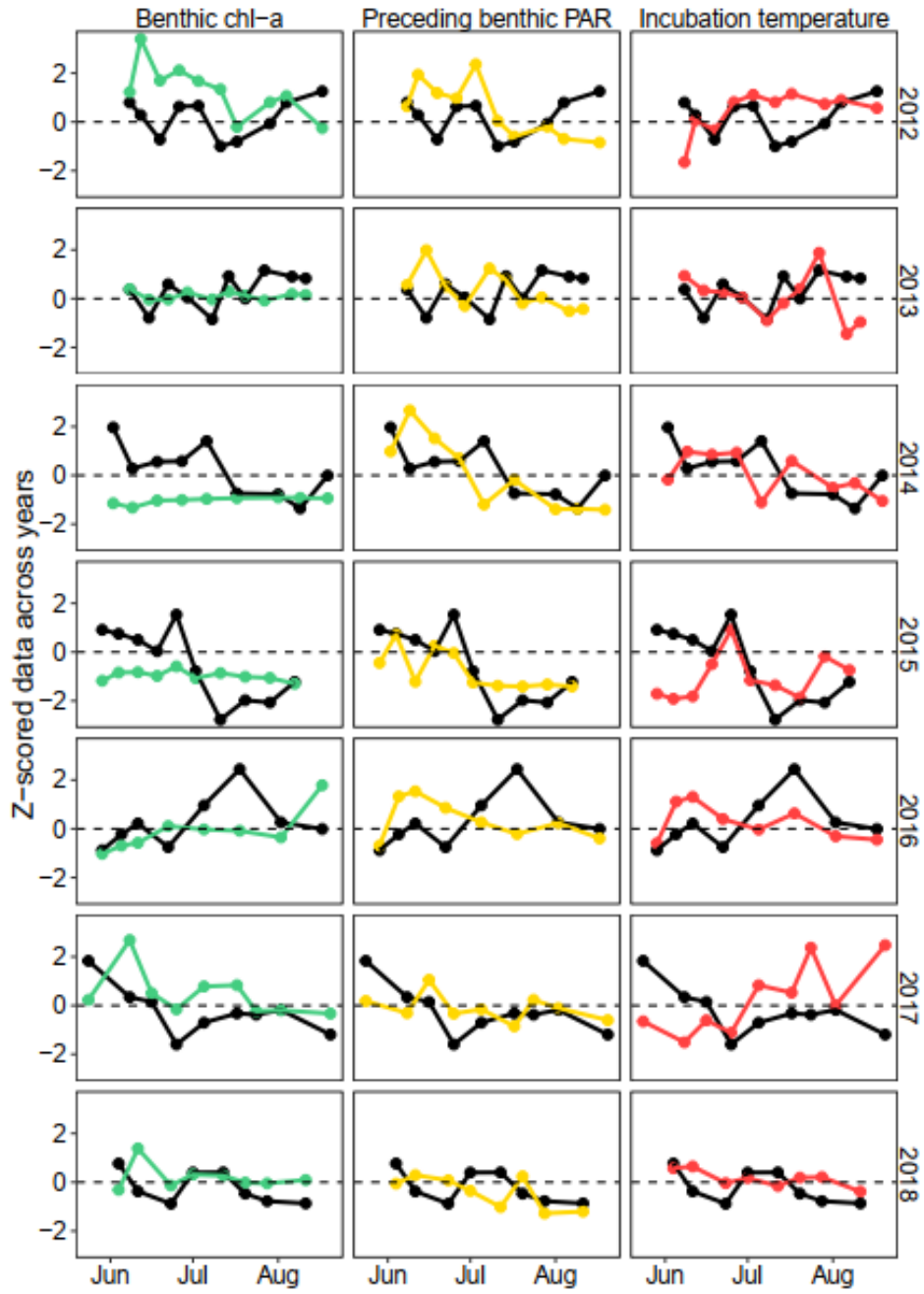

Figure S6. Interannual trends between benthic P_{max} (shown in black) benthic chl-a, preceding benthic light availability, and incubation temperature, which were included as potential explanatory variables of benthic P_{max}. Benthic P_{max} and benthic chl-a were first ln-transformed, and then all variables were Z-transformed across years.

CHAPTER 3

Reconstructing consumer-resource dynamics using carbon stable isotope signatures of archived specimens

Status: in preparation for submission to *Ecology* (as a Report submission-type)

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Running head: Consumer-resource interactions from $\delta^{13}C$

ABSTRACT

Consumer-resource interactions can cause population cycles; however, demonstrating consumer-resource dynamics is often confounded by the absence of data on either consumer or resource. Dramatic population fluctuations of *Tanytarsus gracilentus* (Diptera: Chironomidae) in Lake Mývatn are likely governed by consumer-resource interactions between midge larvae and benthic algae. Here, we analyzed carbon stable isotope signatures of archived *T. gracilentus* specimens collected from 1977-2015 to investigate *T. gracilentus* population dynamics using their $\delta^{13}C$ values. As a known benthic herbivore/detritivore, we expect *T. gracilentus* $\delta^{13}C$ values should reflect algal $\delta^{13}C$ values, which in turn reflect algal primary productivity. Thus, we treat midge $\delta^{13}C$ values as a surrogate for resource availability. We analyzed a time series of 78 midge generations to estimate interactions within and between consumers and resources, while accounting for measurement error and possible preservation effects on isotope values. Statistical results were consistent with our hypothesis treating $\delta^{13}C$ values as a resource availability surrogate. ^{13}C-enrichment, indicating high benthic productivity, was associated with increased *T. gracilentus* abundance, and high *T. gracilentus* abundance was associated with ^{13}C-depletion, consistent with benthic productivity declines. Our study adds new evidence that consumer-resource interactions drive *T. gracilentus* population fluctuations and demonstrates potential stable isotope applications for reconstructing consumer-resource dynamics.

Key words: population fluctuation, Chironomidae, diatoms, benthic primary production, Mývatn

INTRODUCTION

Cyclic population dynamics are widespread and are often caused by consumer-resource interactions (Murdoch et al. 2003). The majority of documented cases, however, appear to

involve prey and predators, rather than primary producers. Confirming the role of consumer-resource interactions in driving population fluctuations, regardless of whether resources are primary producers, is often challenging due to the lack of data for one ecological partner (Einarsson et al. 2016). Thus, in many cases, surrogate resource availability data are necessary to understand consumer population dynamics.

In this study, we examine a consumer population with widely fluctuating abundance and use carbon stable isotope analysis to infer how resource availability is linked to these population fluctuations. *Tanytarsus gracilentus* (Diptera: Chironomidae) is a keystone species in Lake Mývatn, Iceland, and decades of extensive monitoring (1977-present) have shown the population undergoes dramatic fluctuations, spanning 4-5 orders of magnitude, with irregularly timed crashes, occurring every 4-10 years. Our working hypothesis is that consumer-resource interactions drive *T. gracilentus* fluctuations; however, data for their main food source, benthic diatoms, do not exist, making direct time-series analyses of consumer-resource dynamics impossible (Einarsson et al. 2016). Nonetheless, previous studies using resource surrogates support the influence of consumer-resource interactions on midge population dynamics. First, *T. gracilentus* wing lengths (a resource availability proxy) decline in the generations preceding a crash, while there was no relationship between *T. gracilentus* and predator abundance (Einarsson et al. 2002). Second, cyclic interactions between diatom pigments (diatoxanthin) and midge abundance (egg capsules) were found in sediment cores from Mývatn (Einarsson et al. 2016). Third, a consumer-resource model allowing for alternative dynamical states between a relatively stable high point and a high-amplitude population cycle closely matches the observed fluctuations and irregular periodicity in the long-term *T. gracilentus* abundance data (Ives et al. 2008). Finally, there is no evidence that other potential factors (e.g., predators, diseases, climatic

variability) cause midge population crashes. Nonetheless, evidence that interactions with their resources drive the dramatic fluctuations in *T. gracilentus* abundance is circumstantial.

Our primary objective was to determine whether temporal patterns in *T. gracilentus* stable carbon isotope signatures provide additional support linking consumer-resource interactions to their population fluctuations. Carbon stable isotope analysis can reveal consumer resource use and, in some cases, resource productivity. These inferences are based on changes in the ratio between ^{13}C and ^{12}C ($\delta^{13}C$) during biological processes. In aquatic systems, algal $\delta^{13}C$ values reflect primary productivity due to the relationship between inorganic carbon demand and photosynthetic rates. Primary producers preferentially use ^{12}C during photosynthesis because it reacts more quickly than ^{13}C in biochemical reactions (Fry 2006). Because benthic algae have a relatively limited carbon supply due to thick boundary layers (i.e., stagnant water separating periphyton from the overlying water), they more commonly incorporate ^{13}C during fixation or use bicarbonate, resulting in their relatively enriched $\delta^{13}C$ values (Hecky and Hesslein 1995, Hill and Middleton 2006). High rates of primary productivity exacerbate carbon-limitation and are associated with ^{13}C-enrichment because increasing inorganic carbon demand relative to the concentration available reduces the degree of photosynthetic fractionation (Hecky and Hesslein 1995, Finlay 2001). In contrast to carbon fixation, little fractionation occurs between consumers and their diets (Fry 2006). Therefore, primary consumer $\delta^{13}C$ values reflect those of primary producers, raising the potential for consumer $\delta^{13}C$ values to serve as a surrogate for resource availability (e.g., primary productivity; Devlin et al. 2013). Analyzing stable isotopes from archived specimens can provide opportunities to reconstruct the history of consumer resource use and ecosystem productivity (Wainright et al. 1993, Grey et al. 2009, Blight et al. 2015). We analyzed $\delta^{13}C$ signatures of adult *T. gracilentus* specimens collected from 1977-2015 and

examined the relationship between $\delta^{13}C$ values and corresponding population sizes. As *T. gracilentus* is a primary consumer (herbivore/detritivore) with known food sources (benthic diatoms and associated detritus) (Ingvason et al. 2004), temporal variation in $\delta^{13}C$ values should largely reflect isotopic shifts of their resource (benthic primary producers), rather than a change in diet or trophic position (sensu Grey et al. 2009). Thus, we analyze the data under the hypothesis that $\delta^{13}C$ values of *T. gracilentus* mirror changes in benthic productivity, thereby providing a proxy for their resource availability. To supplement our interpretation of $\delta^{13}C$ values, we examine the relationship between benthic productivity and $\delta^{13}C$ values in a supplemental experiment (Appendix 1). If treating $\delta^{13}C$ as a proxy for algal productivity is consistent with consumer-resource dynamics, then this provides evidence to support not only the use of $\delta^{13}C$ as a proxy, but also for the existence of consumer-resource dynamics.

METHODS

Study system, chironomid monitoring, and isotope sampling

Mývatn (65°37'N, 17°00'W; 37 km^2) is a shallow lake in northeast Iceland with a mean depth of 2.5 m (Einarsson et al 2016). Mývatn is naturally eutrophic due to nutrient-rich groundwater inputs and supports high internal primary production, while receiving little external carbon inputs (Jónasson 1979, Einarsson et al. 2004). Benthic algae (especially sediment-associated diatoms) generally dominate whole-lake production; however, phytoplankton (especially cyanobacteria blooms) vary interannually and can contribute to total production (Einarsson et al. 2004; Chapter 2).

Mývatn is Icelandic for "midge lake", and the high abundance of chironomid larvae is a defining ecological feature of the lake. The dominant species, *T. gracilentus*, can comprise 67%

of secondary production (Lindegaard and Jónasson 1979) and undergoes large population fluctuations: larvae exceed densities of 200,000 individuals m^{-2} in high midge years (Lindegaard and Jónasson 1979) and are nearly absent in crash years. *T. gracilentus* larvae construct silk tubes in the sediment and feed on benthic algae and detritus. Two non-overlapping *T. gracilentus* generations generally occur each year, with spring and summer generation adults emerging in late May or early August, respectively.

Since 1977, chironomid abundances at Mývatn have been monitored with window traps located on the lakeshore. Window traps passively capture aerial insects and consist of an open box containing a preservative, situated 1-3 m above the elevation of the water surface (see Gardarsson et al. 2004 for additional details). For this study, the focal window trap is located on the Sydri-Neslond peninsula along the main basin's northern shore and captures a chironomid assemblage characteristic of the profundal habitat (Gardarsson et al. 2004). Window traps were sampled from May to September every one to two weeks. Dipterans were enumerated and identified to species (typically with subsampling). Species abundances were summed for spring and summer (using a cutoff date of ~15-20 July), which distinguishes the two annual *T. gracilentus* emergences (Gardarsson et al. 2004). After sample processing, archived specimens were preserved in 70% ethanol for long-term storage, with the identified individuals contained in glass vials and the unsorted (i.e., non-identified) sample portion contained in plastic containers.

We retrieved archived *T. gracilentus* adults that were collected from 1977-2015 for $\delta^{13}C$ analysis. Typically, we retrieved samples for a single date per emergence, but in some cases, samples from two dates within an emergence were analyzed separately. Material was generally retrieved from the unsorted sample portion, but in some low-midge years, we used individuals from the identified collection. Our target was 60 *T. gracilentus* adults per sample, and when

samples were smaller, we collected as many individuals as possible. We lack $\delta^{13}C$ data for some crash year emergences because we could not obtain enough material for isotopic analysis. Most specimens were retrieved in 2017, but a few were retrieved in 2015. If enough midges remained in the retrieved portions from 2015, we analyzed additional midges from these samples in 2017. This allowed us to compare the variability of sample pairs collected within a window trap on the same date to sample pairs collected from different dates within the same emergence; variability among both types of sample pairs were similar.

Midge adults were exposed to window trap preservatives and 70% ethanol for long-term storage. From 1977-2000, the window trap preservative consisted of 10% formalin, a small amount of detergent, and ethylene glycol (Gardarsson et al. 2004), but in 2000, it was switched to propylene glycol. We examined effects of these preservation methods on isotope values in a small experiment, with associated methods and results reported in Appendix 2.

To prepare samples for isotopic analysis, they were rinsed with deionized water, dried at 60 ºC, homogenized with a pestle, and stored in a desiccator for 24 h. The University of California Davis Stable Isotope Laboratory (Davis, CA, USA) performed the isotopic analysis. Isotope signatures are expressed in delta (δ) notation where $\delta = [(R_{sample} - R_{standard})/R_{standard}] \times 1000$, and $R = {}^{13}C/{}^{12}C$. $\delta^{13}C$ is reported relative to Vienna PeeDee Belemnite. Analytical error standard deviations were 0.09‰ and 0.04‰, respectively, for the 2015 and 2017 analyses. Duplicates run for a subset of homogenized samples had a mean standard deviation of 0.07‰.

Time-series analysis

We used a state-space model to examine the interactions between midge abundance and $\delta^{13}C$ values (Harvey 1989; Ives et al. 2003). When multiple samples within an emergence were analyzed for $\delta^{13}C$, we used the mean value. Prior to analysis, we standardized *T. gracilentus* $\delta^{13}C$

values and log-transformed abundance by subtracting the respective variable's mean from each observation and dividing by the variable's standard deviation (z-transformation).

Consumer-resource dynamics were modeled as

$$x_1(t) = b_{10} + b_{11}[x_1(t-1) - b_{10}] + b_{12}[x_2(t-1) - b_{20}] + \varepsilon_{1t}$$
$$x_2(t) = b_{20} + b_{22}[x_2(t-1) - b_{20}] + b_{21}[x_1(t-1) - b_{10}] + c_{21}U_1(t) + \varepsilon_{2t} \quad (1)$$

where $x_1(t)$ and $x_2(t)$ are the transformed *T. gracilentus* abundance and $\delta^{13}C$ value in generation t; coefficients b_{ij} quantify the effect of $x_j(t-1)$ on $x_i(t)$, such that b_{12} represents the effect of $\delta^{13}C$ on the change in midge abundance and b_{21} represents the effect of midge abundance on the change in $\delta^{13}C$; coefficients b_{11} and b_{22} give the autocorrelation of midge abundance and $\delta^{13}C$ values; b_{i0} scale mean values; and ε_{it} are normal random variables with mean 0 and variance σ_i^2, representing process error for each state variable. Visual inspection of $\delta^{13}C$ data suggested that spring generation midges were ^{13}C-depleted relative to those from the summer generation of the same year. We included a categorical covariate, $U_1(t)$, to account for season (value zero for spring and one for summer), with c_{21} giving the effect of season on $x_2(t)$.

We assumed measurement error for $x_1(t)$ and $x_2(t)$ that is represented by:

$$x_1^*(t) = x_1(t) + \varphi_1(t)$$
$$x_2^*(t) = x_2(t) + c_{22}U_2(t) + \varphi_2(t)$$

where $x_1(t)$ and $x_2(t)$ are the observed transformed abundance of *T. gracilentus* and $\delta^{13}C$ values; $\varphi_1(t)$ is a Gaussian random variable with mean zero and variance ν_1^2 representing observation error for log *T. gracilentus* abundance; and $\varphi_2(t)$ is a Gaussian random variable with mean zero and variance $\nu_2^2/n(t)$ representing observation error for $\delta^{13}C$ values, where $n(t)$ is the number of individuals pooled for analysis. We coded window trap preservative methodology, which changed in 2000, with a categorical covariate $U_2(t)$, which had value zero up to 1999 and one

thereafter. This allows the mean $\delta^{13}C$ to differ by methodology, with c_{22} representing this effect on $\delta^{13}C$ values.

The likelihood function was fit for the model given by equations 1, resulting in estimates for 12 variables: b_{10}, b_{20}, b_{11}, b_{22}, b_{12}, b_{21}, σ_1^2, σ_2^2, v_1^2, v_2^2, c_{21}, c_{22}. To determine the statistical significance of the effect of $\delta^{13}C$ on midge abundance (b_{12}) and the effect of midge abundance on $\delta^{13}C$ (b_{21}), we compared the full model to a reduced 10-parameter model (where $b_{12} = b_{21} = 0$) using a likelihood ratio test (LRT). We also tested the significance of b_{12} and b_{21} separately by comparing the full model to a reduced 11-parameter model where either $b_{12} = 0$ or $b_{21} = 0$ with LRTs. All analyses were conducted in R version 3.6.1 (R Core Team 2019) using code modified from (Ives and Dakos 2012).

RESULTS

The results of the time-series analysis of midge abundance and $\delta^{13}C$ values were consistent with the dynamics that would arise from consumer-resource interactions in which $\delta^{13}C$ values are a proxy for algal production. High $\delta^{13}C$ values tended to precede peak *T. gracilentus* abundances, and $\delta^{13}C$ values tended to decline when midges reached high abundance (Figure 1). The estimate for coefficient b_{12}, which measures the effect of $\delta^{13}C$ on changes in midge abundance, was positive (Table 1), indicating that enriched $\delta^{13}C$ values were associated with increased midge abundance in the following generation. Coefficient b_{21}, which measures the effect of midge abundance on $\delta^{13}C$, was negative, indicating that high midge abundances were associated with decreases in $\delta^{13}C$ values. Coefficients b_{12} and b_{21} were significantly different from zero when analyzed together ($\chi^2(2) = 15.09$; $p = 0.0005$) and separately ($b_{12} = 0$: $\chi^2(1) = 5.21$, $p = 0.022$; $b_{21} = 0$: $\chi^2(1) = 11.62$, $p = 0.0007$).

The model also demonstrated an effect of season (spring vs. summer emergence) on $\delta^{13}C$ values, represented by the positive estimate for c_{21} (Table 1; Figure 1): midges from the summer generation generally had higher $\delta^{13}C$ values relative to those from the spring generation of the same year. The negative estimate for c_{22} suggests that $\delta^{13}C$ values were lower when propylene glycol was the window trap preservative; however, this is inconsistent with our experimental results comparing propylene glycol/ethanol and formalin/ethanol storage effects (Appendix 2). Midge abundance and $\delta^{13}C$ values showed moderate autocorrelation as indicated by b_{11} and b_{22}. The model was able to separate process and measurement error for our two variables (Table 1).

DISCUSSION

We investigated whether the interaction between the midges and their main food resource, benthic algae, could explain the population fluctuations of *T. gracilentus* in Lake Mývatn. Midge abundance in Mývatn has been monitored since 1977, and we used the specimens collected from this monitoring program to obtain $\delta^{13}C$ values. We analyzed this time series assuming that $\delta^{13}C$ values were a proxy for benthic algal production. Benthic algal $\delta^{13}C$ values increase with primary productivity because as inorganic carbon becomes limiting, algae discriminate less against ^{13}C (Hecky and Hesslein 1995, Hill and Middleton 2006); our supplemental experiment examining the link between benthic productivity and $\delta^{13}C$ in sediment from Mývatn supported this interpretation (Appendix 1). In retrospective studies, detecting a mechanism responsible for temporal variation in consumer stable isotope signatures is often challenging because multiple factors may contribute to the observations. For instance, both changes in organism feeding behavior (e.g., dietary or trophic level shifts) and altered isotopic baselines (e.g., environmental conditions or ecosystem perturbations affecting primary producers) can affect consumer isotope values (Wainright et al. 1993, Grey et al. 2009, Blight et

al. 2015). Visual observations, gut contents, and $\delta^{13}C$ signatures of *T. gracilentus* larvae demonstrate their consistent feeding habits and reliance on benthic diatoms and associated detritus (Ingvason et al. 2004; Chapter 4; unpublished data). Thus, we assume that shifts in $\delta^{13}C$ signatures of benthic algae largely contribute to variation in *T. gracilentus* $\delta^{13}C$ values, rather than dietary or trophic level shifts. The results of the time-series analysis were consistent with the hypothesis that midge population fluctuations were driven by interactions between midges and benthic algae. This not only gives evidence for consumer-resource dynamics, but also supports for using $\delta^{13}C$ as a proxy for benthic algal production.

Our results complement other lines of evidence that the dramatic population fluctuations of midges in Mývatn are driven by midge-algal interactions. For example, in a paleoecology analysis, high diatoxanthin (a diatom pigment and proxy for Mývatn's benthic algal biomass) concentrations were associated with increased midge egg capsule abundance (Einarsson et al. 2016). Einarsson et al. (2002) analyzed data from the same monitoring program as our data, focusing on the period 1977-1999 when wing lengths were recorded for male midges. Using wing length as a proxy for food abundance, they showed that food limitation (decreased wing lengths) preceded population crashes. Additionally, in a mesocosm experiment, increased benthic productivity was associated with faster timing to *T. gracilentus* emergence, demonstrating primary production's effects on midge development (Phillips unpublished data). Low to moderate experimental densities of *T. gracilentus* larvae enhance benthic primary production by increasing substrate availability through their tube-building; however, at high *T. gracilentus* densities, consumption of benthic algae may outweigh their substrate-boosting effect and lead to decreased algal productivity (Phillips et al. 2019). In another mesocosm experiment, moderately high *T. gracilentus* larval densities (~85,000 individuals m^{-2}) negatively affected

benthic productivity rates (Phillips unpublished data), which is consistent with our results when $\delta^{13}C$ is treated as a proxy for benthic algal production. Additionally, Einarsson et al. (2016) showed that increased midge abundance was associated with declines in diatom pigments in sediment cores, suggesting that midges may reduce diatom biomass as their population grows. Decreased diatom abundance could reduce competition among algae, and thus, enable higher ^{13}C-discrimination.

While our results are consistent with $\delta^{13}C$ indicating algal productivity, a dietary shift could be an alternative hypothesis for variation in midge $\delta^{13}C$ values. After benthic algae, phytoplankton is the most likely potential carbon source for midges. Because phytoplankton have low $\delta^{13}C$ values relative to benthic algae (Hecky and Hesslein 1995), under this hypothesis our statistical results would imply that high midge abundance would increase midge reliance on phytoplankton. Phytoplankton production is low relative to benthic production in Mývatn except during intermittent cyanobacteria blooms; thus, this explanation would seemingly require increased cyanobacterial blooms caused by high midge densities. However, high *T. gracilentus* densities should hypothetically inhibit, rather than contribute to, cyanobacteria blooms, due to the stabilizing and oxygenating effects of their tube-building, which should decrease the flux of phosphate to the water column (Einarsson et al. 2004). Finally, visual observations of *T. gracilentus* gut contents show consistent feeding habits and reliance on benthic diatoms and associated detritus (Ingvason et al. 2004; unpublished data). Thus, the alternative hypothesis that diet shifts cause changes in $\delta^{13}C$ values does not fit what we know about the biology of Mývatn, in contrast to the hypothesis that $\delta^{13}C$ values reflect benthic algal productivity.

The time-series analysis revealed additional biologically interesting information. While lower than the autocorrelation of *T. gracilentus* abundance, b_{11}, the magnitude of autocorrelation

for $\delta^{13}C$ values, b_{22}, was moderately high. Autocorrelation measures how quickly variables change through time, and relatively high autocorrelation for $\delta^{13}C$ is surprising given the short generation times of algae compared to midges; the rate at which the algal population could grow could potentially greatly reduce autocorrelation. The estimated autocorrelation for $\delta^{13}C$ suggests that other factors than algal population growth rates are important for benthic algal productivity. Possibilities include changes in nutrient reserves in the sediment that limit algal productivity, and the positive effect of midges on algal productivity by providing a solid substrate on which to grow (midge tubes), which change on a similar time scale as midge generations.

The time-series analysis also shows that *T. gracilentus* $\delta^{13}C$ values were typically higher in summer than spring generations, indicated by $c_{21} > 0$. This seasonal difference could reflect more productive algae in summer, as this season provides much greater light availability than spring. Alternatively, the seasonal trend in midge $\delta^{13}C$ values could reflect different $\delta^{13}C$ signatures of inorganic carbon used by algae. Ice-off at Mývatn occurs in late spring (mid-May), but light can penetrate ice once snow has melted, similar to other high-latitude lakes (Karlsson et al. 2008). Thus, benthic photosynthesis likely occurs under the ice in spring. Because ice prevents atmospheric CO_2 influx into lakes (Striegl et al. 2001), the most common carbon source for photosynthesis likely comes from heterotrophic respiration of benthic detritus (Karlsson et al. 2008). This likely influences algal $\delta^{13}C$ values, as CO_2 derived from microbial degradation of organic matter is ^{13}C-depleted compared to atmospheric CO_2 (Hecky and Hesslein 1995).

We investigated how preservation methods similar to those used in Mývatn's long-term monitoring may affect $\delta^{13}C$ values and found more enriched $\delta^{13}C$ values when propylene glycol, rather than formalin, was the initial preservative (Appendix 2). However, this experimental result is inconsistent with the results from the time-series model, which estimated a lower mean $\delta^{13}C$

value when propylene glycol was used. The reason for these conflicting results is unclear, but differences in study organism (black flies in the experiment vs. midges in the time-series) or isotopic signatures of chemical preservatives (long-term monitoring vs. our supplementary experiment) may have contributed to the discrepancy (Appendix 2). While we performed the supplemental experiment to understand how chemical preservation may affect our archived specimens, our statistical approach provided an alternate way to account for this potential artifact, and model results were similar regardless of the preservation covariate's inclusion. The lack of correspondence between our model estimate and supplemental experiment should not incumber interpretation of $\delta^{13}C$ patterns because chemical preservation effects are largely independent of storage duration (Sweeting et al. 2004, Syväranta et al. 2008; Appendix 2), and we were more interested in temporal trends in $\delta^{13}C$ values than the specific signatures per se.

Attempts to link consumer isotopic signatures and population dynamics have been done in an "exploratory" nature (Wainright et al. 1993), but to our knowledge, this is the first study to explicitly examine interactions between consumer abundance and resources based on their isotopic signatures. Our results show strong interactions between consumer populations and algal primary production and provide support that midge-algal interactions cause the 5-orders-of-magnitude *T. gracilentus* population fluctuations in Mývatn. Thus, we have provided new evidence that the dynamics of *T. gracilentus* in Mývatn is one of the rare examples in which interactions between primary consumer and primary producers are strong enough to drive large fluctuations in abundances. In this example, $\delta^{13}C$ values provide a valuable proxy for resource availability when long-term data are nonexistent. Temporal records of stable isotope signatures may similarly lead to valuable insights for other consumer populations.

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LITERATURE CITED

Blight, L. K., K. A. Hobson, T. K. Kyser, and P. Arcese. 2015. Changing gull diet in a changing world: A 150-year stable isotope (d13C, d15N) record from feathers collected in the Pacific Northwest of North America. Global Change Biology 21:1497–1507.

Einarsson, Á., A. Gardarsson, G. M. Gíslason, and A. R. Ives. 2002. Consumer – resource interactions and cyclic population dynamics of Tanytarsus gracilentus (Diptera: Chironomidae). Journal of Animal Ecology 71:832–845.

Einarsson, Á., U. Hauptfleisch, P. R. Leavitt, and A. R. Ives. 2016. Identifying consumer-resource population dynamics using paleoecological data. Ecology 97:361–371.

Einarsson, Á., G. Stefánsdóttir, H. Jóhannesson, J. S. Ólafsson, G. M. Gíslason, I. Wakana, G. Gudbergsson, and A. Gardarsson. 2004. The ecology of Lake Myvatn and the River Laxa: Variation in space and time. Aquatic Ecology 38:317–348.

Finlay, J. C. 2001. Stable carbon isotope ratios of river biota: implications for energy flow in lotic food webs. Ecology 82:1052–1064.

Fry, B. 2006. Stable Isotope Ecology.

Gardarsson, A., Á. Einarsson, G. M. Gíslason, T. Hrafnsdóttir, H. R. Ingvason, E. Jónsson, and J. S. Ólafsson. 2004. Population fluctuations of chironomid and simuliid Diptera at Myvatn in 1977-1996. Aquatic Ecology 38:209–217.

Grey, J., C. T. Graham, J. R. Britton, and C. Harrod. 2009. Stable isotope analysis of archived roach (Rutilus rutilus) scales for retrospective study of shallow lake responses to nutrient reduction. Freshwater Biology 54:1663–1670.

Harvey, A. C. 1989. Forecasting, structural time series models and the Kalman filter. Cambridge University Press, Cambridge, UK.

Hecky, R. E., and R. H. Hesslein. 1995. Contributions of benthic algae to lake food webs as revealed by stable isotope analysis. Journal of the North American Benthological Society 14:631–653.

Hill, W. R., and R. G. Middleton. 2006. Changes in carbon stable isotope ratios during periphyton development. Limnology and Oceanography 51:2360–2369.

Ingvason, H. R., J. S. Ólafsson, and A. Gardarsson. 2004. Food selection of Tanytarsus gracilentus larvae (Diptera: Chironomidae): an analysis of instars and cohorts. Aquatic Ecology 38:231–237.

Ives, A., B. Dennis, K. Cottingham, and S. Carpenter. 2003. Estimating community stability and ecological interactions from time-series data. Ecological Monographs 73:301–330.

Ives, A. R., and V. Dakos. 2012. Detecting dynamical changes in nonlinear time series using locally linear state-space models. Ecosphere 3:58.

Ives, A. R., A. Einarsson, V. A. A. Jansen, and A. Gardarsson. 2008. High-amplitude fluctuations and alternative dynamical states of midges in Lake Myvatn. Nature 452:84–87.

Jónasson, P. M. 1979. The Lake Mývatn ecosystem, Iceland. Oikos 32:289–305.

Karlsson, J., J. Ask, and M. Jansson. 2008. Winter respiration of allochthonous and autochthonous organic carbon in a subarctic. Limnology and Oceanography 53:948–954.

Lindegaard, C., and P. M. Jónasson. 1979. Abundance , population dynamics and production of zoobenthos in Lake Myvatn , Iceland. Oikos 32:202–227.

Murdoch, William W., Cheryl J. Briggs, and Roger M. Nisbet. Consumer-resource dynamics. Vol. 36. Princeton University Press, 2003.

Phillips, J. S., A. R. McCormick, Á. Einarsson, S. N. Grover, and A. R. Ives. 2019. Spatiotemporal variation in the sign and magnitude of ecosystem engineer effects on lake ecosystem production. Ecosphere 10:e02760.

R Core Team 2019. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Striegl, R. G., P. Kortelainen, and J. P. Chanton. 2001. Carbon dioxide partial pressure and 13C content of north temperate and boreal lakes at spring ice melt. Limnology and Oceanography 46:941–945.

Sweeting, C. J., N. V. C. Polunin, and S. Jennings. 2004. Tissue and fixative dependent shifts of δ13C and δ15N in preserved ecological material. Rapid Communications in Mass Spectrometry 18:2587–2592.

Syväranta, J., S. Vesala, M. Rask, J. Ruuhijärvi, and R. I. Jones. 2008. Evaluating the utility of stable isotope analyses of archived freshwater sample materials. Hydrobiologia 600:121–130.

Wainright, S. C., M. J. Fogarty, R. C. Greenfield, and B. Fry. 1993. Long-term changes in the Georges Bank food web: trends in stable isotope compositions of fish scales. Marine Biology 115:481–493.

Table 1. Parameter estimates from the state-space model.

Coefficient	Estimate
b_{10}	-0.99
b_{20}	-0.32
b_{11}	0.84
b_{22}	0.42
b_{12}	0.26
b_{21}	-0.31
c_{21}	1.37
c_{22}	-0.59
σ_1	0.61
σ_2	0.45
υ_1	0.0
υ_2	3.1
LL	-153.94

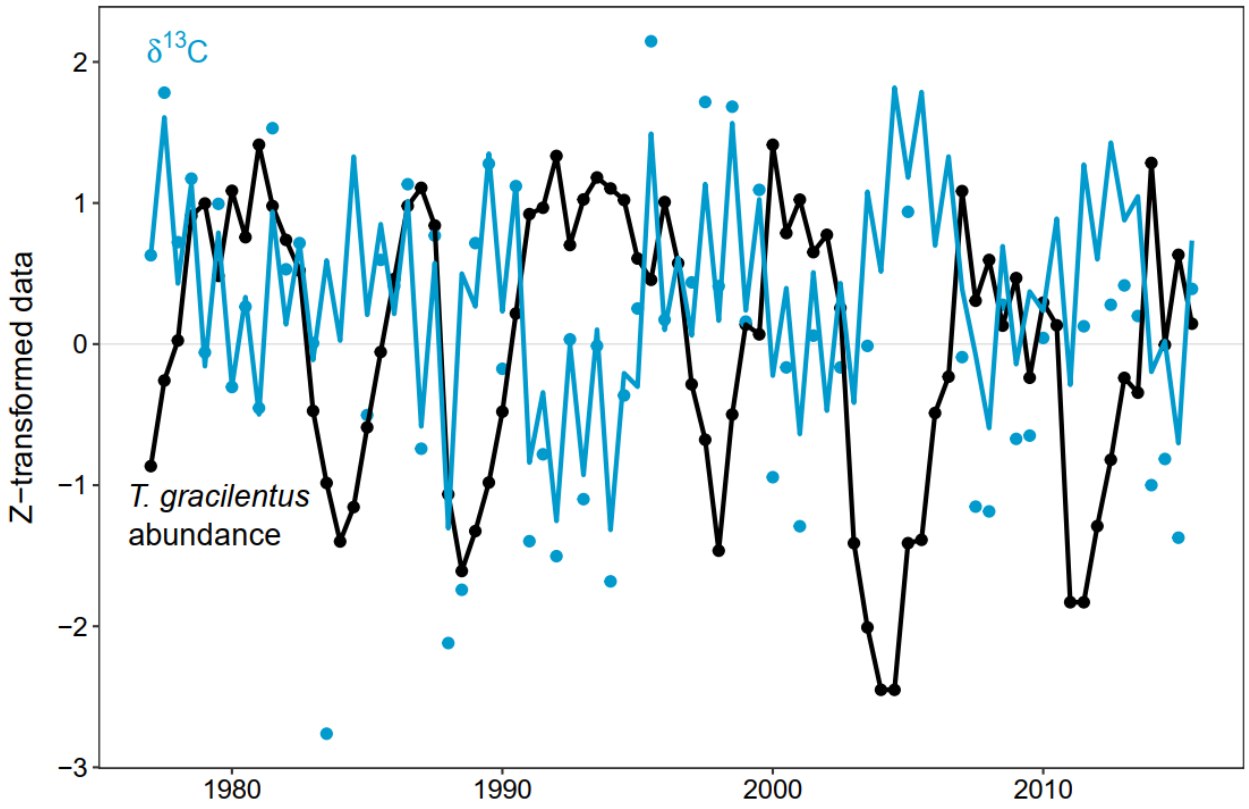

Figure 1. Solid lines show model fits to *T. gracilentus* abundance (black) and $\delta^{13}C$ values (blue). Points give values after standardization.

Appendix C: Supplementary methods and results for Chapter 3 (Part I)

Examining algal $\delta^{13}C$ values across a light gradient

Supplementary Methods

Our study's primary objective was examining consumer-resource interactions using long-term midge abundance data and their corresponding $\delta^{13}C$ values. As *Tanytarsus gracilentus* are primary consumers that rely on benthic algae and detritus, we assume that their $\delta^{13}C$ signatures should reflect variation in $\delta^{13}C$ values of benthic primary producers. Previous studies have demonstrated that enriched primary producer $\delta^{13}C$ values are associated with high biomass and/or primary production because as the demand for inorganic carbon increases relative to its availability, algae lower their discrimination against ^{13}C during photosynthesis (Hecky and Hesslein 1995, Hill and Middleton 2006, Devlin et al. 2013). The goal of this supplemental experiment was to examine whether enriched $\delta^{13}C$ values were associated with benthic productivity in Mývatn.

We chose an experimental approach to help isolate the link between productivity and carbon isotope signatures. We manipulated light as a means of affecting benthic primary production because light is a limiting factor for benthic productivity in many lakes (Vadeboncoeur et al. 2014), including Mývatn (Phillips et al. 2019), and then examined the effect of experimental shading on benthic algal $\delta^{13}C$ values. In July 2018, we collected sediment cores (50 cm height, 5 cm inner diameter) with a Kajak corer from a 2.7-m deep site in the southwest region of Mývatn. The top 15 cm from each core was extruded into clear acrylic tubes (33 cm height, 5 cm inner diameter), such that the vertical structure of the cores remained intact. We plugged the bottom of each 33-cm tube with a circular piece of foam and secured plastic

material around the tube's bottom perimeter. The experimental sediment cores were arranged within an opaque crate that had circular openings to keep the tubes evenly spaced and upright. The sediment surface within each tube was level with the crate's opaque top, with the remaining ~15 cm of each clear tube extending beyond the top of the crate. Thus, sediment below the surface layer was not exposed light, mimicking in situ benthic conditions.

We assigned cores to one of five shading treatments, such that cores were left uncovered (i.e., full light), covered with mosquito netting (1, 4, or 7 layers), or covered with multiple layers of opaque black plastic (i.e., full dark). Each shading treatment had 4 replicates, but one full light core was lost during the experiment. For the shading treatments, we wrapped the upper 15 cm of each tube with the appropriate number of netting or black plastic. Cores were distributed between two crates, such that each crate had two full sets of each shading treatment. Before the start of the experiment, we measured photosynthetically active radiation (PAR; μmol photons m^{-2} s^{-1}) (Li-192 Quantum Underwater Sensor, Li-COR, Lincoln, Nebraska, USA) outside and inside the acrylic tubes of each treatment to determine light reduction imposed by shading,

We deployed the crates on the lake bottom in a nearshore bay with a depth of 1.8 m. Experimental cores were open to exchange with the overlying lake water, though we attached circular plastic discs ~5 cm above each core's opening to block sediment deposition or settling of phytoplankton into the experimental tubes. Partway through the experiment, the crates were moved to the eastern part of the lake (2.6 m depth) because of a dense cyanobacteria bloom that reached the original experiment location.

After one month, we measured benthic metabolism and sampled the surface sediments for stable carbon isotope analysis. We measured net ecosystem production (NEP) and ecosystem respiration (ER) with paired incubations of each experimental tube. Incubations to measure NEP

were performed with the ambient shading treatments on each tube. For the full dark treatment, we rolled the plastic shading down several centimeters for the NEP incubation, such that light availability during the incubation mimicked light availability during the experiment's month-long duration (as tubes were open to the lake, a small amount of light likely reached the sediment through the tube opening). For the ER measurements, we subjected all tubes to complete darkness by wrapping them with multiple layers of black plastic. Metabolism measurements were based on the change in dissolved oxygen (DO) concentrations, which we measured at the start and end of each incubation with handheld ProODO Probes (YSI, Yellow Springs, Ohio, USA). During the incubation periods, cores were sealed airtight with opaque rubber stoppers (sensu Phillips et al. 2019), and crates were deployed at the experimental depth (2.6 m). NEP was calculated as the hourly change in evolved O_2 (mg O_2 L-1 h^{-1}) during incubations with the experimental shade treatments, and ER corresponded to the rate of oxygen consumption (mg O_2 L-1 h^{-1}) during complete darkness. Under the assumption that ER is independent of light, we calculated gross primary production (GPP) for each core as the summed magnitudes of NEP and ER. We converted metabolic rates to areal units by multiplying the volumetric rates by the water column height overlying the sediment in the tube. We estimated PAR availability within the tubes during the NEP incubation based on the benthic light levels and the light reduction capacity of each shade treatment. To examine how GPP responded to the shade treatments, we fit a photosynthesis-irradiance (P-I) curve, using a hyperbolic tangent function, to the measured GPP rates. We examined whether ER differed across shade treatments with a linear model.

After the incubations, we scraped the top 0.75-cm layer from the sediment surface, which should reflect the most photosynthetically active layer of benthic algae (sensu Chapter 4). We sieved the material through 500-µm mesh and collected the filtered material on 20-µm mesh.

Sediment samples were dried at 60 °C. We did not acidify sediment samples because previous results showed that HCl acidification (Harris et al. 2001) did not change the $\delta^{13}C$ signatures of sediment from Mývatn (Chapter 4). Sediment samples were homogenized with plastic pestle, re-dried, stored in a room temperature desiccator for 24 h, and weighed into tin capsules for $\delta^{13}C$ analysis. Stable isotope analysis was performed by the University of California Davis Stable Isotope Facility (Davis, CA, USA) (see Main text: Methods). We examined the relationship between shade treatment and surface sediment $\delta^{13}C$ values with a linear model.

Supplementary Results

Shade treatments were imposed on the experimental cores for one month, but ER did not differ across shade treatments at the end of the experiment ($t_{17} = 1.03$, $p = 0.317$; Figure S1). The response of GPP to light (based on light levels measured during the NEP incubation) were typical of a P-I curve, whereby GPP initially increased with light before plateauing at higher light availability (Figure S1). While we only measured GPP at the end of the experiment, it is reasonable to expect that cores with higher light availability had higher GPP during the duration of the experiment.

Surface sediment $\delta^{13}C$ values were significantly affected by shading treatment; more enriched $\delta^{13}C$ values were associated with treatments providing greater light availability (Figure S2). As ER was not affected by shade treatment, productivity differences across the light treatments likely accounted for the difference in $\delta^{13}C$. This interpretation is consistent with enhanced competition among benthic algae for inorganic carbon under high light conditions, which reduces discrimination against ^{13}C. Overall, results from this supplemental experiment

demonstrate that factors affecting primary production influence $\delta^{13}C$ values of benthic algae in Mývatn, supporting their suitability to serve as a proxy for benthic primary production.

References

Devlin, S. P., M. J. Vander Zanden, and Y. Vadeboncoeur. 2013. Depth-specific variation in carbon isotopes demonstrates resource partitioning among the littoral zoobenthos. Freshwater Biology 58:2389–2400.

Harris, D., W. R. Horwath, and C. van Kessel. 2001. Acid fumigation of soils to remove carbonates prior to total organic carbon or carbon-13 isotopic analysis. Soil Sci. Soc. Am. J. 65:1853–1856.

Hecky, R. E., and R. H. Hesslein. 1995. Contributions of benthic algae to lake food webs as revealed by stable isotope analysis. Journal of the North American Benthological Society 14:631–653.

Hill, W. R., and R. G. Middleton. 2006. Changes in carbon stable isotope ratios during periphyton development. Limnology and Oceanography 51:2360–2369.

Phillips, J. S., A. R. McCormick, Á. Einarsson, S. N. Grover, and A. R. Ives. 2019. Spatiotemporal variation in the sign and magnitude of ecosystem engineer effects on lake ecosystem production. Ecosphere 10:e02760.

Vadeboncoeur, Y., S. P. Devlin, P. B. Mcintyre, and M. J. Vander Zanden. 2014. Is there light after depth? Distribution of periphyton chlorophyll and productivity in lake littoral zones. Freshwater Science 33:524–536.

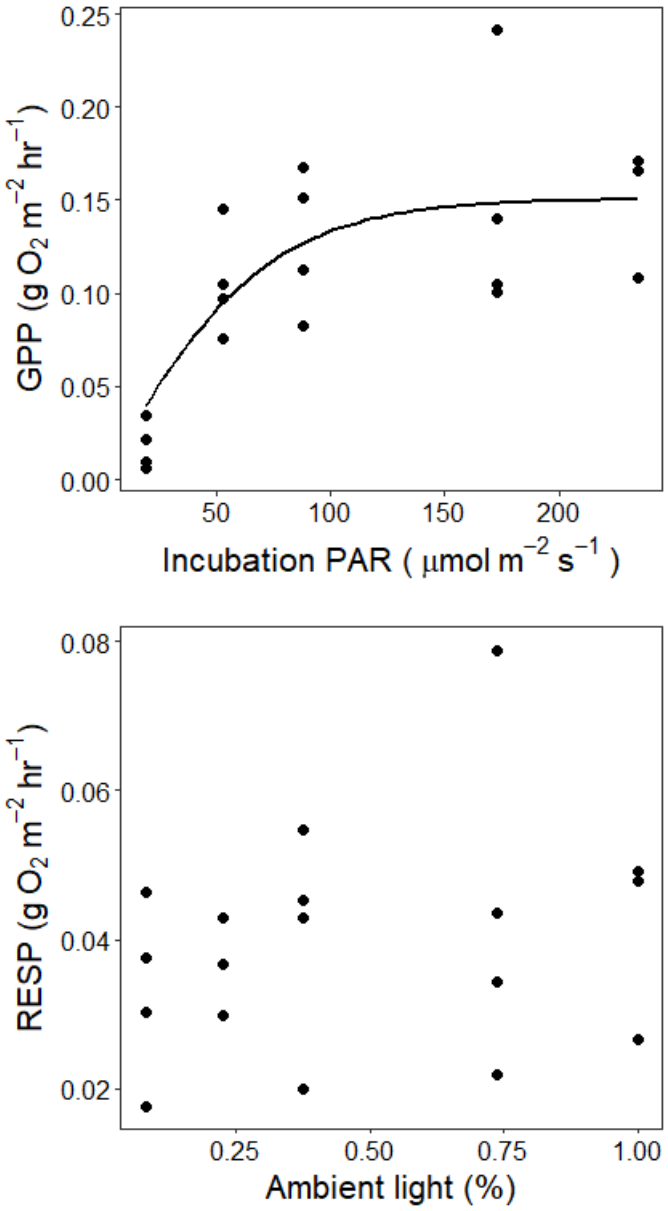

Figure S1. Measured gross primary production and respiration for sediment cores that experienced different shading treatments for one month. Values on the x-axis of the GPP panel depict the light levels experienced by each core during the incubation in which net ecosystem production was measured. The line shows the fit of a hyperbolic-tangent curve to the GPP data.

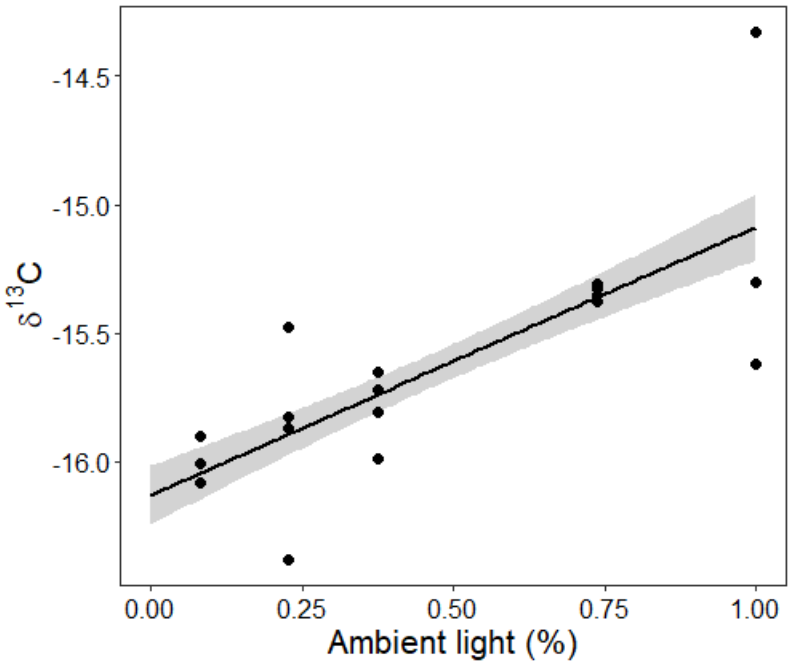

Figure S2. Sediment $\delta^{13}C$ values were more enriched with increasing ambient light availability (i.e., less shading). Points show raw data, and lines correspond to the fit from the linear model with standard errors.

Appendix D: Supplementary methods and results for Chapter 3 (Part II)

Quantifying preservation effects on $\delta^{13}C$ values

Supplementary Methods

The primary objective of our study was comparing the trends in midge abundance and their $\delta^{13}C$ values, such that the temporal trends in $\delta^{13}C$ values were of greater interest than the exact values. However, preservation effects were nonetheless important to consider because window trap preservative methodologies changed in 2000, and chironomid samples experienced long-term storage in ethanol for varying durations (main text: Methods). Thus, we designed a small experiment to examine storage effects on dipteran $\delta^{13}C$ signatures.

When we set up the experiment in early August 2015 there were no emergent chironomids, so we instead collected adult *Simulium* spp. (Diptera: Simuliidae) with sweep-nets near the southwestern shore of Mývatn. Simuliids likely came the River Laxá, which forms Mývatn's outlet. We acknowledge that preservation effects may differ between simuliids and chironomids. However, studies examining the effects of particular chemical preservatives on multiple taxa of a similar group (e.g., different fishes or macroinvertebrates) have generally reported $\delta^{13}C$ shifts in a consistent direction (i.e., either ^{13}C-enrichment or ^{13}C-depletion), even if the magnitude of this effect differs among taxa (Kelly et al. 2006, Syväranta et al. 2008, Xu et al. 2011, Rennie et al. 2012). Thus, chemical preservation likely affects simuliids in a qualitatively similar way to chironomids, although the magnitude of isotopic shifts may differ.

After freezing simuliids for 48 h, we processed control samples and set up preservation treatments. The setup of this experiment was designed to mimic the preservation regime experienced by the archived chironomids which may spend up to two weeks in the window trap

preservative mixture (formalin pre-2000 or propylene glycol post-2000) before they experience longer term storage in ethanol. “Control” samples refer to replicate subsamples (n=4) of 15 simuliids that were randomly collected from the frozen individuals and dried at 60 °C for 48 h before being prepared for isotopic analysis. In 125-mL plastic containers, we set up two preservative treatments (n=3 replicates per treatment): 10% formalin (representing the window trap preservative used prior to 2000) and a 1:1 mixture of propylene glycol and water (representing the window trap preservative used from 2000 onward). Each of the six initial preservative containers received 75 simuliids and 30 mL of preservative solution. After 13 days, flies from all the initial containers were transferred into new 20-mL plastic vials filled with 15 mL of 70% ethanol and 2 mL of the preservation solution (to mirror the potential inoculation of long-term monitoring storage containers by residual window trap preservative). Hereafter, we refer to these preservation treatments as “formalin/ethanol” and “propylene glycol/ethanol.” Following the protocols used for preparing archived *T. gracilentus* specimens (main text: Methods), fifteen simuliids were homogenized and prepared for stable isotope analysis. From each preservation vial, we analyzed pooled samples 1, 27.5, and 53 months (with different simuliids comprising the pools) after the transfer to ethanol.

We performed two statistical analyses to determine whether the $\delta^{13}C$ values of preserved samples 1) differed from control samples and 2) were affected by storage duration. To test whether preservation method affected isotopic signatures, we used a linear model, with $\delta^{13}C$ as the response variable and treatment (3 levels: control, formalin/ethanol, propylene glycol/ethanol) as the predictor. In this analysis, we only included preserved specimens from the first sampling event (1 month post initial transfer), such that this statistical test did not account for any temporal changes in isotopic signatures due to storage duration. To test whether $\delta^{13}C$

values of preserved specimens were affected by storage duration, we omitted the control samples because they were only analyzed for one time point. We used a linear mixed model (LMM) with preservation treatment (2 levels: formalin/ethanol, propylene glycol/ethanol), number of months post initial transfer to ethanol, and their interaction as fixed effects and vial number as a random effect to account repeated sampling of simuliids from each vial.

Supplementary Results

Preservation treatment had a significant effect on $\delta^{13}C$ values ($F_{2, 7} = 11.81$; $p = 0.006$), when comparing controls to preserved specimens sampled 1 month after being transferred from their initial preservatives to ethanol.

Storage duration did not significantly affect $\delta^{13}C$ values of chemically preserved samples (Table S1; Figure S1). Thus, any chemical effects that altered $\delta^{13}C$ signatures likely took place within the first month of storage in ethanol. $\delta^{13}C$ values of the formalin/ethanol appeared more variable than those of the propylene glycol/ethanol treatment (Table S2; Figure S1), though this could partly be due to different individuals comprising pooled samples between sampling events. Type-III F-test results suggested no overall difference in $\delta^{13}C$ values between preservation treatments (Table 1); however, type-II F-test results indicated a significant difference between treatments (Table 1). Both preservation treatments were generally ^{13}C-enriched relative to controls, with the type-II F-test suggesting that the formalin/ethanol treatment caused less enrichment than propylene glycol/ethanol (Table S2; Figure S1).

Supplementary Discussion

Similar to numerous other studies, our experimental results demonstrate that chemical preservation can affect organism $\delta^{13}C$ signatures. Propylene glycol/ethanol and formalin/ethanol treatments generally resulted in ^{13}C-enrichment relative to controls. Storage in ethanol, which was common to both treatments, may have contributed to the similar direction of the $\delta^{13}C$ shifts, despite the different initial chemical preservatives between the treatments.

Many studies have shown enriched $\delta^{13}C$ values as a result of ethanol preservation (Kaehler and Pakhomov 2001, Sweeting et al. 2004, Kelly et al. 2006, Syväranta et al. 2008, Ventura and Jeppesen 2009, Xu et al. 2011); studies generally attribute these effects to degradation of lipids, which are ^{13}C-depleted relative to other tissues (DeNiro and Epstein 1977). Decreased tissue C:N ratios with ethanol preservation support this mechanism (Sweeting et al. 2004, Syväranta et al. 2008). In our experiment, simuliid C:N ratios from control samples were higher than the C:N ratios in any of our preserved samples, though control samples also had greater variability in C:N ratios (Figure S2). Moreover, C:N ratios between the formalin/ethanol and propylene glycol/ethanol treatments are relatively similar (Figure S2). Thus, degradation of lipids is a reasonable explanation for enrichment of simuliid $\delta^{13}C$ values.

We are not aware of previous studies that have investigated the effect of propylene glycol preservation on stable isotope signatures. It is possible that initial storage in propylene glycol had either 1) little effect on $\delta^{13}C$ values such that the subsequent ethanol storage solely contributed to ^{13}C-enrichment or 2) redundant effects on $\delta^{13}C$ as ethanol, such that changes to $\delta^{13}C$ values had already occurred before the transfer to ethanol.

Our experimental formalin/ethanol treatment generally resulted in ^{13}C-enrichment but with a lower magnitude than the propylene glycol/ethanol treatment; though, two formalin/ethanol samples (from different vials) were lower than the mean control $\delta^{13}C$ value at

27.5 or 53 months of storage (Figure S1). A majority of previous studies document that storage in formalin storage causes ^{13}C-depletion (Bosley and Wainright 1999, Kaehler and Pakhomov 2001, Sarakinos et al. 2002, Kelly et al. 2006, Xu et al. 2011, Rennie et al. 2012 but see Feuchtmayr and Grey 2003 who found ^{13}C-enrichment). Several studies have proposed addition of relatively ^{13}C-depleted carbon from the formalin preservative to organism tissues as a mechanism for this ^{13}C-depletion (Kaehler and Pakhomov 2001, Edwards et al. 2002, Sarakinos et al. 2002, Sweeting et al. 2004, Kelly et al. 2006). Thus, samples in our formalin/ethanol treatment may have experienced sequential ^{13}C-depletion (during the initial two weeks in formalin) and ^{13}C-enrichment (during subsequent storage in ethanol) during the preservation process.

The higher degree of ^{13}C-enrichment when propylene glycol rather than formalin was the initial preservative in our experiment is inconsistent with our time series results (main text: Results), which suggested a decrease in the mean δ^{13}C value after 2000, when propylene glycol was the window trap preservation. While we cannot specify with certainty why these results conflict with one another, we outline several possibilities here. First, preservation effects can be taxon-specific (e.g., Edwards et al. 2002; Kelly et al. 2006), such that the different focal organisms (i.e., chironomids vs. simuliids) could have contributed to inconsistencies between our experimental and model results. For instance, if fat content varied between taxa, this could affect the degree of ^{13}C-enrichment from ethanol preservation (Sweeting et al. 2004). However, similar species generally show isotopic shifts in the same direction for a given preservative (see Supplemental Methods). Thus, it is unlikely that taxon-specific responses of chironomids and simuliids would explain the opposing patterns from our experimental and model results. Second, unlike our experiment, in which the initial preservation duration was standardized to 13 days,

chironomids from the long-term monitoring samples were exposed to the initial preservative for varying lengths of time (i.e., from < 1 day to 2 weeks, depending on the time interval between falling into the trap and trap collection). Edwards et al. (2002) found that the length of formalin fixation did not affect ^{13}C-depletion for storage durations longer than 10 days, though, it is unknown whether effects of formalin fixation vary at shorter (i.e., < 10 days) storage durations. Variation in chironomid exposure time to the initial chemical preservative does not provide an obvious means of reconciling the contrasting results from our experiment and model, but it could nonetheless affect ultimate preservation effects. Third, differences in the isotopic composition of the preservatives used in the long-term monitoring and our experiment may have affected the direction and magnitude of isotopic shifts. For instance, formalin $\delta^{13}C$ values can vary among manufacturers, and larger shifts in tissue $\delta^{13}C$ values occur in formalin with more depleted $\delta^{13}C$ values (Edwards et al. 2002). Alternatively, it is possible that the time series results represent a biological change rather than an artifact of preservation methodologies (i.e., there was a decline in the mean $\delta^{13}C$ value of basal resources consumed by *T. gracilentus* after 2000). However, we do not know of a factor that would contribute to this change. Furthermore, it seems unlikely that it would coincidentally align with the change in preservation methodology. In short, we cannot undoubtedly determine an explanation for the discrepancy between the experimental preservation effects and the model results. Nonetheless, this does not hamper the interpretation of our data because we were interested in the temporal trends in $\delta^{13}C$ values rather than reconstructing the actual isotopic signatures. Our results mirror previous studies in suggesting changes to $\delta^{13}C$ values likely occurred early in the preservation process. We did not see a significant change in the $\delta^{13}C$ of preserved samples after being stored for over 4 years; similarly, multiple studies have

demonstrated that isotopic shifts are independent of storage duration (Edwards et al. 2002, Sweeting et al. 2004, Syväranta et al. 2008, Xu et al. 2011, Rennie et al. 2012).

References

Bosley, K. L., and S. C. Wainright. 1999. Effects of preservatives and acidification on the stable isotope ratios (15N:14N, 13C:12C) of two species of marine animals. Canadian Journal of Fisheries and Aquatic Sciences 56:2181–2185.

DeNiro, M. J., and S. Epstein. 1977. Mechanism of carbon isotope fractionation associated with lipid synthesis. Science 197:261–263.

Edwards, M. S., T. F. Turner, and Z. D. Sharp. 2002. Short- and long-term effects of fixation and preservation on stable isotope values (d13C, d15N, d34S) of fluid-preserved museum specimens. Copeia 4:1106–1112.

Feuchtmayr, H., and J. Grey. 2003. Effect of preparation and preservation procedures on carbon and nitrogen stable isotope determinations from zooplankton. Rapid Communications in Mass Spectrometry 17(23):2605-2610.

Kaehler, S., and E. A. Pakhomov. 2001. Effects of storage and preservation on the Δ13C and Δ15N signatures of selected marine organisms. Marine Ecology Progress Series 219:299–304.

Kelly, B., J. B. Dempson, and M. Power. 2006. The effects of preservation on fish tissue stable isotope signatures. Journal of Fish Biology 69:1595–1611.

Rennie, M. D., T. Ozersky, and D. O. Evans. 2012. Effects of formalin preservation on invertebrate stable isotope values over decadal time scales. Canadian Journal of Zoology 90:1320–1327.

Sarakinos, H. C., M. L. Johnson, and M. J. Vander Zanden. 2002. A synthesis of tissue-preservation effects on carbon and nitrogen stable isotope signatures. Canadian Journal of Zoology 80:381–387.

Sweeting, C. J., N. V. C. Polunin, and S. Jennings. 2004. Tissue and fixative dependent shifts of δ13C and δ15N in preserved ecological material. Rapid Communications in Mass Spectrometry 18:2587–2592.

Syväranta, J., S. Vesala, M. Rask, J. Ruuhijärvi, and R. I. Jones. 2008. Evaluating the utility of stable isotope analyses of archived freshwater sample materials. Hydrobiologia 600:121–130.

Ventura, M., and E. Jeppesen. 2009. Effects of fixation on freshwater invertebrate carbon and nitrogen isotope composition and its arithmetic correction. Hydrobiologia 632:297–308.

Xu, J., Q. Yang, and M. Zhang. 2011. Preservation effects on stable isotope ratios and consequences for the reconstruction of energetic pathways. Aquatic Ecology 45:483–492.

Table S1. Statistical results for the LMM examining the effect of storage duration on $\delta^{13}C$ values of samples under different preservation treatments (formalin/ethanol, propylene glycol/ethanol). Results are shown from Type-III and Type-II F-tests with Kenward-Roger denominator degrees of freedom.

Term	**Type III**		**Type II**	
Months in storage	$F_{1, 10.00} = 3.20$	$P = 0.104$	$F_{1, 10.00} = 1.84$	$P = 0.205$
Preservation trmt	$F_{1, 13.43} = 2.67$	$P = 0.126$	$F_{1, 4.00} = 17.27$	$P = 0.014$
Months in storage x Preservation trmt	$F_{1, 10.00} = 1.38$	$P = 0.267$		

Table S2. Differences in the $\delta^{13}C$ values ($\Delta\ \delta^{13}C$) between preservation treatments and the mean control value (-26.71‰). Values shown are the mean ± SD for each treatment at 1, 27.5, or 53 months post transfer from the initial preservative to ethanol.

	Δ $\delta^{13}C$		
Preservation Treatment	**1 month**	**27.5 months**	**53 months**
formalin/ethanol	0.64 ± 0.09	0.25 ± 0.76	-0.21 ± 1.16
propylene glycol/ethanol	1.32 ± 0.52	1.51 ± 0.17	1.26 ± 0.28

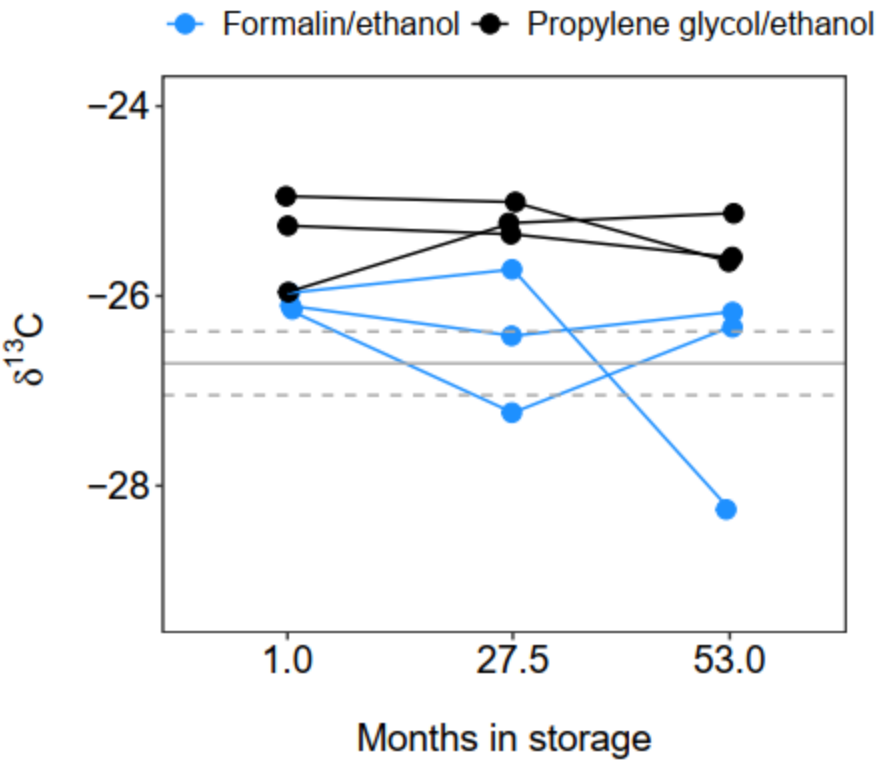

Figure S1. $\delta^{13}C$ values of chemically preserved simuliids samples. Points shows the raw data, with colored lines connecting samples from the same vial over time. The solid horizontal line shows the mean $\delta^{13}C$ value of the control samples, and the dashed horizontal lines show 1 standard deviation above and below this mean control value.

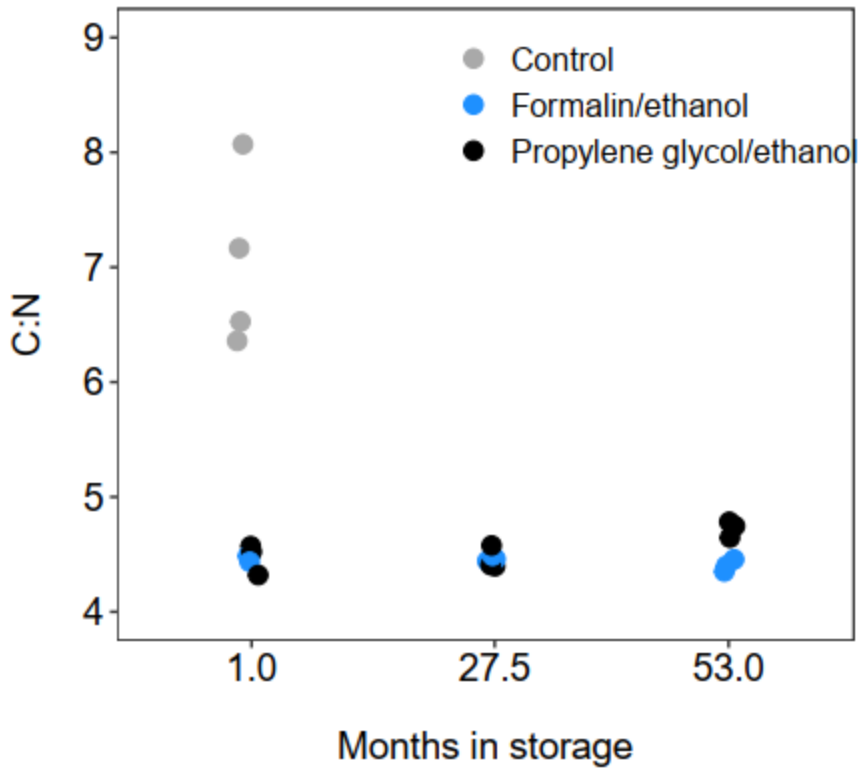

Figure S2. C:N ratios of control and chemically preserved simuliid samples. Points are slightly jittered horizontally, which partially obscures that controls were processed at month "0".

CHAPTER 4

Resource partitioning between midge larvae inferred from stable isotope analysis

Status: in preparation for submission to *Ecology*

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Running head: Resource use of coexisting consumers

Keywords: sediment, tube-building, benthos, primary production, *Tanytarsus*, *Chironomus*

ABSTRACT

Resource partitioning among ecologically similar species may weaken interspecific competitive and facilitate species coexistence and co-occurrence in the same habitat. *Tanytarsus gracilentus* and *Chironomus islandicus* (Diptera: Chironomidae) larvae coexist at high abundances in Lake Mývatn, Iceland. Both species have similar biologies, feeding from within silk tubes in the sediment on similar benthic resources. Furthermore, both species show large, roughly synchronized population fluctuations, implying that they rely on the same fluctuating resources. These points pose the question of how these species coexist at high densities, and in this study, we investigate how these species co-occur. We first examine whether larvae partition habitats across different sites in the lake. The abundances of both species are positively correlated through space, even at the scale of 20 cm^2. Thus, there is no evidence of spatial partitioning. To investigate resource partitioning, we inferred differences in *T. gracilentus* and *C. islandicus* diets through interspecific comparisons of larval $\delta^{13}C$ values and comparisons of these signatures to those of surface sediments. Additionally, we assessed interspecific differences in $\delta^{13}C$ over multiple generations from archived adult specimens collected from 1977-2015. Relative to *C. islandicus*, *T. gracilentus* $\delta^{13}C$ values were significantly higher for larvae ($\Delta\delta^{13}C$ = 5.39 ± 1.84‰) and adults (2.12 ± 1.47‰), suggesting differences in their resource use. Stable carbon isotopes also show that neither species indiscriminately feeds on surface sediments. Relative to surface sediments, *T. gracilentus* larvae had higher $\delta^{13}C$ values (1.84 ± 0.96‰), suggesting that they selectively feed on ^{13}C-enriched resources (i.e., the most productive algal cells). In contrast, *C. islandicus* had lower values than surface sediments (-2.87 ± 1.95‰), suggesting that *C. islandicus* feed within deeper sediment layers or selectively consume resources with low $\delta^{13}C$ signatures from surface sediments. Overall, our results suggest *T.*

gracilentus and *C. islandicus* partition their use of sediment-associated resources, which may in part explain their coexistence at high densities at very local scales.

INTRODUCTION

Interspecific competition can influence population dynamics and the spatial distribution of species. In the classical formulation of resource partitioning, the strength interspecific competition is inversely related to their overlap in resource use (MacArthur and Levins 1967, Pacala and Roughgarden 1982). Therefore, resource partitioning can facilitate the coexistence of ecologically similar species (Pianka 1974). Examples of resource partitioning among similar species include using resources at temporally separate times (temporal partitioning), occupying distinct habitats (spatial partitioning), and specializing on different subsets of the resource (resource use partitioning) (Schoener 1974).

Interspecific competition can affect abundances of the participant species. For instance, negative correlations among ecologically similar species are often associated with interspecific competition (Schmitt 1985, Morin 2011), which in the most extreme cases may result in the competitive exclusion of one species from a given area (e.g., Connell 1961). Additionally, a decline in the abundance of one species (e.g., due to its specific response to an abiotic factor) may result in compensatory shifts in the abundance of a competing species (Tilman 1996, Micheli et al. 1999, Klug et al. 2000). While negative correlations among species are often considered an expectation of interspecific competition, competing species may also be expected to show synchronous dynamics and positive correlation in abundance. Because competing species share the same resources, resource fluctuations will affect competing species similarly, leading to positive correlations in abundance (Korpimäki 1994, Ives et al. 1999, Micheli et al.

1999, Ripa and Ives 2003). Synchronous fluctuations between competitors are especially likely when their interactions with resources are strong enough to drive consumer-resource population cycles; in this case, the cyclic dynamics of one competitor will entrain the cyclic dynamics of the other, leading to positive correlations in their densities through time (Ripa and Ives 2003).

In this study, we compared the abundances and resource use of two chironomid species, *Tanytarsus gracilentus* and *Chironomus islandicus* (Diptera: Chironomidae), that coexist at high abundances in Lake Mývatn, Iceland. The two species have similar biologies; both construct silk tubes within the sediment from which they feed and in which they are protected from invertebrate and vertebrate predators. Furthermore, both species show large population fluctuations, across five orders of magnitude for *T. gracilentus* and three for *C. islandicus*; population crashes occur episodically every 4-10 years (Einarsson et al. 2002, 2004, Ives et al. 2008; unpublished data). Multiple lines of evidence point to consumer-resource interactions with benthic diatoms as the driver of dramatic *T. gracilentus* population fluctuations (Einarsson et al. 2002, 2016, Ives et al. 2008; Chapter 3). *C. islandicus* fluctuations have been synchronous with *T. gracilentus* since at least 1977, when long-term monitoring of Mývatn's chironomid populations began, and this synchrony suggests that *T. gracilentus* and *C. islandicus* share similar benthic resources (Einarsson et al. 2002, 2004, Ives et al. 2008; Gardarsson et al. 2004). Given the biological similarities and synchronous dynamics of *T. gracilentus* and *C. islandicus*, it is pertinent to examine whether resource use overlap influences their population fluctuations through interspecific competition or, alternatively, whether resource use partitioning facilitates their coexistence at high densities.

Previous studies based on gut content analyses suggest high resource overlap between *T. gracilentus* and *C. islandicus*. Both species have been described as unselectively feeding on diatoms and detritus from surface sediments (Ólafsson 1987 as reported within Einarsson et al. 2004), and *T. gracilentus* gut contents were largely similar to the sediment surface, with selectivity for detritus or algae showing only small seasonal and ontogenetic variation (Ingvason et al. 2002, 2004). Fatty acid analysis has also supported *T. gracilentus* reliance on diatoms, as well as bacteria that are likely associated with detritus (Ingvason 2002); there are no corresponding fatty acid profiles of *C. islandicus* from Mývatn. The apparent similarities in *T. gracilentus* and *C. islandicus* diets give supporting evidence that shared resources may underlie the high-amplitude and synchronized *T. gracilentus* and *C. islandicus* fluctuations in Mývatn. However, they also pose the question of how *T. gracilentus* and *C. islandicus* coexist.

We investigated spatial partitioning and resource use partitioning as possible explanations for the coexistence of *T. gracilentus* and *C. islandicus*. Although *T. gracilentus* and *C. islandicus* populations fluctuate synchronously across generations in the monitoring data from Mývatn (Gardarsson et al. 2004), these long-term data are based on the counts of emergent adults and integrate midge abundances over much of the lake. This leaves the possibility that *T. gracilentus* and *C. islandicus* larvae occur in spatially distinct areas of the lake. Therefore, we compared larval *T. gracilentus* and *C. islandicus* abundances in Mývatn to assess their spatial correlation at multiple scales. We investigated possible differences in resource use of each species with stable carbon isotope analysis, which provides information about assimilated resources because there is relatively little difference in the ratio of $^{13}C/^{12}C$ isotopes between a consumer and its diet (Rounick and Winterbourn 1986). Even though reported gut contents for *T. gracilentus* and *C. islandicus* are very similar, interspecific comparisons of $\delta^{13}C$ might reveal differences in

resource use that are not apparent from gut contents, as stable isotope signatures provide an integrated record of assimilation by consumers (Rounick and Winterbourn 1986). We used adult *T. gracilentus* and *C. islandicus* specimens collected between 1977-2015 to investigate interspecific differences in $\delta^{13}C$ values over multiple generations. We also compared $\delta^{13}C$ values of *T. gracilentus* and *C. islandicus* larvae to each other and to surface sediment values to identify interspecific differences in resource selectivity. We examined *T. gracilentus* and *C. islandicus* larval $\delta^{13}C$ values across a depth gradient, as depth-associated patterns of resource availability and isotopic signatures can affect macroinvertebrate $\delta^{13}C$ values (Hershey et al. 2006, Solomon et al. 2008, Devlin et al. 2013). Lastly, we examined sediment $\delta^{13}C$ values at distinct layers to determine whether basal resource $\delta^{13}C$ signatures differ according to their vertical position within the benthic habitat.

METHODS

Study system

Lake Mývatn is located in northeastern Iceland (65°37'N, 17°00'W) and has high primary production, while receiving little external carbon inputs (Jónasson 1979). The lake is shallow, with a mean depth of 2.3 m in the main south basin. The smaller north basin is naturally shallower (mean natural depth 1 m), but diatomite mining from 1967-2004 created depressions >5 m deep (Einarsson et al. 2004). Much of the benthic substrate consists of sediment composed of diatomaceous frustules, organic matter, sand, and tephra (Jónasson 1979). Benthic algae (mainly epipelic diatoms) comprise a majority of whole-lake production, though phytoplankton production varies spatially and temporally within and across years (Einarsson et al. 2004; Chapter 2). Thus, available food sources for sediment-dwelling primary consumers are likely

limited to benthic algae, settled phytoplankton, detritus (potentially from benthic and pelagic sources), and sediment-associated microorganisms.

Benthic pathways dominate Mývatn's energy flow, and midge larvae are the dominant consumers in the lake (Jónasson 1979). *T. gracilentus* and *C. islandicus* larval densities may exceed 500,000 and 19,000 individuals m^{-2} (Lindegaard and Jónasson 1979, Thorbergsdóttir et al. 2004). Due to the higher individual biomass of *C. islandicus*, both species contribute strongly to secondary production, with *T. gracilentus* and *C. islandicus* respectively comprising 67% and 24% of zoobenthic production, in years with high midge abundances (Lindegaard and Jónasson 1979). While the two species are ecologically similar with respect to their habitat and tube-dwelling, they differ in life histories. *T. gracilentus* has two adult emergences per year in spring (late May) and summer (early August); the entire population generally emerges at once, although in some summer generations (e.g., 2015), a proportion of the population may remain in the larval stage and emergence in the following spring. *C. islandicus* individuals typically spend a year as larvae, though they may develop over two years (Lindegaard and Jónasson 1979). An annual *C. islandicus* emergence precedes or coincides with the spring *T. gracilentus* emergence.

Larval abundance and isotope sample collection

We routinely sample midge larvae at multiple sites in Mývatn as part of a long-term monitoring project. From 2015-2019, larvae were sampled from six sites 3-4 times per year (from late May to mid-August). Cores were collected with a Kajak corer (diameter = 5 cm), with replicate (n = 3-5, but typically n = 3) cores for each sampling event. The top 2-cm sediment layer was extruded from each core and sieved through 63-µm mesh and the remaining sediment was sieved through 125-µm mesh. Finer mesh is used for the top sediment to capture first instar

T. gracilentus larvae which reside in this layer. After recombining the material retained on the 63-µm and 125-µm mesh sieves for a given core, samples were subsampled to a target of 50-100 larvae, and midges were hand-picked and stored in 70% ethanol. Larvae were taxonomically identified to the tribe level (Tanytarsini and Chironomini, to which *T. gracilentus* and *C. islandicus* respectively belong) using a dissecting microscope. For the areas sampled in the lake, *T. gracilentus* and *C. islandicus* make up the vast majority of Tanytarsini and Chironomini.

During the summers of 2016-2018, we collected midge larvae and surface sediment for stable isotope analysis from sites ranging from 2.5 to 5.1 m in depth. In 2016, sample collection focused on the six sites from which larval abundance data were collected (see above), but in subsequent years, isotope sample collection focused on the main long-term monitoring station at Mývatn (depth = 3.3 m; Ives 2013) and sites in the lake's north basin. A complete list of isotope samples, including collection dates and locations, is contained in the Appendix (Table S1).

For surface sediment samples collected during 2016-2018, we used the top 0.75-cm of sediment from Kajak cores to represent the most photosynthetically active layer of benthic algae. These samples do not represent a pure sample of algal material and also contain the surface sediment microbial assemblage and detritus, although similar sampling techniques have been used to characterize benthic resources in other studies (Karlsson and Byström 2005, Devlin et al. 2013). In 2019, we collected additional cores in order to characterize $\delta^{13}C$ values at different layers within the sediment. Three cores were collected from two of the sites previously sampled for sediment isotope samples (with depths of 2.5 m and 4.25 m). A 0.75-cm layer of sediment was collected from the surface of these cores (as above), as well as 0.75-cm layers that were collected 5 cm and 10 cm below the sediment's surface. To remove midges and their tubes from

sediment samples, we sieved material through 500-µm mesh. The filtered material was collected on 20-µm mesh and transferred to a tin weigh boat for drying.

To collect midge larvae for stable isotope analysis, we sieved separate Kajak cores through 125-µm or 250-µm mesh and picked individuals from the remaining material into tap water, where they stayed for 48 hr at 4 °C to clear their guts. We used a dissecting microscope when necessary to distinguish Tanytarsini and Chironomini larvae. Because *T. gracilentus* and *C. islandicus* are the dominant members of these two groups, we refer to Tanytarsini and Chironomini larvae as *T. gracilentus* and *C. islandicus*, respectively. We collected mainly third and fourth instar individuals which are visually distinct from earlier instars. We pooled larvae for stable isotope analysis, with the number pooled dependent on individual mass and availability. Samples included a minimum of 8 *T. gracilentus* and 2 *C. islandicus* individuals, but we aimed to use more individuals when possible (up to ~100 *T. gracilentus* and ~30 *C. islandicus*). When possible, we analyzed larvae from replicate cores, but in many instances, replicates had to be combined from a site to reach the necessary biomass for isotopic analysis. Sediment and larvae samples were dried at 60 °C.

Adult abundance and isotope sample collection

Aerial insect populations at Mývatn have been monitored since 1977 with window traps (see Gardarsson et al. 2004 for additional details). Gardarsson et al. (2004) demonstrated the synchrony of *T. gracilentus* and *C. islandicus* population fluctuations from 1977-1996 based on these adult counts; here, we include more recent data to examine the correlation in *T. gracilentus* and *C. islandicus* adult counts from 1977-2015. The focal window trap used in this study is located on the peninsula (Sydri-Neslond) that separates Mývatn's south and north basins, and

captures a midge assemblage characteristic of profundal habitats (Gardarsson et al. 2004), in which *T. gracilentus* and *C. islandicus* have been shown to dominate zoobenthic communities (Lindegaard and Jónasson 1979). All adult midges were identified to species.

We obtained *T. gracilentus* and *C. islandicus* adult specimens from archived long-term monitoring samples for this trap (sensu Chapter 3) to examine interspecific differences in stable carbon isotope signatures. We aimed to collect 60 *T. gracilentus* and 30 *C. islandicus* individuals per sample, and when this was not possible, we collected as many individuals as we could. Adult midges were exposed to chemical preservation in the window traps (originally a mixture of 10% formalin, detergent, and ethylene glycol that was later switched to propylene glycol in 2000) and long-term storage in ethanol. Preservation treatments can affect organism $\delta^{13}C$, but generally these effects occur early in the preservation process, such that chemical preservation effects on isotope signatures should be largely unaffected by storage time (Sweeting et al. 2004, Syväranta et al. 2008). Thus, while chemical preservation may have affected the actual $\delta^{13}C$ values of archived midge specimens, comparisons between *T. gracilentus* and *C. islandicus* signatures are nonetheless informative, under the assumption that $\delta^{13}C$ values of both taxa respond to chemical preservation in a similar manner (Syväranta et al. 2008, Rennie et al. 2012). Adult samples were rinsed in deionized water and dried at 60 °C before isotopic analysis.

Analytical methods: *stable isotope analysis*

We homogenized sediment, larvae, and adult samples using plastic pestles. All samples were then re-dried, kept in a desiccator at room temperature for 24 h, and weighed into tin capsules for isotopic analysis. Analyses of stable carbon isotope ratios were conducted by the University of California Davis Stable Isotope Facility (Davis, CA, USA). Isotope ratios are

expressed using delta (δ) notation (units ‰), where $\delta = [(R_{sample} - R_{standard})/R_{standard}] \times 1000$, and $R = {}^{13}C/{}^{12}C$. The reference material for carbon was Vienna Pee Dee Belemnite. Samples were run by this facility in multiple years, with the analytical errors (reported as standard deviations) for $\delta^{13}C$ ranging from 0.02-0.09‰. To quantify error associated with sample preparation, duplicates were analyzed for a subset of samples, which produced standard deviations for $\delta^{13}C$ of 0.029‰ for sediment, 0.189‰ for midge larvae, 0.079‰ for midge adults.

To assess the influence of inorganic carbonates on sediment $\delta^{13}C$ signatures, we performed an HCl acidification following the methods of Harris et al. (2001) on a subset of sediment samples from 2018 and compared the isotopic signatures of acid-fumigated samples to non-treated samples from the same core. There was no difference in $\delta^{13}C$ between acid-fumigated and non-treated sediment (paired t-test: $t_7 = -1.25$, $p = 0.252$). Thus, we present stable isotope signatures from non-acidified sediment samples.

Statistical analysis

We performed two types of analyses for larval midges: analyses of the spatial correlation in *T. gracilentus* and *C. islandicus* larval abundances, and analyses comparing their $\delta^{13}C$ values. To analyze the spatial correlations, we used a descriptive approach to examine whether a high abundance of one species was positively or negatively associated with the abundance of the other. Using five years of data from six sites, we compared the correlation in abundances of both species at three levels: individual cores, aggregated site-date combinations, and aggregated site-year combinations. The spatial extent of a single core is 20 cm^2 (the area sampled by a Kajak corer), and replicate cores within a site encompass a few square meters. When calculating the correlation between abundances at the core level, we standardized abundances for each species

by subtracting the taxon-specific mean abundance for each site-date combination. When calculating the correlation between abundances aggregated at the site-date level, we standardized abundances for each species by subtracting the taxon-specific mean abundance for each site-year combination. We also examined the correlation in adult *T. gracilentus* and *C. islandicus* counts from 1977-2015, using data corresponding only to the early part of summer, as this captures the spring emergence of *T. gracilentus*, which coincides with the annual *C. islandicus* emergence. We Spearman's rank correlation coefficient (ρ) for all correlations.

To analyze sediment and larval stable isotope signatures, we aggregated data by date-site combinations. We performed paired analyses to compare $\delta^{13}C$ values between 1) *T. gracilentus* and *C. islandicus* larvae, 2) *T. gracilentus* larvae and surface sediment, and 3) *C. islandicus* larvae and surface sediment. For these analyses, we were interested in paired differences between samples collected at the same time from the same location. Because all three sample types were not always available for a given date-site combination, we conducted multiple analyses, rather than one analysis consisting of all data. For each analysis, we subset our data to include paired samples for the comparison of interest. In most cases, samples of both types from a given site were collected on the same date, but for cases in which the dates did not match exactly, we temporally paired sample types as closely as we could from the same site. Generally, this was a difference of 1-5 d, but in 2016, five *T. gracilentus* samples and six *C. islandicus* samples collected in late May were paired with sediment collected two weeks later. For each analysis, we investigated differences in $\delta^{13}C$ values using paired t-tests and report the differences in isotope values ($\Delta\delta^{13}C$) for each sample pair. We performed an analogous analysis on $\delta^{13}C$ values for paired *T. gracilentus* and *C. islandicus* adults collected from the same emergence.

Because chemical preservation may have affected adult midge $\delta^{13}C$ values, we only report the differences between paired adult samples ($\Delta\delta^{13}C$) rather than the actual $\delta^{13}C$ signatures.

We used a linear model to explore how site depth affected $\delta^{13}C$ values of midge larvae and surface sediment. In this analysis, we included all data for each sample type for all sampled site-date combinations from 2016-2018 (i.e., regardless of whether it was part of an above paired analysis). Using the cores collected in 2019, we examined how layer depth within the sediment affected $\delta^{13}C$ values with a linear mixed effect model (LMM), with site and layer depth as fixed effects and core as a random effect. The LMM was fit using the 'lme4' package (Bates et al. 2015), and statistical results are reported from an F-test with Kenward-Roger denominator degrees of freedom using the 'car' package (Fox and Weisberg 2019). All statistical analyses were performed in R version 3.6.1 (R Core Team 2019).

RESULTS

T. gracilentus *and* C. islandicus *abundance*

T. gracilentus and *C. islandicus* larval abundances were positively correlated at the levels of individual cores ($\rho = 0.23$; Fig 1a), aggregated site-date combinations ($\rho = 0.43$; Fig 1b), and aggregated site-year combinations ($\rho = 0.61$; Fig 1c). Thus, high *T. gracilentus* abundance was positively associated with high *C. islandicus* abundance at these spatial scales. Adult counts of *T. gracilentus* and *C. islandicus* were also positively correlated in the long-term monitoring data from 1977-2015 ($\rho = 0.78$; Fig 1d). Similar to previously published results from 1977-1996 (Gardarsson et al. 2004), this demonstrates the strong positive association in abundance of the two species across many generations.

Stable isotope values

Across all dates and sites, mean ± SD $\delta^{13}C$ values were -14.66 ± 1.67‰ for *T. gracilentus* larvae, -19.69 ± 2.74‰ for *C. islandicus* larvae, and -16.89 ± 1.58‰ for surface sediment. Paired *T. gracilentus* and *C. islandicus* larval samples significantly differed in their $\delta^{13}C$ values (t_{11} = 10.18, $p < 0.001$). *T. gracilentus* consistently had higher $\delta^{13}C$ values than *C. islandicus* (Fig 2a), with a mean difference in their $\delta^{13}C$ signatures ($\Delta\delta^{13}C$) of 5.39 ± 1.84‰. Relative to paired surface sediment samples, *T. gracilentus* larvae had significantly higher $\delta^{13}C$ values (t_9 = 6.07, $p < 0.001$; Fig 2b), with a mean $\Delta\delta^{13}C$ of 1.84 ± 0.96‰. *C. islandicus* larvae had significantly lower $\delta^{13}C$ values compared to paired surface sediments (t_{22} = -7.07, $p < 0.001$; Fig 2c), with a mean $\Delta\delta^{13}C$ of -2.87 ± 1.95‰. Similar to the larval analysis, *T. gracilentus* adults had significantly higher $\delta^{13}C$ values than *C. islandicus* from the same emergence (t_{30} = 8.04, $p < 0.001$), with $\Delta\delta^{13}C$ = 2.12 ± 1.47‰. In contrast to the larval comparison which involved samples collected from the same site, sampled adults likely originated from many locations in the lake, and consequently the adult comparison captures population-level $\delta^{13}C$ differences.

T. gracilentus larvae, *C. islandicus* larvae, and surface sediment $\delta^{13}C$ values decreased with increasing depth (Fig 3). However, there was a significant sample type × depth interaction (Table 1), such that the *C. islandicus* $\delta^{13}C$ signatures appeared to decrease more strongly with depth than *T. gracilentus* or sediment.

Layer depth within the sediment significantly affected $\delta^{13}C$ values ($F_{1, 11}$ = 11.64, $p = 0.006$); within a sediment core, the top 0.75-cm layer typically had higher $\delta^{13}C$ values than the layers from 5 cm or 10 cm below the sediment's surface (Fig 4). This demonstrates vertical patterns of sediment $\delta^{13}C$ values within the benthic habitat. The depth of the site from which cores were collected also affected $\delta^{13}C$ values ($F_{1, 4}$ = 85.34, $p = 0.001$); sediment from the

deeper site had significantly lower $\delta^{13}C$ values than the shallower site (Fig 4), mirroring our results from the above site-depth analysis.

DISCUSSION

In this study, we compared the correlation in abundance and stable isotope values for two chironomid species that occur at high densities in Lake Mývatn. All evidence points to consumer-resource interactions as the driver behind *T. gracilentus* population fluctuations, and *C. islandicus* fluctuates in synchrony *T. gracilentus*, implying that both species are limited by the same resources. Previous studies have shown little difference in gut contents between species, and both species construct tubes in the sediment where they feed on benthic algae and detritus that is largely derived from benthic production. Thus, the coexistence of *T. gracilentus* and *C. islandicus* in Mývatn is puzzling. To understand this coexistence, we first examined correlations between the species' larval abundances to see if they were partitioned in space across the lake. At all scales we could investigate – comparing individual 20-cm^2 cores, comparing aggregated samples taken on a given sampling date across sites, and comparing annual values for each site – *T. gracilentus* and *C. islandicus* abundances were positively correlated. Therefore, there was no evidence of spatial partitioning across different areas of the lake. We then analyzed larval and adult stable isotopes to look for differences in resource use that gut content analyses may not reveal. *T. gracilentus* and *C. islandicus* had consistently different $\delta^{13}C$ values, with *T. gracilentus* having values higher than surface sediment and *C. islandicus* having lower values. The stable carbon isotope results suggest partitioning of food resources at fine spatial scales.

The degree of enrichment in *T. gracilentus* $\delta^{13}C$ values compared to surface sediments provides insight into their feeding strategy. While trophic fractionation generally causes

increases in consumer $\delta^{13}C$ values relative to their resources, it is unlikely the sole factor contributing to the pattern between *T. gracilentus* larvae and surface sediments. ^{13}C-trophic fractionation in aquatic consumers across multiple studies averaged 0.47‰ and was lower in herbivores compared to carnivores (Vander Zanden and Rasmussen 2001). In studies involving midge larvae, Doi et al. (2006) and Goedkoop et al. (2006) report mean ^{13}C-trophic fractionation values of 0.3‰ and 0.5‰, respectively. Thus, among our paired samples, the high *T. gracilentus* larvae $\delta^{13}C$ values compared to surface sediment (mean $\Delta\delta^{13}C = 1.84$‰) is greater than expected by trophic fractionation alone. Enriched algal $\delta^{13}C$ signatures are associated with high rates of benthic primary production because increased photosynthetic rates can induce inorganic carbon limitation and lead to lower discrimination against the heavy ^{13}C isotope during carbon fixation (Hecky and Hesslein 1995, Hill and Middleton 2006, Devlin et al. 2013). This suggests that *T. gracilentus* larvae selectively consume the most productive algal material within the photosynthetically active layer (e.g., rapidly growing diatom cells), an explanation which Devlin et al. (2013) proposed to explain the high $\delta^{13}C$ values of benthic grazers relative to surface sediment. The tube-building behavior of *T. gracilentus* may facilitate their selection of ^{13}C-enriched benthic resources. For instance, *T. gracilentus* larvae increase benthic gross primary production, as their tubes provide additional substrate for diatoms (Phillips et al. 2019). If *T. gracilentus* rely heavily on productive algal material that they 'garden' from their tubes (sensu Ings et al. 2012), it could elevate their $\delta^{13}C$ values above the bulk surface sediment.

The depleted *C. islandicus* $\delta^{13}C$ values relative to surface sediments (mean $\Delta\delta^{13}C = -2.87$‰) is also consistent with selectivity for certain resources (Solomon et al. 2008), although a different selectivity than *T. gracilentus*. We suggest three possible explanations for the low *C. islandicus* $\delta^{13}C$ values. First, *C. islandicus* may consume microbes that process organic matter at

the sediment surface. Aerobic heterotrophic bacteria may discriminate against ^{13}C, especially when carbon is non-limiting (McGoldrick et al. 2008), and surface sediments at Mývatn have relatively high organic matter (23 ± 3%) and carbon (6.8 ± 2.4%) content (unpublished data). Selectivity for such microbes may affect consumer $\delta^{13}C$ values; for example, low $\delta^{13}C$ values of *C. tentans* relative their detrital food source was attributed to the assimilation of microbial biomass growing on the detritus (McGoldrick et al. 2008). Second, studies have attributed low midge $\delta^{13}C$ values to the consumption of methanotrophic bacteria, which have very depleted $\delta^{13}C$ values due to high fractionation during biogenic methane production and additional discrimination against ^{13}C when methanotrophs exploit biogenic methane (Bunn and Boon 1993, Grey et al. 2004, Hershey et al. 2006). Although our *C. islandicus* $\delta^{13}C$ values are not near the observed values (e.g., -40‰) for consumers with substantial dietary contributions from methanotrophs (Bunn and Boon 1993, Grey et al. 2004), even small reliance on a highly depleted resource could affect consumer $\delta^{13}C$ values. Third, filter-feeding and deposit-feeding *Chironomus* may rely on pelagic-sourced carbon by consuming phytoplankton from the water column or recently deposited phytodetritus from the sediment's surface (Johnson 1985, 1987, Gullberg et al. 1997, Doi et al. 2006). In Mývatn, differences in $\delta^{13}C$ values between paired surface sediment and pelagic samples were low (2.65 ± 1.71‰, unpublished data) compared to other studies (7.6‰ in Doi et al. 2010; 11.7-15.3‰ in Chételat et al. 2010), which limits our power to address whether *C. islandicus* uses pelagic-derived detritus. Nonetheless, we compared larval *C. islandicus* $\delta^{13}C$ values to those from pelagic samples (paired by site and collected 0-9 d from the larvae) and found variable differences (mean $\Delta\delta^{13}C \pm SD = -0.69 \pm 2.09$‰) and, thus, no conclusive evidence supporting use of pelagic-derived detritus.

Differences in the vertical distributions and tube-building behaviors of *T. gracilentus* and *C. islandicus* may also explain their partitioning of sediment resources. *Tanytarsus* larvae build vertical tubes arranged against one another that extend above the sediment surface, such that larvae generally remain in the top 2 cm of sediment (Heinis et al. 1994, Chaloner and Wotton 1996, Ólafsson and Paterson 2004). *T. gracilentus* feeding on productive diatoms from these tubes explains their enriched $\delta^{13}C$ values. In contrast, *C. islandicus* in Mývatn have burrows extending 10-15 cm below the sediment surface (Herren et al. 2017), and other studies have similarly documented *Chironomus* spp. at sediment depths of 7-12 cm (Heinis et al. 1994, Charboneau and Hare 1998). The depth of *C. islandicus* burrows might make rapidly growing diatoms less important in their diets. Additionally, sediment $\delta^{13}C$ values were depleted 5 cm and 10 cm below the sediment's surface relative to the top 0.75-cm layer from the same core, potentially due to the retention of recalcitrant organic matter fractions during decomposition (Meyers and Ishiwatari 1993, Lehmann et al. 2002). If *C. islandicus* forage 5-10 cm below the sediment's surface, they likely encounter resources with relatively low $\delta^{13}C$ values. Thus, if *T. gracilentus* and *C. islandicus* feeding activities correspond to the contrasting vertical distributions of their tubes, this would facilitate the partitioning of sediment-associated resources vertically within a habitat, despite the lack of spatial partitioning demonstrated by positive correlations in larval abundances across the lake.

In addition to differences in resource use, it is worth considering whether life cycle differences (e.g., larval development time) between *C. islandicus* and *T. gracilentus* affected comparisons of their $\delta^{13}C$ values. Isotope signatures represent a time-integrated account of consumer assimilation, such that there is a time lag before a consumer's isotopic signature reaches equilibrium with its diet. Therefore, a consumer's $\delta^{13}C$ value may not match its current

diet if it switches resources or if its resource has temporally variable $\delta^{13}C$ signatures. This raises the possibility that the longer larval life stage of *C. islandicus* may allow their isotopic signatures to integrate resource $\delta^{13}C$ variability across a longer time span than *T. gracilentus* individuals and contribute to our observed interspecific differences in $\delta^{13}C$ values. Despite the differences between *C. islandicus* and *T. gracilentus* body mass, which in part determines isotopic turnover in consumers, the expected $\delta^{13}C$ half-lives for both taxa (based on quantitative relationships reported within Thomas and Crowther 2015 and Vander Zanden et al. 2015 and individual body masses from Herren et al. 2017) are relatively short (i.e., on the order of days, rather than weeks or months). Consequently, *C. islandicus* should come to steady state (96.9% isotopic replacement, Thomas and Crowler 2015) with any hypothetical isotopic shifts in basal resources within 2 weeks of *T. gracilentus*. Along these lines, in a reciprocal transplant experiment, $\delta^{13}C$ values of *Chironomus* spp. and *Stictochironomus rosenschoeldi* larvae matched their new sediment within one month (Hershey et al. 2006), suggesting that chironomid larvae equilibrate to isotopic shifts of available resources relatively quickly. Therefore, we suggest life cycle differences are not strong enough to explain the consistent differences between *T. gracilentus* and *C. islandicus* $\delta^{13}C$ values; we instead attribute them to contrasting patterns of resource use.

Despite the interspecific differences in $\delta^{13}C$ values, *T. gracilentus* and *C. islandicus* larval $\delta^{13}C$ values decreased across a depth gradient. This suggests that $\delta^{13}C$ of both consumers in part reflects the isotopic characteristics of the sediment, as surface sediment $\delta^{13}C$ also decreased with depth. The declines in $\delta^{13}C$ values of *T. gracilentus*, *C. islandicus* and surface sediment likely reflect decreasing primary productivity at deeper depths of the lake (Devlin et al. 2013). This pattern is consistent with our interpretation of high $\delta^{13}C$ values of *T. gracilentus* reflecting their use of rapidly growth diatoms on their tubes. Nonetheless, other studies have

attributed the negative relationship between depth and $\delta^{13}C$ values to increased phytoplankton deposition (Karlsson and Byström 2005, Doi et al. 2006) or increased prevalence of organic matter emanated from respired carbon and methanogenesis (Solomon et al. 2008).

Overall, our data show that *T. gracilentus* and *C. islandicus* use resources differently. In contrast, we found no evidence of spatial partitioning across the lake, even at the scale of individual sediment cores and cores taken within a few meters of each other at the same site. Previous studies on gut contents, feeding behavior observations, and knowledge of the biologies of these two species, suggest that *T. gracilentus* and *C. islandicus* would utilize similar resources, but their $\delta^{13}C$ values showed striking and consistent differences. Contrasting selectivity for resources within the sediment surface or foraging at different vertical layers in the sediment likely contribute to their resource partitioning and may explain how *T. gracilentus* and *C. islandicus* coexist. *T. gracilentus* abundance affects the structure and physicochemical conditions in benthic habitats, which may in part explain the coupled fluctuations of *T. gracilentus* and *C. islandicus*. However, their synchronized population fluctuations likely imply dynamic coupling of their resources. This could be due to overlap in their diets, while not being substantial enough to result in similar $\delta^{13}C$ values. Alternatively, they may have non-overlapping resources that are dynamically coupled and fluctuate in synchrony. For example, fluctuations in benthic diatoms might be coupled to fluctuations in organic matter availability or heterotrophic activity. This would reconcile resource use partitioning and synchronized fluctuations of *T. gracilentus* and *C. islandicus*. While we do not know the exact mechanism by which *T. gracilentus* and *C. islandicus* partition resources, $\delta^{13}C$ values have illuminated this aspect of their ecologies, and our results demonstrate that stable isotope analyses may provide unexpected insights into interactions among species.

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AUTHOR CONTRIBUTIONS

All authors contributed to data collection. ARM prepared samples for stable isotope analysis. ARM conducted the data analysis with input from ARI and JSP. ARM wrote the first draft of the manuscript with all authors providing input for subsequent drafts.

REFERENCES

Bates, D., M. Maechler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models using lme4. Journal of Statistical Software 67(1):1-48.

Bunn, S. E., and P. I. Boon. 1993. What sources of organic carbon drive food webs in billabongs? A study based on stable isotope analysis. Oecologia 96:85–94.

Chaloner, D. T., and R. S. Wotton. 1996. Tube Building by Larvae of 3 Species of Midge (Diptera: Chironomidae). Journal of the North American Benthological Society 15:300–307.

Charboneau, P., and L. Hare. 1998. Burrowing Behavior and Biogenic Structures of Mud-Dwelling Insects. Journal of the North American Benthological Society 17:239–249.

Chételat, J., L. Cloutier, and M. Amyot. 2010. Carbon sources for lake food webs in the Canadian High Arctic and other regions of Arctic North America. Polar Biology 33:1111–

1123.

Connell, J. H. 1961. The influence of interspecific competition and other factors on the distribution of the barnacle Chthamalus stellatus. Ecology 42:710–723.

Devlin, S. P., M. J. Vander Zanden, and Y. Vadeboncoeur. 2013. Depth-specific variation in carbon isotopes demonstrates resource partitioning among the littoral zoobenthos. Freshwater Biology 58:2389–2400.

Doi, H., E. Kikuchi, S. Shikano, and S. Takagi. 2010. Differences in nitrogen and carbon stable isotopes between planktonic and benthic microalgae. Limnology 11:185–192.

Doi, H., E. Kikuchi, S. Takagi, and S. Shikano. 2006. Selective assimilation by deposit feeders: Experimental evidence using stable isotope ratios. Basic and Applied Ecology 7:159–166.

Einarsson, Á., A. Gardarsson, G. M. Gíslason, and A. R. Ives. 2002. Consumer – resource interactions and cyclic population dynamics of Tanytarsus gracilentus (Diptera: Chironomidae). Journal of Animal Ecology 71:832–845.

Einarsson, Á., U. Hauptfleisch, P. R. Leavitt, and A. R. Ives. 2016. Identifying consumer-resource population dynamics using paleoecological data. Ecology 97:361–371.

Einarsson, Á., G. Stefánsdóttir, H. Jóhannesson, J. S. Ólafsson, G. M. Gíslason, I. Wakana, G. Gudbergsson, and A. Gardarsson. 2004. The ecology of Lake Myvatn and the River Laxa: Variation in space and time. Aquatic Ecology 38:317–348.

Fox, J., and S. Weisberg. 2019. An R Companion to Applied Regression, Third Edition. Thousand Oaks, CA: Sage.

Gardarsson, A. 2006. Temporal processes and duck populations: examples from Myvatn. Hydrobiologia 567:89–100.

Gardarsson, A., Á. Einarsson, G. M. Gíslason, T. Hrafnsdóttir, H. R. Ingvason, E. Jónsson, and J.

S. Ólafsson. 2004. Population fluctuations of chironomid and simuliid Diptera at Myvatn in 1977-1996. Aquatic Ecology 38:209–217.

Goedkoop, W., N. Akerblom, and M. H. Demandt. 2006. Trophic fractionation of carbon and nitrogen stable isotopes in Chironomus riparius reared on food of aquatic and terrestrial origin. Freshwater Biology 51:878–886.

Grey, J., A. Kelly, S. Ward, N. Sommerwerk, and R. I. Jones. 2004. Seasonal changes in the stable isotope values of lake-dwelling chironomid larvae in relation to feeding and life cycle variability. Freshwater Biology 49:681–689.

Gullberg, K. R., W. Goedkoop, and R. K. Johnson. 1997. The fate of diatom carbon within a freshwater benthic community - a microcosm study. Limnology and Oceanography 42:452–460.

Harris, D., W. R. Horwath, and C. van Kessel. 2001. Acid fumigation of soils to remove carbonates prior to total organic carbon or carbon-13 isotopic analysis. Soil Sci. Soc. Am. J. 65:1853–1856.

Hecky, R. E., and R. H. Hesslein. 1995. Contributions of benthic algae to lake food webs as revealed by stable isotope analysis. Journal of the North American Benthological Society 14:631–653.

Heinis, F., J.-P. Sweerts, and E. Loopik. 1994. Micro-environment of chironomid larvae in the littoral and profundal zones of Lake Maarsseveen I, The Netherlands. Archiv für Hydrobiologie 130:53–67.

Herren, C. M., K. C. Webert, M. D. Drake, M. J. Vander Zanden, A. Einarsson, A. R. Ives, and C. Gratton. 2017. Positive feedback between chironomids and algae creates net mutualism between benthic primary consumers and producers. Ecology 98:447–455.

Hershey, A. E., S. Beaty, K. Fortino, S. Kelly, M. Keyse, C. Luecke, W. J. O'Brien, and S. C. Whalen. 2006. Stable isotope signatures of benthic invertebrates in arctic lakes indicate limited coupling to pelagic production. Limnology and Oceanography 51:177–188.

Hill, W. R., and R. G. Middleton. 2006. Changes in carbon stable isotope ratios during periphyton development. Limnology and Oceanography 51:2360–2369.

Ings, N. L., A. G. Hildrew, and J. Grey. 2012. 'House and garden': larval galleries enhance resource availability for a sedentary caddisfly. Freshwater Biology 57:2526–2538.

Ingvason, H. R. 2002. The food-ecology of Tanytarsus gracilentus in Lake Myvatn.

Ingvason, H. R., J. S. Ólafsson, and A. Gardarsson. 2002. Temporal pattern in resource utilization of Tanytarsus gracilentus larvae. Internationale Vereinigung fuer Theoretische und Angewandte Limnologie Verhandlungen 28:1041–1045.

Ingvason, H. R., J. S. Ólafsson, and A. Gardarsson. 2004. Food selection of Tanytarsus gracilentus larvae (Diptera: Chironomidae): an analysis of instars and cohorts. Aquatic Ecology 38:231–237.

Ives, A. R. 2013. LTREB Biological Limnology at Lake Mývatn 2012-current. LTER Network Information System Repository.

Ives, A. R., A. Einarsson, V. A. A. Jansen, and A. Gardarsson. 2008. High-amplitude fluctuations and alternative dynamical states of midges in Lake Myvatn. Nature 452:84–87.

Ives, A. R., K. Gross, and J. L. Klug. 1999. Stability and Variability in Competitive Communities. Science 286:542–545.

Johnson, R. K. 1985. Feeding efficiencies of Chironomus plumosus (L.) and Chironomus anthracinus (Zett.) in mesotrophic Lake Erken. Freshwater Biology 15:605–612.

Johnson, R. K. 1987. Seasonal variation in diet of Chironomus plumosus (L.) and C. anthracinus

Zett. (Diptera: Chironomidae) in mesotrophic Lake Erken. Freshwater Biology 17:525–532.

Jónasson, P. M. 1979. The Lake Mývatn ecosystem, Iceland. Oikos 32:289–305.

Karlsson, J., and P. Byström. 2005. Littoral energy mobilization dominates energy supply for top consumers in subarctic lakes. Limnology and Oceanography 50:538–543.

Klug, J. L., J. M. Fischer, A. R. Ives, and B. Dennis. 2000. Compensatory dynamics in planktonic community responses to pH perturbations. Ecology 81:387–398.

Korpimäki, E. 1994. Rapid or delayed tracking of multi-annual vole cycles by avian predators? Journal of Animal Ecology 63:619–628.

Lehmann, M. F., S. M. Bernasconi, A. Barbieri, and J. A. McKenzie. 2002. Preservation of organic matter and alteration of its carbon and nitrogen isotope composition during simulated and in situ early sedimentary diagenesis. Geochimica et Cosmochimica Acta 66:3573–3584.

Lindegaard, C., and P. M. Jónasson. 1979. Abundance , population dynamics and production of zoobenthos in Lake Myvatn , Iceland. Oikos 32:202–227.

MacArthur, R., and R. Levins. 1967. The limiting similarity, convergence, and divergence of coexisting species. The American Naturalist 101:377–385.

McGoldrick, D. J., D. R. Barton, M. Power, R. W. Scott, and B. J. Butler. 2008. Dynamics of bacteria – substrate stable isotope separation: dependence on substrate availability and implications for aquatic food web studies. Can J Fish Aquat Sci 65:1983–1990.

Meyers, P. A., and R. Ishiwatari. 1993. Lacustrine organic geochemistryman overview of indicators of organic matter sources and diagenesis in lake sediments 20:867–900.

Micheli, F., K. L. Cottingham, J. Bascompte, O. N. Bjørnstad, G. L. Eckert, J. M. Fischer, T. H. Keitt, B. E. Kendall, J. L. Klug, and J. A. Rusak. 1999. The dual nature of community

variability. Oikos 85:161–169.

Morin, P. J. (2011). Community Ecology. 2nd Edition.Wiley-Blackwell. Chichester, West Sussex, UK.

Ólafsson, J. S. 1987. Food of Chirnomidae (Diptera) in Lake Mývatn. Unpublished thesis, for honours degree, Department of Biology. University of Iceland, 62 pp. (In Icelandic, with English abstract).

Ólafsson, J. S., and D. M. Paterson. 2004. Alteration of biogenic structure and physical properties by tube-building chironomid larvae in cohesive sediments. Aquatic Ecology 38:219–229.

Pacala, S., and J. Roughgarden. 1982. Resource partitioning and interspecific competition in two two-species insular Anolis lizard communities. Science 217:444–446.

Phillips, J. S., A. R. McCormick, Á. Einarsson, S. N. Grover, and A. R. Ives. 2019. Spatiotemporal variation in the sign and magnitude of ecosystem engineer effects on lake ecosystem production. Ecosphere 10:e02760.

Pianka, E. R. 1974. Niche overlap and diffuse competition. Proceedings of the National Academy of Sciences 71:2141–2145.

R Core Team (2019). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rennie, M. D., T. Ozersky, and D. O. Evans. 2012. Effects of formalin preservation on invertebrate stable isotope values over decadal time scales. Canadian Journal of Zoology 90:1320–1327.

Ripa, J., and A. R. Ives. 2003. Food web dynamics in correlated and autocorrelated environments. Theoretical Population Biology 64:369–384.

Rounick, J. S., and M. J. Winterbourn. 1986. Stable carbon isotopes and carbon flow in ecosystems. BioScience 36:171–177.

Schmitt, R. J. 1985. Competitive interactions of two mobile prey species in a patchy environment. Ecology 66:950–958.

Schoener, T. W. 1974. Resource partitioning in ecological communities. Science 185:27–39.

Solomon, C. T., S. R. Carpenter, J. J. Cole, and M. L. Pace. 2008. Support of benthic invertebrates by detrital resources and current autochthonous primary production: results from a whole-lake 13C addition. Freshwater Biology 53:42–54.

Sweeting, C. J., N. V. C. Polunin, and S. Jennings. 2004. Tissue and fixative dependent shifts of δ13C and δ15N in preserved ecological material. Rapid Communications in Mass Spectrometry 18:2587–2592.

Syväranta, J., S. Vesala, M. Rask, J. Ruuhijärvi, and R. I. Jones. 2008. Evaluating the utility of stable isotope analyses of archived freshwater sample materials. Hydrobiologia 600:121–130.

Thomas, S. M., and T. W. Crowther. 2015. Predicting rates of isotopic turnover across the animal kingdom: a synthesis of existing data. Journal of Animal Ecology 84:861–870.

Thorbergsdóttir, I. M., S. Reynir Gíslason, H. R. Ingvason, and Á. Einarsson. 2004. Benthic oxygen flux in the highly productive subarctic Lake Myvatn, Iceland: In situ benthic flux chamber study. Aquatic Ecology 38:177–189.

Tilman, D. 1996. Biodiversity: population versus ecosystem stability. Ecology 77:350–363.

Vander Zanden, M. J., M. K. Clayton, E. K. Moody, C. T. Solomon, and B. C. Weidel. 2015. Stable Isotope Turnover and Half-Life in Animal Tissues : A Literature Synthesis. PLoS ONE 10:e0116182.

Vander Zanden, M. J., and J. B. Rasmussen. 2001. Variation in d15N and d13C trophic fractionation: Implications for aquatic food web studies. Limnology and Oceanography 46:2061–2066.

Table 1. F-test results from the linear model testing for the effects of sample type (*Tanytarsus gracilentus* larvae, *Chironomus islandicus* larvae, and surface sediments), site depth, and their interaction on $\delta^{13}C$ values.

	SS	df_{num}	df_{resid}	F	P-value
$\delta^{13}C$					
Sample type	0.81	2	62	0.22	0.803
Depth	111.83	1	62	60.73	< 0.001
Sample type × Depth	13.60	2	62	3.69	0.031

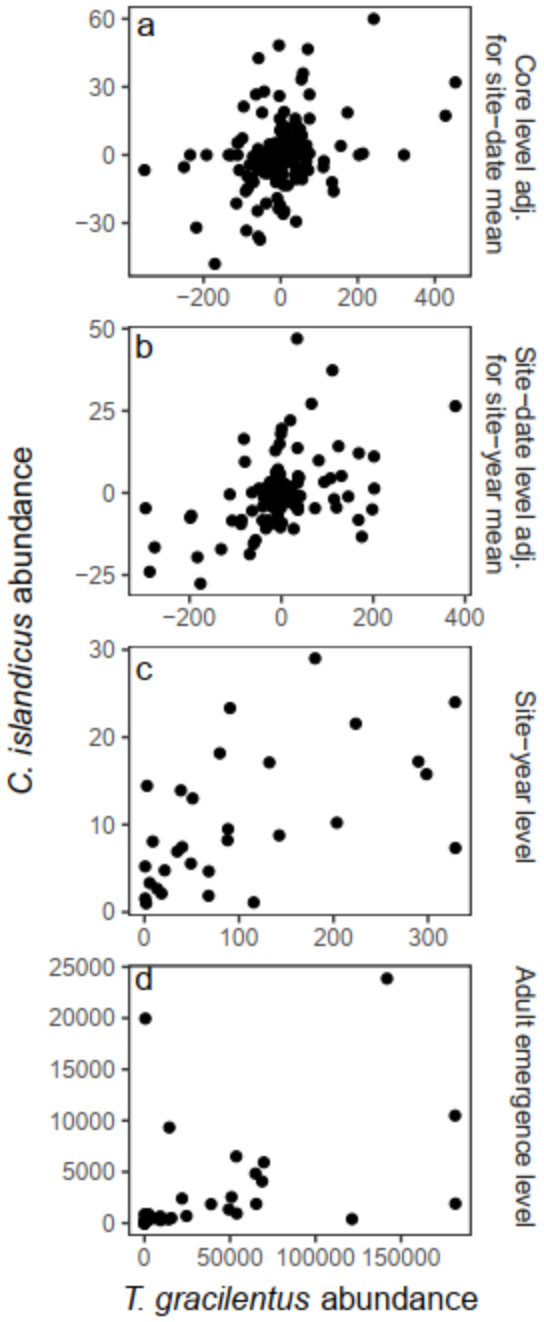

Figure 1. Comparisons of *T. gracilentus* and *C. islandicus* abundances for the levels of a) larvae in individual cores, b) larvae aggregated for site-date combinations, c) larvae aggregated for site-year combinations, and d) adult counts from long-term population monitoring. Larval abundances are based midge densities within a sediment core (20 cm^2) and were standardized by

the mean abundance for each site-date combination (in panel a) or each site-year combination (in panel b).

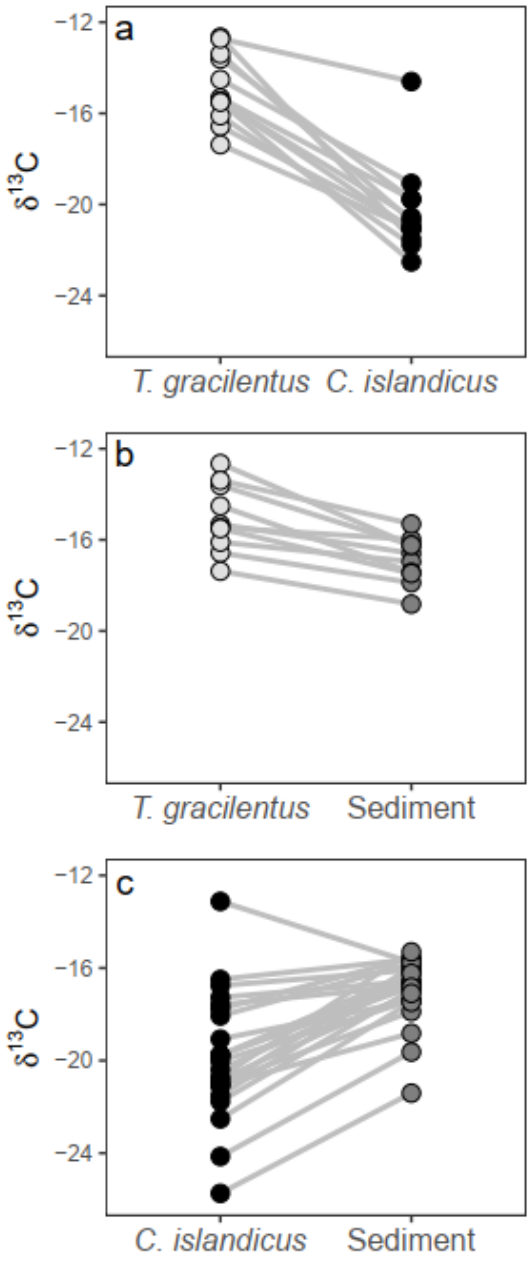

Figure 2. Comparisons of $\delta^{13}C$ (‰) values among paired a) *T. gracilentus* and *C. islandicus* larvae, b) *T. gracilentus* larvae and surface sediment, and c) *C. islandicus* larvae and surface sediment. Lines connect samples from a specific date-site combination that were compared to one another in each paired analysis.

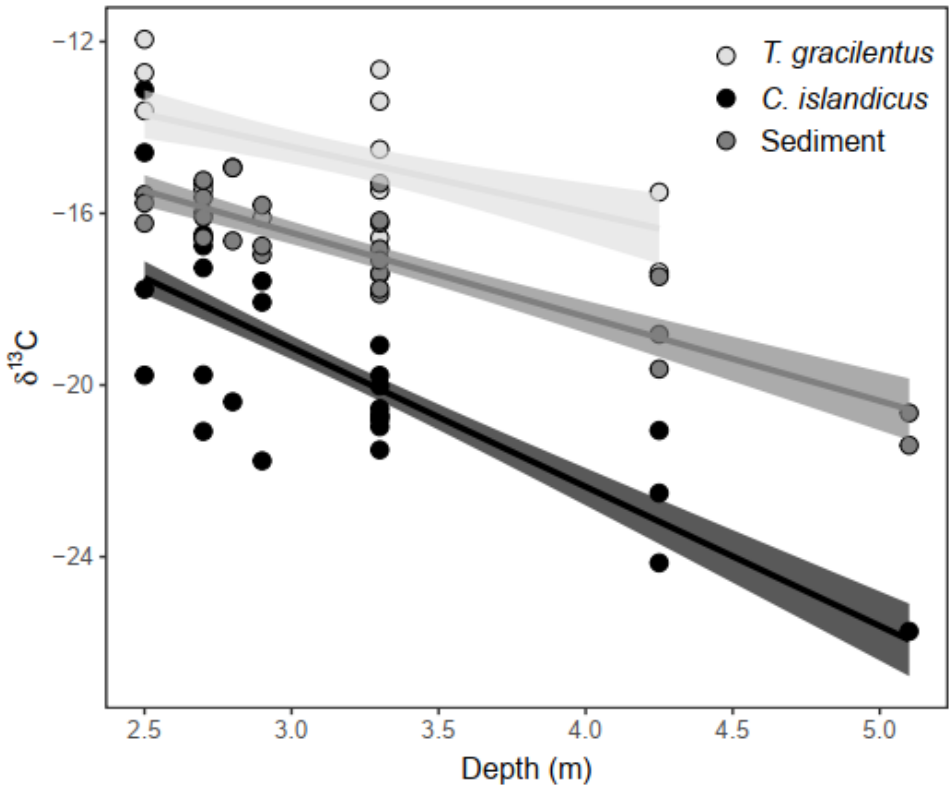

Figure 3. The $\delta^{13}C$ (‰) values of *T. gracilentus* larvae, *C. islandicus* larvae, and surface sediments across the site depths. Points show raw data, and lines correspond to the linear model fit with standard errors.

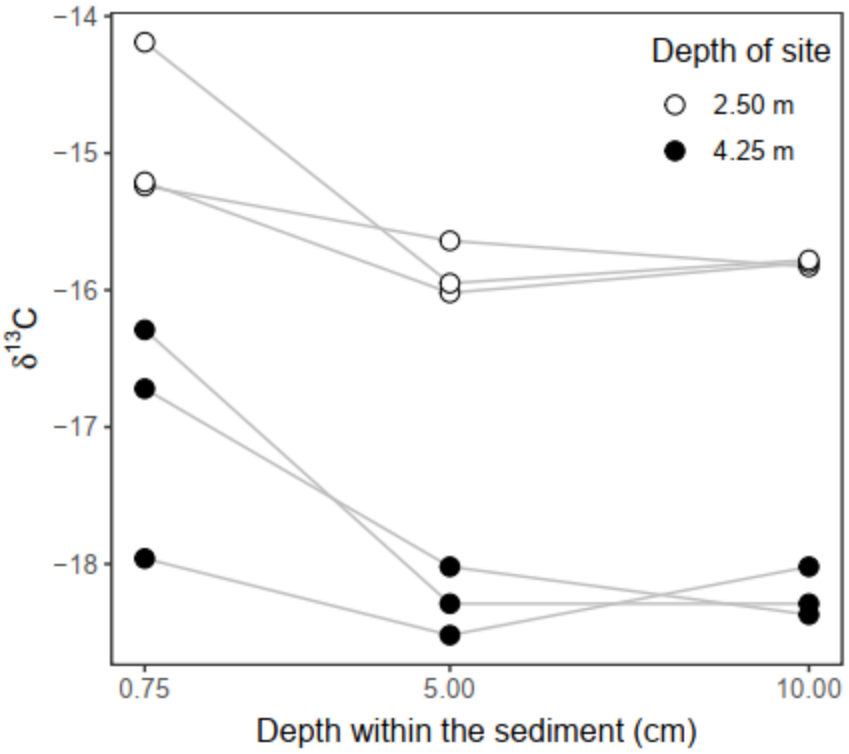

Figure 4. Sediment $\delta^{13}C$ (‰) values were analyzed for multiple layers within individual cores. Cores were collected from two sites with depths 2.50 and 4.25 m. Within each core, sediment layers were sampled at 0.75 cm (i.e., the surface layer), 5 cm, and 10 cm below the sediment's surface. Each point shows a sample, with lines connecting layers from within an individual core.

Appendix E: Supplemental table for Chapter 4

Table S1. List of larvae and sediment samples collected for stable isotope analysis.

Sample type	Sample date	Site depth (m)	Site name	n	$\delta^{13}C$ (‰)
C. islandicus	5/29/2016	2.7	E1	1	-19.76
C. islandicus	5/29/2016	3.3	E3	1	-20.97
C. islandicus	5/30/2016	2.7	E2	1	-21.08
C. islandicus	5/30/2016	2.9	E4	1	-21.77
C. islandicus	5/31/2016	2.5	E5	1	-19.77
C. islandicus	5/31/2016	2.8	E6	1	-20.39
C. islandicus	6/26/2016	2.7	E1	1	-17.27
C. islandicus	6/26/2016	3.3	E3	1	-19.07
C. islandicus	6/26/2016	2.9	E4	1	-17.58
C. islandicus	6/27/2016	2.7	E2	1	-16.76
C. islandicus	6/27/2016	2.5	E5	1	-17.77
C. islandicus	8/11/2016	2.7	E1	1	-16.49
C. islandicus	8/11/2016	3.3	E3	1	-20.02
C. islandicus	8/11/2016	2.9	E4	1	-18.07
C. islandicus	8/11/2016	2.5	E5	1	-13.12
C. islandicus	5/24/2017	3.3	E3	1	-20.55
C. islandicus	6/25/2017	3.3	E3	1	-20.71
C. islandicus	7/17/2017	3.3	E3	1	-21.51
C. islandicus	8/2/2017	3.3	E3	1	-20.72
C. islandicus	6/28/2018	4.25	Reyk	4	-22.52 (0.26)
C. islandicus	7/17/2018	4.25	Reyk	4	-21.06 (1.35)
C. islandicus	7/25/2018	3.3	E3	3	-19.77 (1.68)
C. islandicus	8/10/2018	5.1	Grim	1	-25.74
C. islandicus	8/10/2018	4.25	Reyk	4	-24.14 (1.09)
C. islandicus	8/12/2018	3.3	E3	2	-20.81 (0.82)
C. islandicus	8/12/2018	2.5	E5	3	-14.58 (1.06)
T. gracilentus	5/29/2016	2.7	E1	1	-15.32
T. gracilentus	5/29/2016	3.3	E3	1	-16.57
T. gracilentus	5/30/2016	2.7	E2	1	-15.46
T. gracilentus	5/30/2016	2.9	E4	1	-16.10
T. gracilentus	5/31/2016	2.5	E5	1	-13.61
T. gracilentus	6/26/2016	3.3	E3	1	-14.51
T. gracilentus	5/24/2017	3.3	E3	1	-15.46
T. gracilentus	7/17/2017	3.3	E3	1	-12.65
T. gracilentus	8/2/2017	3.3	E3	1	-13.39
T. gracilentus	6/28/2018	4.25	Reyk	3	-15.51 (0.16)
T. gracilentus	7/3/2018	2.5	E5	3	-11.95 (0.15)
T. gracilentus	7/17/2018	4.25	Reyk	4	-17.37 (0.09)

T. gracilentus	8/17/2018	2.5	E5	3	-12.72 (0.10)
Surface sediment	6/13/2016	2.7	E1	1	-16.59
Surface sediment	6/13/2016	2.7	E2	1	-15.98
Surface sediment	6/13/2016	3.3	E3	1	-17.88
Surface sediment	6/13/2016	2.9	E4	1	-16.96
Surface sediment	6/13/2016	2.5	E5	1	-16.23
Surface sediment	6/13/2016	2.8	E6	1	-16.64
Surface sediment	6/26/2016	2.7	E1	1	-16.57
Surface sediment	6/26/2016	3.3	E3	1	-17.42
Surface sediment	6/26/2016	2.9	E4	1	-16.76
Surface sediment	6/26/2016	2.5	E5	1	-15.56
Surface sediment	6/27/2016	2.7	E2	1	-16.09
Surface sediment	6/27/2016	2.8	E6	1	-14.93
Surface sediment	8/9/2016	2.9	E4	1	-15.81
Surface sediment	8/9/2016	2.5	E5	1	-15.76
Surface sediment	8/9/2016	2.8	E6	1	-14.94
Surface sediment	8/10/2016	2.7	E1	1	-15.63
Surface sediment	8/10/2016	2.7	E2	1	-15.23
Surface sediment	8/10/2016	3.3	E3	1	-17.40
Surface sediment	7/17/2017	3.3	E3	1	-16.23
Surface sediment	8/2/2017	3.3	E3	1	-15.30
Surface sediment	8/14/2017	3.3	E3	1	-17.76
Surface sediment	6/23/2018	4.25	Reyk	1	-17.47
Surface sediment	6/24/2018	3.3	E3	1	-16.17
Surface sediment	7/17/2018	5.1	Grim	1	-20.65
Surface sediment	7/17/2018	4.25	Reyk	1	-18.82
Surface sediment	7/25/2018	3.3	E3	1	-16.83
Surface sediment	8/10/2018	5.1	Grim	1	-21.40
Surface sediment	8/10/2018	4.25	Reyk	1	-19.63
Surface sediment	8/13/2018	3.3	E3	1	-17.09