

The contributions of benthic and pelagic cyanobacterial nitrogen fixation to the nitrogen budget
of Lake Mývatn, Iceland

by

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TABLE OF CONTENTS

ABSTRACT.....	ii
INTRODUCTION.....	iv
CHAPTER 1: Benthic-pelagic coupling of nitrogen fixation during a pelagic cyanobacteria bloom in a shallow, nitrogen-limited lake.....	1
Appendix A: Supplementary Materials for Chapter 1.....	35
CHAPTER 2: Benthic primary producers regulate pelagic nitrogen fixation by mediating benthic nutrient flux in a shallow lake.....	37
CHAPTER 3: Patterns of lake-scale benthic and pelagic nitrogen fixation and retention in Lake Mývatn.....	74
Appendix B: Supplementary Materials for Chapter 3.....	108

ABSTRACT

Cyanobacterial nitrogen (N) fixation can be an important source of N in N-limited aquatic ecosystems, but rates of cyanobacterial N fixation can vary greatly within an ecosystem over space and time. Drivers of variation in cyanobacterial N fixation rates have been studied extensively in recent years. However, the implications of variation in these drivers for the distribution of N fixation and retention of fixed N within an ecosystem remains poorly understood.

Lake Mývatn, Iceland, is a shallow, N-limited ecosystem wherein the abundances of benthic and pelagic cyanobacterial N fixers, as well as environmental factors that control cyanobacterial N fixation, are highly variable. This variation, combined with the existence of few external N sources and sinks, make Mývatn a useful system in which to study variation in the distribution and retention of N.

Here, I explore the causes and consequences of variation in benthic and pelagic N fixation in Mývatn. In Chapter 1, I document the relationship between rates of benthic and pelagic cyanobacterial N fixation. Generally, rates of benthic and pelagic N fixation were negatively related. Benthic N fixation decreased in response to shading by pelagic cyanobacteria, and there was evidence for a reciprocal effect of benthic cyanobacteria or other benthic primary producers on pelagic N fixation. In Chapter 2, I identify mechanisms by which benthic primary producers may regulate pelagic N fixation in the water column above. Benthic primary producers were associated with decreased phosphate release from the benthos, and phosphate release was positively associated with pelagic N fixation rates. This suggests that benthic primary producers may influence pelagic N fixation indirectly via their effects on benthic nutrient release. In

Chapter 3, I use data and observations from Chapters 1 and 2 to estimate annual N fixation across years with variable benthic and pelagic N fixer abundances. Pelagic N fixation had the potential to be much greater than benthic N fixation in Mývatn. I explore the implications of this pelagic-benthic distribution of N fixation for ecosystem N availability by estimating the likely retention of N fixed in the pelagic zone using a dynamic N budget model. Patterns of ecosystem N cycling were best explained when two-thirds of N fixed in the pelagic zone was retained within the benthos. This reveals that pelagic N fixation supplies N that could, at least potentially, support non-diazotrophic primary production, which in Mývatn is primarily benthic.

INTRODUCTION

In aquatic ecosystems wherein external nitrogen (N) supply is low relative to phosphorus (P), primary production is often limited by N (Schindler, 1977; Elser et al., 2007; Howarth et al., 1988). However, N limitation can stimulate internal N supply via N fixation (Schindler 1977; Hayes et al. 2019; Zehr and Capone 2020; Fulweiler et al. 2025). In freshwater lakes, cyanobacterial N fixation is particularly common (Marcarelli et al. 2022) and can alleviate N limitation directly by allowing primary production in cyanobacteria (Paerl, 2017) or indirectly by providing N to primary producers that do not fix N (Scott & McCarthy, 2010; Grantz et al., 2014). Therefore, cyanobacterial N fixation can be important for sustaining primary production in N-limited lakes (Schindler, 1977; Howarth et al., 1988; Marcarelli et al., 2022).

Rates of cyanobacterial N fixation within N-limited lakes can vary greatly over space and time (Scott et al. 2008; Marcarelli et al., 2022). This variation can be driven by variation in environmental factors such as light intensity (Severin & Stal, 2008) and nutrient availability (Howarth et al., 1988; Smith 1990), which can influence the abundance of N fixers (Howarth et al., 1988; Schindler, 1977) and the rate of N fixation within N fixing cyanobacteria (Higgins et al. 2001; Severin & Stal, 2008; Marcarelli et al., 2022). While environmental drivers of N fixation rates have been studied extensively (Howarth et al., 1988; Marcarelli et al., 2022), the effect of spatiotemporal variation in these drivers on the distribution and fate of fixed N within a lake remains poorly understood (Vitousek et al. 2002; Scott & McCarthy, 2010).

In shallow N-limited lakes, cyanobacterial N fixers can colonize the benthic surface or the water column (Einarsson et al., 2004; Scott & Marcarelli, 2012). However, the availability of light and nutrients can be uneven across these habitats. Light availability tends to be relatively

high in the water column, while nutrient availability tends to be relatively high at the benthic surface (Genkai-Kato et al. 2012; Jäger and Diehl 2014). Competition for these unevenly distributed resources can lead to a negative relationship between primary production by benthic and pelagic primary producers (Vadeboncouer et al., 2001; McCormick et al., 2021). However, whether there is a similar negative relationship between benthic and pelagic cyanobacterial N fixation remains unknown.

Classical whole-lake N budgets estimate N fixation as the deficit between N supply and loss (Schindler, 1977; Ólafsson, 1979; Jónsson, 2016). However, this approach has limitations: (i) it does not account for temporal variation in N dynamics; (ii) it does not identify where in the lake N fixation occurs; and (iii) it provides no information about the retention of fixed N. Thus, while classical static N budgets are fundamental to understanding overall patterns of N supply and loss in lakes, they fail to address spatiotemporal variation in N fixation or the fate of fixed N, which are critical to understanding the relationship between N fixation and N availability for primary producers (Vitousek et al., 2002; Scott et al., 2019; Marcarelli et al., 2022).

Determining the relationship between benthic and pelagic N fixation, and mechanisms underlying any potential interactions between them, could improve our understanding of the ecological implications of shifts in benthic and pelagic N fixer abundances. For example, if the relationship between benthic and pelagic N fixation is negative, then accounting for this negative interaction could improve estimates of both within-zone and whole-ecosystem N fixation. Furthermore, improved understanding of the distribution of N fixation within an ecosystem could help to explain the contribution of N fixation to ecosystem N availability and primary production. The ability of cyanobacterial N fixation to increase N availability for all primary producers depends, in part, on the retention of fixed N within a lake (Scott & McCarthy, 2010;

Grantz et al., 2014). If the retention of fixed N depends on whether it is fixed in the pelagic vs. benthic zone, then N retention and, potentially, whole-ecosystem N availability, may depend more on where N is fixed than how much N is fixed at the scale of the entire ecosystem (Scott & McCarthy, 2010).

Lake Mývatn in northeastern Iceland is an ideal system in which to investigate the distribution of benthic and pelagic N fixation as well as the retention of fixed N. Mývatn is a shallow, N-limited lake that supports abundant benthic and pelagic cyanobacterial N fixers (Jónasson and Aðalsteinsson, 1979; Einarsson et al., 2004). Furthermore, Mývatn has relatively few sources of external N supply and N loss, easing estimates in external N supply, N loss, and the N deficit potentially accounted for by N fixation (Ólafsson, 1979b; Jónsson, 2016). The estimated rate of N fixation in the lake is high based on the deficit between external N supply and N loss from classical static N budgets (Ólafsson, 1979b; Jónsson, 2016). However, N fixation in benthic or pelagic cyanobacterial N fixers in the lake had not been previously quantified directly. Light and nutrient availability varies greatly within the lake over space and time (Ólafsson 1979a,b; Einarsson et al. 2004; McCormick et al. 2021), both within and across benthic and pelagic zones (Jónasson & Aðalsteinsson, 1979; Ólafsson 1979a,b; McCormick et al., 2021). Further, the abundance of benthic and pelagic cyanobacterial N fixers varies greatly over space and from year to year (Einarsson et al., 2004; McCormick et al. 2021). Despite this, drivers of variation in benthic and pelagic cyanobacterial N fixation in the lake have not been identified in Mývatn.

Here, I investigate the causes and consequences of variation in benthic and pelagic cyanobacterial N fixation in Mývatn. In Chapter 1, I quantify local N fixation rates in both zones across multiple sites over the course of a pelagic cyanobacterial bloom. Benthic N fixation

decreased as pelagic cyanobacterial abundance and pelagic N fixation increased, likely due to increased shading by pelagic cyanobacteria. When standardized by the biomass of pelagic cyanobacteria, pelagic N fixation was consistent within sites over time while different among sites. This suggests that site characteristics determine the capacity for N fixation within pelagic cyanobacteria in the water column despite variation in pelagic cyanobacterial abundance.

In Chapter 2, I explore whether benthic primary producers indirectly influence pelagic N fixation via their effects on nutrient fluxes from the sediment. I conducted paired benthic nutrient flux and pelagic N fixation assays during cyanobacterial blooms in Lake Mývatn. Sites with greater phosphate release from the sediment were associated with higher pelagic N fixation rates. In turn, phosphate release was negatively associated with dissolved oxygen concentration at the sediment-water interface, which was positively related to benthic primary producer abundance. These results suggest that benthic primary producers indirectly regulate pelagic N fixation in the water column above through their effects on benthic nutrient fluxes.

In Chapter 3, I utilize data and observations from Chapters 1 and 2 to estimate lake-scale benthic and pelagic N fixation. Further, I evaluate the retention of fixed N using an updated static N budget for the lake and a dynamic N budget that accounts for spatiotemporal variation in N cycling. In my updated static N budget, I show that the annual amount of N fixation required to explain the deficit between external N supply and loss varies greatly from year to year, driven largely by variation in N loss via outflow and midge emergences. By extrapolating recent direct measurements of benthic and pelagic N fixation to the lake scale under scenarios of different benthic and pelagic N fixer abundances, I show that the pelagic zone has a much higher potential for N fixation than the benthic zone. I then use a dynamic N budget, calibrated with N loss data from the outlet river Laxá, to show that about two-thirds of organic N fixed in the pelagic zone

must be retained in the benthos to explain patterns of observed N loss. These results suggest that N fixed in pelagic cyanobacterial blooms may ultimately support benthic primary production in Mývatn.

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CHAPTER 1

Benthic-pelagic coupling of nitrogen fixation during a pelagic cyanobacteria bloom in a shallow, nitrogen-limited lake*

Abstract

In shallow aquatic ecosystems, N_2 fixation can occur in benthic and pelagic primary producers, and primary producers in either zone may limit N_2 fixation in the other by reducing the exchange of limiting resources. For example, N_2 fixing primary producers in the pelagic zone may reduce light availability in the benthos, while N_2 fixing primary producers in the benthos may reduce nutrient release from the sediment to the pelagic zone. To investigate the interaction between benthic and pelagic N_2 fixation, we took paired measurements of N_2 fixation by cyanobacterial epiphytes of *Cladophorales* mats and pelagic cyanobacteria in shallow, N-limited Lake Mývatn, Iceland. Across four sites and dates in summer 2022, we estimated the N_2 fixation capacity (N_{max}) of both benthic and pelagic cyanobacteria using N_2 fixation-irradiance (N-I) curves. By pairing N-I curve parameter estimates with estimates of in situ light intensity, we estimated the total areal rate of benthic and pelagic N_2 fixation. Light attenuation increased across sites as the cyanobacteria bloom progressed, reducing benthic light intensity and decreasing benthic N_{max} . There was also a possible reciprocal effect of the benthos on pelagic N_2 fixation, as biomass-standardized pelagic N_{max} differed consistently across sites over time, although the cause of this is unknown. Over the study, pelagic N_2 fixation accounted for 87-100% of total areal in situ N_2 fixation. Together, these results suggest that benthic and pelagic phototrophic N_2 fixation are

coupled in Lake Mývatn, and, at least during pelagic cyanobacterial blooms, phototrophic N₂ fixation is dominated by the pelagic zone.

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Introduction

In shallow aquatic ecosystems (i.e., lakes, estuaries), ecosystem processes performed by primary producers in benthic and pelagic zones are often coupled. Here, we use the term coupling in a broad sense to describe situations in which a process operating in one zone affects the same process in the other, with these effects possibly but not necessarily being reciprocal. Many cases of benthic-pelagic coupling involve benthic and/or pelagic primary producers mediating the exchange of limiting resources such as nutrients and light to the other zone (Schindler and Scheurel 2002; Jäger and Diehl 2014). For example, nutrient cycling can be coupled when benthic primary producers reduce the release of nutrients from the sediment to the water column, or when pelagic primary producers increase the sedimentation of autochthonous organic matter (Sand-Jensen & Borum 1991; Kemp et al. 1999; Vasconcelos et al. 2018). Primary production can be coupled when pelagic primary producers shade the benthos, or when benthic primary producers decrease the release of nutrients to the water column (Hansson 1992; Vadeboncouer et al. 2001, 2003). Benthic-pelagic coupling of nutrient cycling and primary production, among other ecosystem processes, have been widely studied and found to have large effects on ecosystem structure and function in many shallow lakes (Vander Zanden and Vadeboncouer 2020) and shallow coastal ecosystems (Griffiths et al. 2017). Atmospheric nitrogen (N_2) fixation by diazotrophic (N_2 fixing) primary producers (i.e., free-living cyanobacteria and algae with cyanobacterial endosymbionts) is an ecosystem process that may be similarly coupled in these systems due to the dependence of phototrophic N_2 fixation on the availability of nutrients (Howarth et al. 1988) and light (Severin and Stal 2008) (i.e., coupled resources). Nonetheless, we

know of no study investigating the coupling between benthic and pelagic phototrophic N₂ fixation.

Phototrophic N₂ fixation often represents an important contribution to the nitrogen (N) budget of N-limited aquatic ecosystems (Zehr and Capone 2020), which draws attention to the abiotic factors that control the rate of N₂ fixation. Abiotic drivers include the availability of nutrients and light which often affect both the abundance and per capita N₂ fixation rate of phototrophic N₂ fixers, and thereby determine N₂ fixation rates in aquatic ecosystems (Vitousek et al. 2002). Low ratios of dissolved inorganic nitrogen (DIN) to dissolved inorganic phosphorus (DIP) tend to increase the abundance and/or per capita N₂ fixation rate of diazotrophs in an ecosystem (Schindler 1977). Low light tends to limit N₂ fixation in primary producers due to the dependence of phototrophic N₂ fixation on recent or contemporaneous photosynthesis for ATP (Postgate 1998).

The availability of nutrients and light for primary producers can differ between pelagic and benthic zones due to the effects of primary producers in the opposite zone. Phototrophic N₂ fixation in the benthos may be limited by light due to light attenuation through the water column and, therefore, an increase in the abundance of primary producers in the pelagic zone may decrease benthic N₂ fixation (Jäger and Diehl 2014). Conversely, phototrophic N₂ fixation in the pelagic zone may be limited by nutrients and, if pelagic N₂ fixation depends on nutrient flux from the benthos, then uptake of nutrients by benthic primary producers will decrease nutrient availability for pelagic N₂ fixation. Benthic-pelagic coupling of phototrophic N₂ fixation via these mechanisms is likely to vary over space and time due to variation in the abundance of

benthic and pelagic phototrophic diazotrophs. In the pelagic zone, N₂ fixing cyanobacteria form blooms that vary in spatial extent, severity, and/or duration from year to year (Ho et al. 2019). In the benthic zone, benthic cyanobacteria associated with algal mats can occur in discrete patches or over wide areas (Scott and Marcarelli 2012), with patterns changing markedly among years (Einarsson et al. 2004, Fig. 15).

Lake Mývatn is a large (37 km²), shallow (mean depth= 2.3 m) lake in northeastern Iceland. It is characterized by strong benthic-pelagic coupling of primary production (McCormick et al. 2021), and its shallow depth allows sunlight to typically reach the benthic surface (Einarsson et al. 2004). These characteristics, combined with N-limitation (molar N:P ratio of external nutrient supply ~2:1) (Ólafsson 1979), make Lake Mývatn an ideal site to investigate the dynamics of phototrophic N₂ fixation across benthic and pelagic zones. In Lake Mývatn, pelagic phototrophic N₂ fixation is primarily carried out by diazotrophic *Dolichospermum* spp., which dominate phytoplankton blooms that vary in severity, extent, and duration from year to year (Einarsson et al. 2004). In the benthic zone, diazotrophic epiphytes, including endosymbionts of *Epithemia* spp. and free-living cyanobacterial *Calothrix* spp., are associated with mat-forming filamentous green algae (Cladophorales); these mats vary annually in extent and thickness (Einarsson et al. 2004). Although heterotrophic N₂ fixers may contribute to N₂ fixation, we have found no measurable heterotrophic N₂ fixation in the experiments reported in the Results or in studies not presented here (K. R. Book unpubl.). Because the lake is shallow and the benthic surface is typically illuminated, benthic algae typically account for the majority of ecosystem-level primary production (McCormick et al. 2021). However, the majority of primary production shifts from the benthos to the pelagic zone during phytoplankton blooms due to bloom-induced shading of

benthic algae (McCormick et al. 2021). Nonetheless, the trade-off between benthic and pelagic primary production is roughly equal, so total primary production remains roughly constant during phytoplankton blooms. Here, we investigate whether similar benthic-pelagic shifts occur in phototrophic N_2 fixation as were observed in primary production.

We investigated the relationship between benthic and pelagic phototrophic N_2 fixation by taking paired measurements of benthic and pelagic N_2 fixation over the course of a phytoplankton bloom in the summer, 2022. Pelagic N_2 fixation was measured in the phytoplankton assemblage and benthic N_2 fixation was measured in Cladophorales mats. While these assemblages include both non- N_2 fixing and N_2 fixing algae, our N_2 fixation measurements reflect the activity of the cyanobacterial N_2 fixers (i.e. pelagic *Dolichospermum* spp. and diazotrophic epiphytes of Cladophorales) and for simplicity we will refer to the assemblages as pelagic and benthic cyanobacteria. On four dates, we sampled benthic and pelagic cyanobacteria at four sites. We estimated the maximum N_2 fixation capacity in benthic and pelagic cyanobacteria, N_{max} , by measuring their rate of N_2 fixation over a gradient of light levels and fitting N_2 fixation-irradiance (N-I) curves; this also enabled us to parameterize the relationship between light intensity and N_2 fixation. We simultaneously monitored in situ light intensity to estimate the rate of light attenuation over the water column and predict the amount of light available for cyanobacteria at all depths. By pairing in situ light intensity predictions with N-I curve parameter estimates for benthic and pelagic cyanobacteria, we calculated the total areal rate of benthic and pelagic N_2 fixation in situ for each site and sample date.

We expected that the capacity for N₂ fixation in pelagic cyanobacteria would increase as their concentration increased over the course of a bloom. We also expected that the bloom would cause shading of the benthos and thereby decrease benthic N₂ fixation. These expectations for bloom-induced shading on benthic N₂ fixation follow from earlier findings about the effects of bloom-induced shading on benthic primary production in the lake (McCormick et al. 2021). Our expectations for possible benthic effects on pelagic N₂ fixation were less certain. In prior monitoring assays (not presented) at Lake Mývatn, we found that the thickness of Cladophorales mats was negatively correlated with the flux of phosphorus from the sediment to the water column (K. R. Book unpubl.). Therefore, we suspected that Cladophorales mats could potentially limit the availability of phosphorus or other nutrients for pelagic cyanobacteria. Our final expectation was that an increase in areal in situ N₂ fixation in the pelagic zone during blooms would be offset by a decrease in areal in situ benthic N₂ fixation as found for primary production (McCormick et al. 2021). However, we did not know whether this offset would be great enough to keep the total areal rate of in situ N₂ fixation the same, or whether the pelagic cyanobacteria bloom would lead to a change in the total areal rate of N₂ fixation.

Methods

Study area

Lake Mývatn is a large, shallow lake in northeast Iceland (65°40'N, 17°00'W) (Einarsson et al. 2004). During the ice-free months (May - October), the lake does not stratify, and the water temperature fluctuates around the mean of 12°C in close response to air temperature, often

changing 3-5 degrees over the course of a week when weather conditions shift (Ólafsson 1979). Because the lake is shallow, sunlight typically reaches the benthic surface. However, water transparency decreases notably during phytoplankton blooms which frequently occur in the summertime (Jónasson and Aðalsteinsson 1979; McCormick et al. 2021).

The lake is naturally eutrophic and highly productive due to the high concentration of inorganic nutrients in groundwater springs that feed the lake along its eastern shoreline (mean N = 0.08 mg L⁻¹, mean P = 0.05 mg L⁻¹, Ólafsson 1979). However, the supply of N (1.4 g m⁻² year⁻¹) relative to P (1.5 g m⁻² year⁻¹) is very low (2:1 molar ratio) and falls below the Redfield ratio of 16:1, suggesting N limitation (Redfield 1958). Internal loading via the release of recycled organic matter from the sediment may supply substantial N (1.89 g NH₄ m⁻² y⁻¹) and P (0.13 g PO₄ m⁻² y⁻¹) to benthic and pelagic primary producers in the lake (Gíslason et al. 2004). However, estimates of N (-15.8 to 12.1 mg N m⁻² d⁻¹) and P (-18.2 to 17.8 mg P m⁻² d⁻¹) release vary over space and time, and are often negative (i.e., net nutrient uptake) due at least in part to bioassimilation by benthic primary producers (Thorbergdóttir and Gíslason 2004). N₂ fixation by primary producers is expected to be important for the annual N budget of the lake but has not been quantified directly (Ólafsson 1979; Einarsson et al. 2004). N₂ fixing *Dolichospermum* spp., which dominate episodic phytoplankton blooms, are thought to account for almost all pelagic N₂ fixation (Jónasson and Aðalsteinsson 1979; Á.E. pers. comm.). We suspect that lake-wide N₂ fixation in the benthic zone is dominated by N₂ fixing epiphytes of the mat-forming filamentous green algae *Cladophora glomerata* and *Aegagropila linnaei* (Cladophorales). In areas near springs, we have observed mats of diazotrophic *Anabaena* sp. and *Nostoc* spp., and macrophytes with diazotrophic epiphytes also occur; however, these are localized, and we performed our

experiments in the main basin of Lake Mývatn where the sediment is diatomaceous ooze and Cladophorales mats are often widespread. There are no published studies characterizing the epiphyte assemblage of Cladophorales mats in Lake Mývatn, but they are dominated by diatoms of the genus *Epithemia* which contain N₂ fixing cyanobacterial endosymbionts; cyanobacteria belonging to the genus *Calothrix* spp. can also regularly occur (K.R. Book unpubl.). We have observed no non-diazotrophic cyanobacteria in the phytoplankton or Cladophorales epiphyte assemblage.

Measuring N₂ fixation rates

On 18 July, 2 August, 13 August, and 20 August, 2022, we measured N₂ fixation in both benthic and pelagic cyanobacteria at four permanent sampling sites (E1, E3, E4, and E5; Ives et al. 2021) across Lake Mývatn (Fig. 1). Sites differed in depth (E1= 2.7 m, E3= 3.3 m, E4= 2.9 m, and E5= 2.5 m). Cladophorales mats were present at E1, E4, and E5, but absent at E3, resulting in no benthic N₂ fixation measurements at that site. Due to challenging weather conditions, we could not complete measurements of N₂ fixation in both zones at all sites on every sample date. We characterized N₂ fixation in pelagic cyanobacteria a total of 11 times and in benthic cyanobacteria a total of 9 times.

To collect pelagic cyanobacteria, we used a modified Schindler trap to sample phytoplankton from a standardized depth range of 0-2 m. For benthic cyanobacteria, we collected Cladophorales mat samples by taking 5-6 sediment cores (area of sediment core= 20 cm²) using a Kajak corer (KC Denmark) and gently removing all Cladophorales present at the sediment

surface. We also collected and filtered (0.45 mm) water taken from an integrated sample of the water column (0-2 m) at each site for subsequent incubations of benthic cyanobacteria. All samples were stored in cool, darkened vessels on the boat and transported to the lab within two hours. In the lab, we gently rinsed the Cladophorales mat samples over 63-mm mesh to remove loosely clinging sediment and phytoplankton. We transferred visually equal aliquots of Cladophorales to 5-6 (the same number of sediment cores taken) replicate gas-tight, septum-equipped 160-mL incubation bottles, ensuring each bottle contained the average amount of Cladophorales found in a single sediment core (i.e., the average amount of Cladophorales per 20 cm² of the benthic surface). We then filled benthic incubation bottles to 125 mL with filtered water from the same site. For pelagic cyanobacteria, we gently mixed the sampled phytoplankton and filled 5-6 replicate incubation bottles with ~125 mL of the phytoplankton suspension.

After stocking the incubation bottles, we placed them in outdoor water baths maintained at roughly 12°C. To estimate the rate of N₂ fixation at light saturation (N_{\max}) in benthic and pelagic cyanobacteria and parameterize the relationship between the rate of N₂ fixation and light intensity (see *Estimating N₂ Fixation Capacity*), we incubated samples from each site across a gradient of light intensities ranging from no light to ambient incident light intensity. On each sample date, incubations consisted of one bottle per light intensity treatment per zone (benthic and pelagic) per site.

We achieved light intensity treatments by affixing shades that reduced ambient light intensity at the surface of the water bath by pre-determined proportions. We determined these proportions by measuring light intensity (PAR; $\mu\text{moles photons m}^{-2} \text{ s}^{-1}$) at the surface of the water bath and

under each shade treatment on the bottom of the bath using a light meter (Li-192 Quantum Underwater Sensor, Li-COR, Lincoln, Nebraska, USA). To determine the light experienced by each incubation bottle, we measured ambient light intensity at the surface of the water bath every 5 minutes during the incubation and multiplied the average light intensity by the appropriate proportion. Ambient light intensity varied among sample dates due to weather, resulting in differing ranges of light intensities experienced by the incubation bottles across sample dates.

We used acetylene reduction assays (ARAs) (Flett et al. 1976; Capone 1993) to estimate the rate of N₂ fixation in each incubation bottle. To initiate the two-hour ARAs, we injected 15 mL of acetylene gas, which we generated by adding water to calcium carbide, into each bottle and immediately placed it in a water bath under the appropriate light intensity treatment. Initial and final gas samples were taken from gently inverted bottles at hours 0 and 2, and these samples were analyzed within 48 h using a gas chromatograph (model 8610; SRI Instruments, Torrance, California, USA) equipped with a flame ionization detector (HayeSep® T, 80/100 mesh). We estimated the rate of N₂ fixation ($\mu\text{g N}_2 \text{ h}^{-1}$) in each sample by converting the rate of acetylene reduction to the rate of N₂ fixation using a 3:1 molar ratio (Capone 1993).

We standardized the rate of N₂ fixation in each incubation bottle in two ways. First, we determined pelagic volumetric rates by dividing pelagic rates by the volume of phytoplankton suspension incubated (cm³) and benthic areal rates by dividing benthic rates by the area of sediment surface that it represented (20 cm²). Since we stocked the incubation bottles with ambient amounts of benthic and pelagic cyanobacteria, these volumetric/areal rates reflect variation in the capacity for N₂ fixation due to differences in the abundance of benthic and

pelagic cyanobacteria across sites and over time. They also allowed us to estimate total areal in situ N_2 fixation in each zone (See *Estimating areal in situ N_2 fixation*). Second, we determined biomass-standardized rates by dividing N_2 fixation rates by proxies of cyanobacterial biomass in each incubation bottle. For pelagic cyanobacteria, we approximated biomass by measuring the concentration of phycocyanin in the phytoplankton suspension ($mg\ cm^{-3}$) using a fluorometric probe (FluoroSense Handheld Fluorometer, Turner Designs, San Jose, CA, USA) and multiplying this concentration by the volume of phytoplankton suspension incubated (cm^3). This approach likely provided a reasonable estimate of cyanobacterial biomass because phycocyanin concentration is closely correlated with cyanobacterial biovolume in taxonomically homogenous cyanobacteria-dominated assemblages (Bertone et al. 2017). For benthic cyanobacteria, we estimated biomass by measuring the wet weight of Cladophorales after pressing the sample with a small weight for 15 seconds to remove excess water. While this metric overrepresents the biomass of N_2 fixers by including the biomass of Cladophorales, non- N_2 fixing epiphytes, and water, it should still accurately reflect variation in the abundance of N_2 fixing epiphytes across sites and over time because epiphyte biomass is linearly related to Cladophorales biomass in Lake Mývatn (I.S. Hart unpubl.), N_2 fixers dominate these epiphyte assemblages (K.R. Book unpubl.), and variation in water weight was standardized prior to measurements. Standardizing rates by biomass allowed us to observe changes in the biomass-specific capacity for N_2 fixation across sites and over time under the assumption that our proxies of cyanobacteria biomass effectively reflect cyanobacterial abundance.

Estimating N_2 fixation capacity

We estimated the N_2 fixation capacity (N_{\max} ; the rate of N_2 fixation at light saturation) of benthic and pelagic cyanobacteria by fitting an adapted Michaelis-Menten equation,

$$N_{\text{fix}} = N_{\max} \cdot \alpha I / (N_{\max} + \alpha I) \quad (1)$$

(Staal et al. 2002), to the volumetric/areal and biomass-standardized N_2 fixation-light intensity relationships for each zone, site, and sample date. In equation 1, N_{fix} is the standardized rate of N_2 fixation, I is the average light intensity over the course of the incubation, and α is the slope of the curve where light intensity approaches zero. We fit equation 1 using nonlinear least squares with the `nls()` function in R (v. 2023.12.1+402; R Core Team 2022).

Monitoring ambient conditions

Over the study, we monitored in situ light intensity (lux) at three depths: at the water surface, 0.5 m below the surface, and 0.5 m above the sediment surface. Measurements were taken every half hour using HOBO loggers (Onset Computer Corporation, Bourne, MA, USA) suspended perpendicularly from weighted buoy lines at each site. We converted light intensity from lux to PAR using a 56:1 conversion ratio (Thimijan and Heins 1983).

We estimated the mean daily rate of light attenuation (m^{-1}) for each site and sample date by regressing the mean daily log-transformed light intensity against water depth. Using these light attenuation estimates, we predicted mean daily light intensity at 1-cm intervals from the water surface to the benthic surface (i.e. the maximum depth of the site). Mean daily pelagic light

intensity represents predicted light intensity over the water column up to 1 cm above the benthic surface. Mean daily benthic light intensity represents light intensity at the benthic surface. Given the variability in ambient in situ surface light intensity among sample dates, we standardized predicted light intensities to a common surface light intensity of 400 PAR when calculating in situ N_2 fixation; at Lake Mývatn during the summertime, an ambient PAR of 400 is typical of cloudy afternoons. We also calculated mean daily temperature over the water column using temperature data collected from the same HOBO loggers.

On 29 June and 6 August, we collected and filtered (0.45 μm) a pelagic water sample from an integrated sample of the water column (0-2 m) at each site. These samples were frozen on the day of sampling and later analyzed for NO_3^- , NH_4^+ , and PO_4^{3-} using ion chromatography (Dionex ICS-2100 and Dionex ICS-1100, ThermoFisher Scientific, Waltham, MA, USA). The lower detection limit for all analytes was assessed using serial dilution assays and determined to be 0.0001 mg L^{-1} , which is low relative to the standard for environmental samples (Agency for Toxic Substances and Disease Registry 2017).

Estimating areal in situ N_2 fixation

We estimated total areal rates of phototrophic N_2 fixation (N_{fix} ; $\text{mg N}_2 \text{ cm}^{-2} \text{ h}^{-1}$) in situ for benthic and pelagic zones by applying the adapted Michaelis-Menten equation (equation 1) along with the parameter estimates (N_{max} and α) from the N-I curves and predicted in situ light intensities (I). We estimated areal in situ pelagic N_2 fixation by summing estimates of N_{fix} for every 1-cm interval over the entire water column. This estimate accounts for self-shading by

phytoplankton over the water column. We estimated total areal in situ benthic N_2 fixation by calculating N_{fix} at the predicted benthic light intensity derived from our ambient light measurements. To quantify uncertainty in our estimates of N_2 fixation due to uncertainty in our N-I curve parameter estimates, we bootstrapped 1,000 samples for each curve and calculated bootstrapped standard errors.

Results

Ambient conditions

The concentration of pelagic cyanobacteria in the phytoplankton (μg phycocyanin L^{-1}) increased across sites over the study until 20 August when it decreased at the two sites that we were able to sample (E4 and E5) (Fig. 2A). The density of benthic cyanobacteria (mg Cladophorales wet weight cm^{-2}) was the same over time within sites and varied consistently among sites (Fig. 2B). At all sites, as pelagic cyanobacteria concentration increased, mean light attenuation increased (Fig. 3A) resulting in a decrease in both mean pelagic light intensity (Fig. 3B) and mean the benthic light intensity (Fig. 3C). In addition to increased light attenuation, the decrease in light intensity at the benthic surface was due to cloudy weather and decreased day length in August. All sites experienced a period of complete benthic darkness from 28 July to 17 August except E5 (Fig. 3C). This period of relatively low light attenuation and relatively high benthic light intensity at E5 coincided with a period of low pelagic cyanobacteria concentration at that site relative to the other sites (Fig. 2A).

The mean daily temperature of the water column was similar on average across sites (E1= 11.6°C, E3= 11.4°C, E4= 11.5°C, and E4= 11.1°C) but ranged from approximately 8°C to 15°C over time due to variation in air temperature (Supporting Information Fig. S1).

Benthic and pelagic N₂ fixation capacity

The rate of N₂ fixation in pelagic and benthic cyanobacteria increased with light intensity up to a saturation point at 100-200 PAR (Fig. 4, Fig. S2). The capacity for areal benthic and volumetric pelagic N₂ fixation at light saturation (N_{max}) varied among sites and over time (Fig. 5A).

Volumetric N_{max} in the pelagic zone increased over time until August 13 and then decreased markedly at the two sites which could be sampled on the final date 20 August (Fig. 5A).

However, when pelagic N_{max} was standardized by the proxy for pelagic cyanobacterial biomass (mg phycocyanin in the incubation bottle), biomass-standardized N_{max} within a site changed much less than the non-standardized rates (Fig. 5B as compared to Fig. 5A). Although pelagic biomass-standardized N_{max} changed little within sites over time, there were consistent differences among sites of greater than 2-fold. To provide a possible explanation for variation in pelagic biomass-standardized N_{max}, we measured the concentration of dissolved inorganic nutrients in the pelagic zone at each site before the study in late June and during the study in early August. However, pelagic dissolved inorganic nutrient concentrations were mostly below the analytical detection limit, making it difficult to determine their effect on pelagic N₂ fixation (Supporting Information Table S1). Nonetheless, the site with the highest biomass-standardized pelagic N_{max} over time, E3, had no Cladophorales mats (Fig. 5B).

In the benthic zone, both areal and biomass-standardized N_{\max} decreased over time (Fig. 5C, 5D). Benthic biomass-standardized N_{\max} was notably higher at E5 than E1 and E4 on 13 August but decreased by 20 August. The high biomass-standardized N_{\max} at E5 relative to E1 and E4 coincided with a period of severe light attenuation and low benthic light intensity at E1 and E4 relative to E5 (Fig. 3; Fig. 5D).

Overall, there was a negative relationship between volumetric pelagic N_{\max} and areal benthic N_{\max} ($\rho = -0.66$, Spearman rank correlation; Fig. 6). This negative relationship was driven by a decrease in benthic N_{\max} over time and differences in pelagic N_{\max} among sites (Fig. 5A, 5B). The limited number of sites and time points makes it difficult to partition the relative contributions of variation among sites and variation over time in explaining this negative relationship.

Areal in situ benthic and pelagic N_2 fixation

Corresponding to the increase in pelagic cyanobacteria biomass (Fig. 2A), estimated areal in situ N_2 fixation in the pelagic zone increased across sites over time from 0.031 to 0.202 $\mu\text{g } N_2 \text{ cm}^{-2} \text{ h}^{-1}$ (Fig. 7A). Estimated areal in situ benthic N_2 fixation ranged from 0.00 to 0.005 $\mu\text{g } N_2 \text{ cm}^{-2} \text{ h}^{-1}$ and decreased over time (Fig. 7B). Furthermore, pelagic cyanobacteria accounted for 87-100% of total areal in situ N_2 fixation which ranged from 0.036 $\mu\text{g } N_2 \text{ cm}^{-2} \text{ h}^{-1}$ at E1 on 18 July to 0.202 $\mu\text{g } N_2 \text{ cm}^{-2} \text{ h}^{-1}$ at E3 on 2 August (Fig. 7C). Because the estimated areal in situ N_2 fixation rate of the pelagic zone was much greater than that of the benthic zone, the decrease in benthic N_2 fixation

did little to diminish the increase in total areal N_2 fixation at high pelagic cyanobacteria concentrations.

Discussion

In this study, we measured the rate of N_2 fixation by benthic and pelagic primary producers over the course of a pelagic cyanobacteria bloom in a shallow, N-limited lake. The capacity for N_2 fixation at light saturation (N_{max}) increased in pelagic cyanobacteria as it decreased in the N_2 fixing epiphytes of benthic Cladophorales mats. The increase in pelagic N_{max} was driven by an increase in pelagic cyanobacteria biomass rather than up-regulation of N_2 fixation, as indicated by generally stable biomass-standardized rates of N_2 fixation (Fig. 5B). Although there was little change in biomass-standardized pelagic N_{max} over time within sites, there were consistent differences in pelagic N_{max} among sites, suggesting that sites differed in characteristics that affect the rate of N_2 fixation. In contrast, benthic N_{max} decreased over the course of the pelagic bloom even though the biomass of benthic Cladophorales mats remained the same. This indicates that benthic N_2 fixers in Cladophorales mats had lower per capita N_2 fixation rates and/or decreased abundance in Cladophorales mats, both of which could have been caused by shading by the pelagic zone. Despite the decrease in benthic N_2 fixation with increasing light attenuation, the total areal in situ N_2 fixation increased due to increased pelagic N_2 fixation during the pelagic cyanobacteria bloom.

The total areal amount and location of N_2 fixation shifted over the course of the pelagic cyanobacteria bloom. Areal N_2 fixation in the pelagic zone was always greater than areal N_2

fixation in the benthic zone, although it accounted for a lower percentage of total areal N_2 fixation earlier than later in the bloom (Fig. 7C). Areal pelagic N_2 fixation was high relative to benthic N_2 fixation because pelagic fixation was integrated over the water column. These results contrast with the findings about primary production from McCormick et al. (2021) who showed that reciprocal shifts in the benthic-pelagic partitioning of primary production during pelagic blooms in Lake Mývatn offset each other, resulting in little change in total primary production.

Both benthic N_{max} and estimated in situ N_2 fixation decreased over the course of the study, likely due to prolonged bloom-induced light limitation. The community of N_2 fixing epiphytes in Cladophorales mats is dominated by endosymbiotic (spheroid bodies of *Epithemia* spp.) and free-living (*Calothrix* spp.) cyanobacteria that depend on contemporaneous photosynthesis for the energy required for N_2 fixation (Higgins et al. 2001; Moulin et al. 2024). This dependence explains, at least in part, why the N_2 fixation-irradiance (N-I) curves (Fig. 4) show similar reductions under low light conditions as found for primary production (McCormick et al. 2021, Fig. 1). Using the N-I curve to estimate in situ N_2 fixation, the decreased light intensity at the benthic surface was sufficient to shut off N_2 fixation under heavy pelagic bloom conditions. However, the dependence of N_2 fixation on contemporaneous photosynthesis does not explain the reduction in N_{max} over the summer, as N_{max} was measured at light saturation. A possible explanation for the reduced rates of N_{max} is that prolonged light limitation reduced the abundance or per capita N_2 fixation rate of epiphytes in Cladophorales mats, or changed epiphyte community composition (Scott and Marcarelli 2012). Higgins et al. (2001) found that N_2 fixation capacity per mg of heterocyst biomass decreased with depth (i.e., light) in *Calothrix*-dominated epilithic periphyton communities of Lake Malawi, implicating physiological changes within

Calothrix cells as the mechanism of N₂ fixation reduction. Gettel et al. (2013) observed lower N₂ fixation capacities in epipelagic primary producers in enriched oligotrophic Alaskan lakes that experienced an increase in light attenuation and decrease in benthic light intensity, in contrast to unenriched reference lakes.

In contrast to benthic N₂ fixation, both N_{max} and estimated in situ N₂ fixation increased in the pelagic zone over the course of the study up to the last sample date when the pelagic cyanobacteria bloom began to collapse lake wide. These increases were driven by an increase in cyanobacteria biomass (as measured by our proxy for pelagic cyanobacterial biomass, mg of phycocyanin); with higher N₂ fixer biomass, the possible rate of N₂ fixation per unit of volume increased. This led to increases in estimated areal in situ pelagic N₂ fixation despite the negative effect of pelagic cyanobacteria biomass on light intensity over the water column; the net effect of the bloom on pelagic N₂ fixation was positive despite the likelihood of very low pelagic N₂ fixation rates over much of the water column due to self-shading. Physiological changes in pelagic cyanobacteria did not explain these patterns, since biomass-standardized N_{max} changed little over time within sites.

Pelagic biomass-standardized N_{max} differed consistently among sites. These differences in N_{max} suggest differences in pelagic conditions such as nutrient availability that are potentially affected by benthic processes. Unfortunately, pelagic concentrations of phosphate, a likely candidate for nutrient limitation of pelagic N₂ fixation, were mostly below detection limit in our study. While this suggests that phosphorus was limiting, it removed any signal to understand differences among sites in pelagic N₂ fixation. The site with the highest pelagic N_{max}, E3, was the only site

without Cladophorales mats (Fig. 4), and prior assays have shown lower nutrient flux rates from the sediment into pelagic water in the presence of Cladophorales (K. R. Book unpubl.). In a previous study in Lake Mývatn, the flux of N and P across the sediment-water interface was highly variable over space and time due at least in part to nutrient assimilation by benthic primary producers (Thorbergsdóttir and Gíslason 2004), which is also found in other lakes (Ortiz and Wilkinson 2021) and estuaries (Bartoli et al. 2018). In general, the primary mechanism by which benthic primary producers affect pelagic primary producers is by mediating the flux of nutrients from the sediment to the water column (Carlton and Wetzel 1988; Hansson 1988). Furthermore, due to the high biomass and surface area of Cladophorales mats relative to epipelagic algae, nutrient assimilation by benthic primary producers is likely to be higher at sites with Cladophorales than sites without (Thybo-Christensen et al. 1993). Thus, based on our findings and previous studies on Lake Mývatn and other systems, we think that the most likely explanation of consistent differences in pelagic biomass-standardized N_2 fixation is variation in nutrient release from the sediment mediated by Cladophorales. Nonetheless, we have no direct evidence that this is the case, and consistent site differences might have other unknown explanations.

Benthic and pelagic cyanobacterial blooms are increasing in severity, duration, and extent in ecosystems worldwide (Huisman et al. 2018). As a result, the magnitude and location of phototrophic N_2 fixation within these ecosystems is likely to change. Our study has demonstrated the importance of considering both benthic and pelagic phototrophic N_2 fixation when determining the effect of ecosystem changes on N_2 fixation, as benthic and pelagic phototrophic N_2 fixation may be coupled in shallow ecosystems where they both occur. As a result, an

increase pelagic N₂ fixation may be offset by a decrease in benthic N₂ fixation (and vice versa). In our study, increased pelagic N₂ fixation during a pelagic cyanobacteria bloom more than compensated for decreased benthic N₂ fixation in Cladophorales mats on an areal basis and over a short time scale. However, the net effect of a shift in the partitioning of benthic and pelagic N₂ fixation on ecosystem-level phototrophic N₂ fixation over a longer period will depend on the abundance and N₂ fixation rate of primary producers in each zone integrated over space and time. Additionally, if N₂ fixed in one zone is retained at a different rate than N₂ fixed in the other, shifts in the partitioning of N₂ fixation could also impact the relationship between N₂ fixation and N acquisition within an ecosystem. Therefore, attention should be given not only on the magnitude and location of N₂ fixation, but also the fate of that fixed nitrogen.

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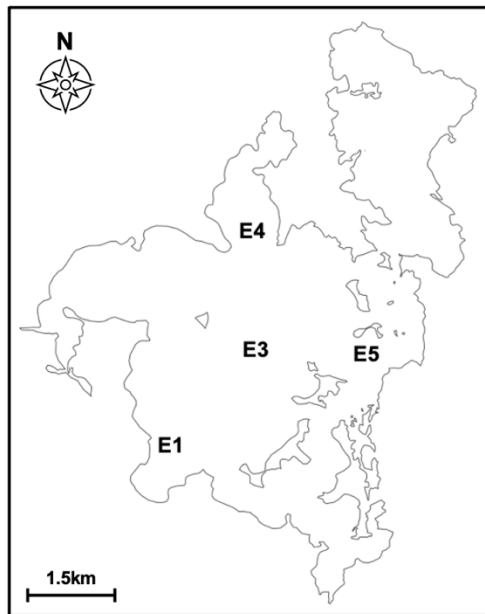
Figures and Tables

Figure 1. Map of Lake Mývatn showing study site locations.

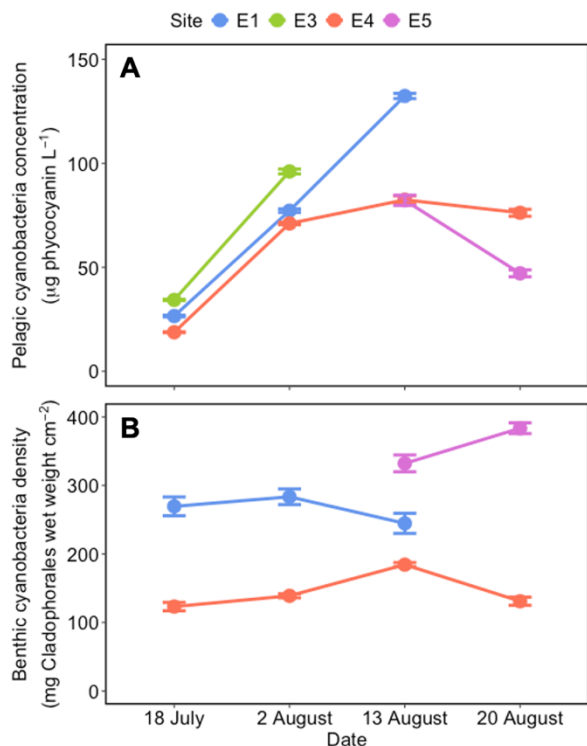


Figure 2. Changes in mean benthic cyanobacterial density and pelagic cyanobacterial concentration across sites and sample dates in 2022. (A) Pelagic cyanobacterial concentration was approximated by measuring the concentration of phycocyanin (mg L^{-1}) in incubation bottles ($n= 5-6$) stocked with an ambient suspension of phytoplankton and (B) benthic cyanobacterial density was approximated by measuring the wet weight of Cladophorales in incubation bottles ($n= 5-6$) where the amount of Cladophorales in an incubation bottle corresponded to 20 cm^2 of the lake's benthic surface. Benthic cyanobacteria were not present and therefore not measured at E3. Bars represent standard error.

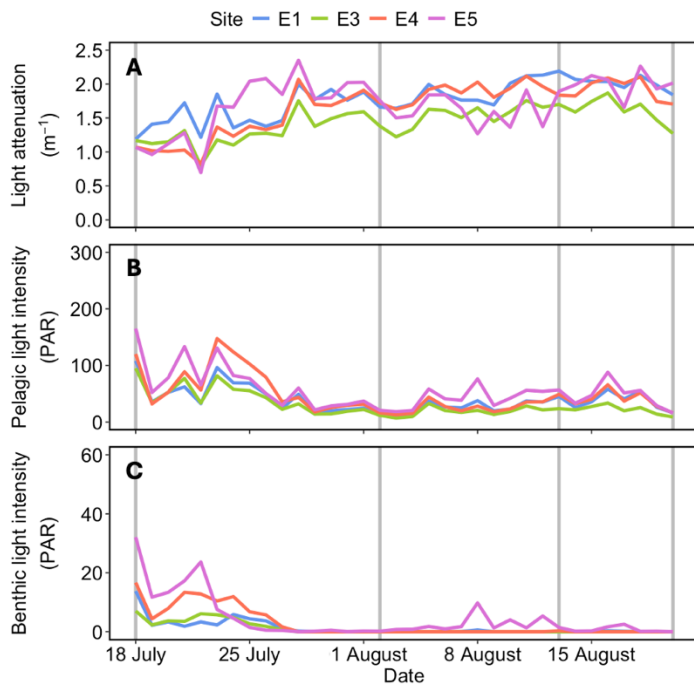


Figure 3. Changes in (A) mean rate of light attenuation, (B) mean pelagic light intensity, and (C) mean benthic light intensity across sites and over the study period in 2022. The vertical lines indicate dates on which benthic and pelagic cyanobacteria were sampled.

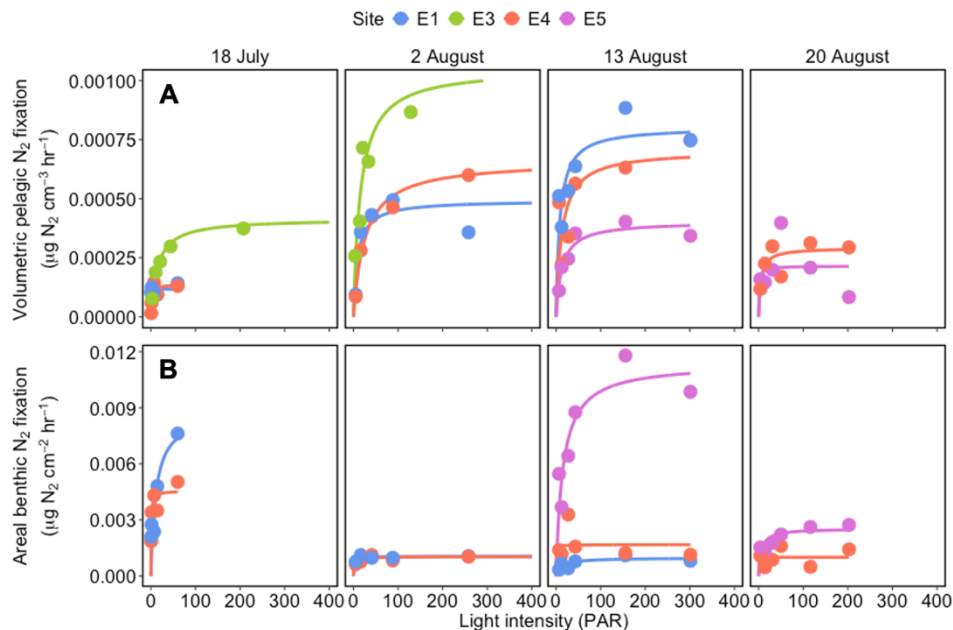


Figure 4. N₂ fixation-irradiance (N-I) curves fit to the relationship between light intensity and volumetric/areal N₂ fixation rates in (A) pelagic and (B) benthic cyanobacteria across sites and sample dates during the study period in 2022. Curves were fit separately for each site and sample date. Pelagic rates were standardized by the volume of phytoplankton suspension incubated (cm³) and benthic rates were standardized by the area of sediment core (cm²) such that rates reflect variation in the biomass of cyanobacteria across sites and sample dates. See Supporting Information Fig. S2 for N-I curves fit to biomass-standardized rates of N₂ fixation.

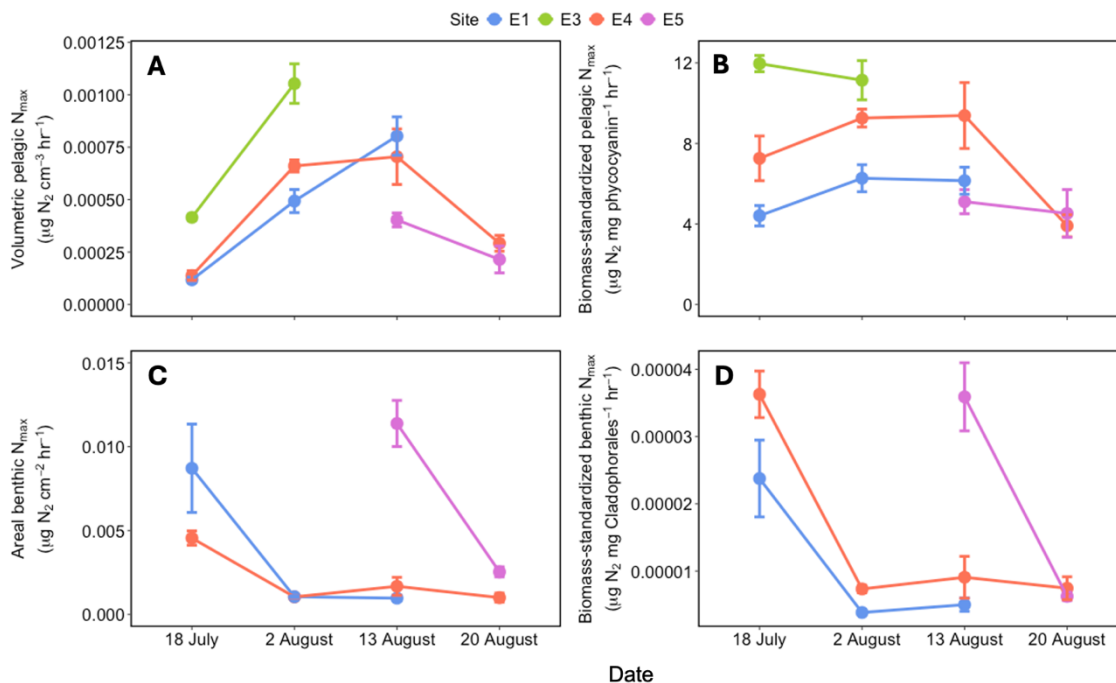


Figure 5. Changes in mean N_{max} of pelagic (A, B) and benthic (C, D) cyanobacteria across sites and sample dates during the study period in 2022. N_{max} estimates were standardized by (A, C) sample volume/area or (B, D) biomass. N_{max} estimates were estimated from the fitted N-I curves in Fig. 4 (A, B) and Supporting Information Fig. S2 (C, D). Bars represent standard error of the estimate.

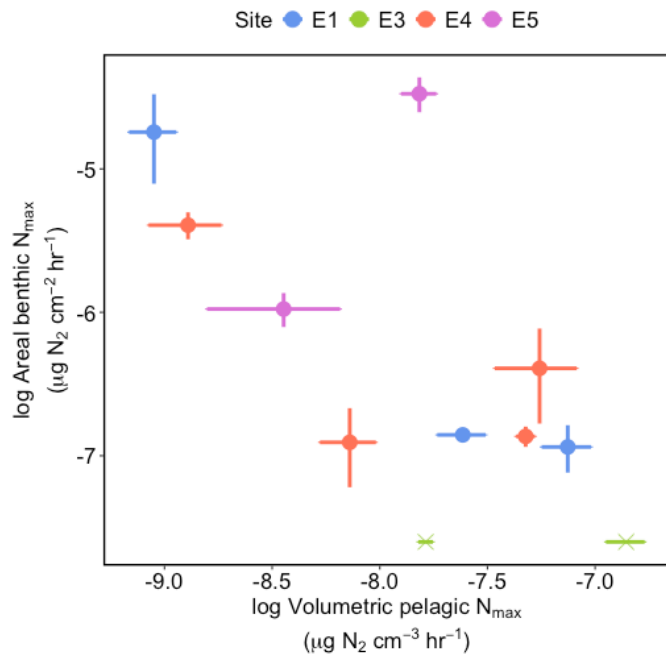


Figure 6. The overall relationship between mean volumetric pelagic N_{\max} and mean areal benthic N_{\max} across sites on a log-log scale. In order to plot pelagic N_{\max} estimates for E3 where benthic cyanobacteria were absent, benthic N_{\max} was assumed to be 10^{-3} and indicated with an “x”. Bars represent standard errors.

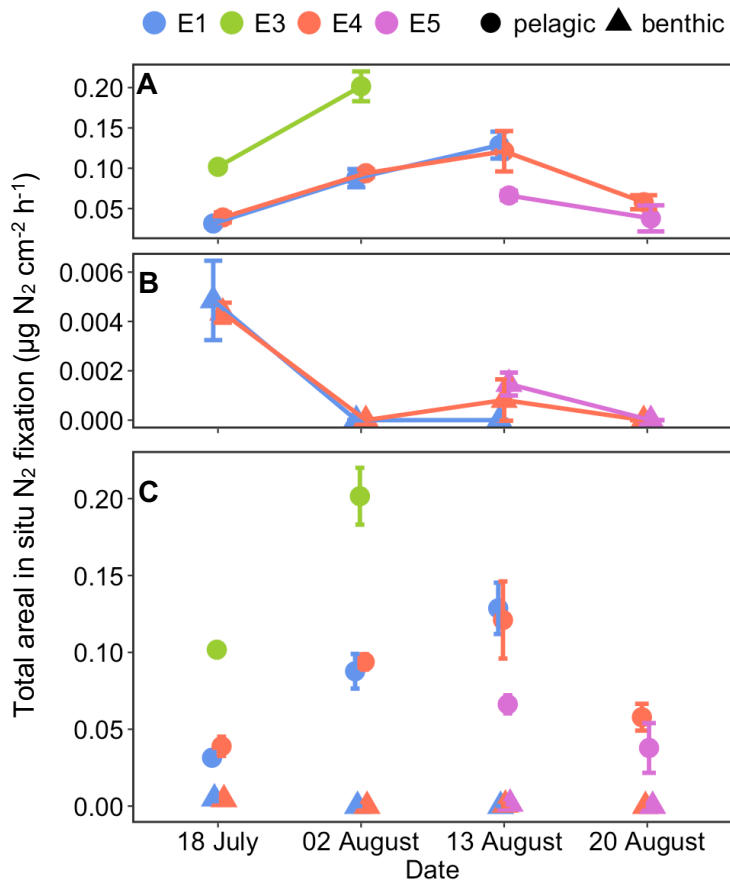


Figure 7. Changes in total areal in situ N₂ fixation across sites and sample dates during the study period 2022. (A) Pelagic and (B) benthic total areal in situ N₂ fixation were estimated by pairing pelagic and benthic N-I curve parameter estimates for each site and sample date with estimates of in situ light intensity over the water column (pelagic) and at the benthic surface (benthic) under a standard surface light intensity of 400 PAR. (C) The relative contributions of benthic and pelagic N₂ fixation to total areal N₂ fixation can be seen when benthic and pelagic rates are plotted on the same scale. Bars represent standard errors.

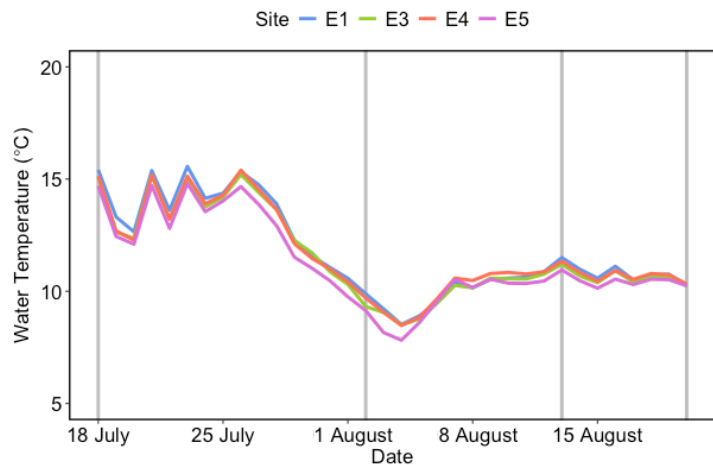


Figure S1. Changes in mean temperature of the water column at each site over the study period in 2022. The vertical lines indicate dates on which benthic and pelagic cyanobacteria were sampled.

CHAPTER 2

Benthic primary producers regulate pelagic nitrogen fixation by mediating benthic nutrient flux
in a shallow lake*

Abstract

Nutrient availability is an important determinant of N fixation rates in pelagic cyanobacteria. In shallow aquatic ecosystems, benthic primary producers can control nutrient release from the sediment to the pelagic zone, yet the relationship between benthic primary producers and pelagic N fixation is not well-understood. Here, we explore the potential role of benthic primary producers in regulating pelagic N fixation rates in Lake Mývatn, Iceland, via their effects on nutrient flux rates from the sediment. In paired benthic nutrient flux and pelagic N fixation assays, we showed that sites with increased benthic phosphate release were associated with increased pelagic N fixation. In turn, during assays of flux rates, benthic phosphate release was negatively associated with DO concentration at the sediment-water interface, which was positively associated with benthic primary producer abundances. Together, these relationships suggest that benthic primary producers indirectly influence pelagic N fixation rates in Mývatn via their effects on benthic nutrient flux rates.

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Introduction

In nitrogen-limited lakes and oceans, nitrogen (N) fixation by pelagic cyanobacteria can support ecosystem-level primary production (Fulweiler et al., 2025; Hayes et al., 2019; Schindler, 1977; Zehr & Capone, 2020). However, the rate of N fixation in pelagic cyanobacteria may vary in response to many environmental factors (Marcarelli et al., 2022; Vitousek et al., 2002) including the availability of N and phosphorus (P), and their ratio, N:P (Howarth et al., 1988; Smith, 1990). The release of stored nutrients from the sediment (i.e., internal loading) can significantly impact the concentrations of N and P, and N:P, in the water column (Søndergaard, Jensen, and Jeppesen 2003; Spears et al. 2008). However, nutrient fluxes across the sediment-water interface are often highly dynamic (Spears et al. 2012; Yang et al. 2013; Thorbergsdóttir and Gíslason 2004), changing in magnitude and direction over space and time in response to both abiotic and biotic factors. Due to their spatiotemporal variation, understanding the influence of benthic nutrient fluxes on pelagic N fixation requires repeated measurements of both processes through space and time. This presents a challenge for studies on the relationship between benthic nutrient fluxes and pelagic N fixation. To our knowledge, this relationship is not well-documented (*but see* Hendzel, Hecky, and Findlay 1994) despite the consensus that internal loading is one of the factors controlling N fixation in freshwater ecosystems (Howarth et al., 1988; Scott et al., 2008)

In shallow lakes with adequate benthic light availability, benthic primary producers are known to be important drivers of variation in benthic nutrient flux rates (Carlton & Wetzel, 1988; Dalsgaard, 2003; Hansson, 1988; Thorbergsdóttir & Gíslason, 2004). Due to diffusion, high interstitial nutrient concentrations relative to the overlying water can lead to high rates of nutrient

release from the sediment (Callender & Hammond, 1982; Lerman, 1979; Mortimer, 1942). However, by assimilating nutrients as they are released from the sediment, benthic primary producers can decrease the flux of N and/or P, shifting the absolute or relative supply of these nutrients to the water column above (Levine and Schindler 1992; Bartoli, Nizzoli, and Viaroli 2003; Spears et al. 2008). Benthic primary producers can also increase or decrease nutrient release rates via their effect on benthic abiotic factors including dissolved oxygen (DO) concentration (Carlton & Wetzel, 1988). Through photosynthesis, benthic primary producers tend to increase DO at the sediment-water interface (Carlton & Wetzel, 1987; Revsbech et al., 1983) which can decrease phosphate (PO_4^{3-}) release by increasing iron-bound phosphate stability (Mortimer, 1942) and increase the release of nitrate (NO_3^-) relative to ammonium (NH_4^+) by promoting nitrification (Rysgaard et al., 1994). Thus, it is well understood that benthic primary producers can regulate the abundance and rate of primary production of phytoplankton by altering benthic nutrient flux rates through these and other mechanisms (Genkai-Kato et al., 2012; Jäger & Diehl, 2014).

The effect of nutrient flux regulation by benthic primary producers on the rate of pelagic cyanobacterial N fixation is less well-understood. N fixation is energetically expensive for cyanobacteria (Horne & Fogg, 1970; Tilzer, 1987), and therefore rates of N fixation are often tightly coupled with rates of pelagic primary production (Severin & Stal, 2008). Nonetheless, N fixation may also depend on the absolute and/or relative availability of N because some cyanobacteria can turn off N fixation (Howarth et al., 1988) and/or non-N fixing phytoplankton can outcompete N fixing cyanobacteria (Tilman et al., 1982; Levine & Schindler, 1999; Schindler et al., 2012) when N availability is high. Furthermore, N fixation is known to be more

temperature-dependent than primary production (Welter et al., 2015) which could break down the relationship between rates of N fixation and rates of primary production. Thus, effects of benthic nutrient fluxes on the rate of N fixation in pelagic cyanobacteria could differ from their effects on primary production.

Lake Mývatn is a shallow, polymictic lake in northeast Iceland. Due to the high concentration of dissolved inorganic nutrients in the groundwater springs that feed the lake along its eastern shoreline, the lake is eutrophic and highly productive (Einarsson et al. 2004). However, the molar supply of N relative to P is low (2:1) relative to the Redfield ratio (16:1) (Redfield, 1934), which suggests that non-diazotrophic (i.e. non-N fixer) primary production in the lake should be N-limited. Potentially in response to this N-limitation, blooms of diazotrophic pelagic cyanobacteria (*Dolichospermum* spp.) frequently occur during the summertime (Jónasson and Aðalsteinsson 1979; Einarsson et al. 2004). During these blooms, the limiting nutrient in the water column can shift to P across much of the lake (Botsch and Book et al. in prep). Pelagic N fixation in these blooms is understood to be an important contribution to the N budget of the lake (Ólafsson 1979; Jónsson 2016; Book et al. in prep). However, the capacity for N fixation within pelagic cyanobacteria varies over space and time, potentially in response to spatiotemporal variation nutrient supply from the benthos (Book et al. in review).

In Mývatn, variation in benthic nutrient fluxes are known to arise, in part, from variation in the abundance and/or community composition of benthic primary producers (Thorbergsdóttir & Gíslason, 2004). Several pieces of evidence suggest that this variation in benthic primary producers may alter the abundance and rate of primary production in pelagic cyanobacteria

locally (McCormick et al. 2021; Botsch and Book et al. in prep) and at the scale of the whole lake (Einarsson et al. 2004). However, the degree to which benthic primary producers influence N fixation rates in pelagic cyanobacteria through their effects on benthic nutrient flux has not been explored directly.

Here, we investigate the relationship between benthic nutrient flux and pelagic N fixation in Mývatn by conducting paired benthic nutrient flux and pelagic N fixation assays across the lake during pelagic cyanobacterial blooms in 2022 and 2023. Because benthic primary producers are known to influence benthic nutrient flux in Mývatn (Thorbergsdóttir & Gíslason, 2004) and elsewhere (Carlton & Wetzel, 1988; Hansson, 1988; Dalsgaard, 2003), we also explore the potential role of benthic primary producers in regulating pelagic N fixation indirectly via their effect(s) on benthic nutrient flux. The few previous studies that have linked benthic nutrient fluxes to pelagic N fixation have done so at the scale of entire lakes (Hendzel et al., 1994); in contrast, here we investigate whether within-lake variation in benthic nutrient fluxes can explain variation in pelagic N fixation rates at different locations within the same lake. We do this by characterizing benthic primary producer abundances, as well as DO concentration in the water overlying the sediment surface, which is influenced by benthic photosynthesis, in each benthic nutrient flux assay.

Methods

Study site

Lake Mývatn is a large (37 km²), shallow (mean depth = 2.3 m) lake in northeastern Iceland. Due to its shallow depth and frequent wind-induced mixing (Ólafsson, 1979a), the lake does not stratify during the ice-free season. The lake lies over a lava field that was created 2,300 years ago and is impervious to ground-water seepage except through the springs along the eastern shore. The lava is covered by a thick (up to 4 m) layer of diatomaceous sediment (Einarsson 1982), the surface of which is colonized by abundant benthic primary producers. Epipellic microalgae, primarily diatoms, and mat-forming green algae (Cladophorales), which host diverse epiphyte communities, are the dominant benthic primary producer assemblages across much of the lake, although their abundances vary over space and time (Einarsson et al. 2004; Botsch and Book et al. in prep). Rates of benthic primary production tend to be high in the lake (Einarsson et al. 2004; McCormick et al. 2021). However, blooms of the pelagic N-fixing cyanobacteria *Dolichospermum* spp., which occur to varying extents, densities, and durations during the summertime (Einarsson et al. 2004), can reduce benthic primary production via shading (McCormick et al., 2021). Abundance-standardized rates of N fixation within *Dolichospermum* blooms vary over space within the lake, potentially in response to persistent site characteristics (Book et al. in review). While both water column and interstitial nutrient concentrations typically indicate N limitation of primary production (molar N:P <16:1) in the lake, the water column appears to be P-limited during *Dolichospermum* blooms (Botsch and Book et al. in prep). Internal loading of N and P vary over space and time likely due to the effects of benthic primary producers on nutrient uptake and/or oxygen concentrations at the sediment-water interface (Thorbergssdóttir & Gíslason, 2004). Nutrient concentrations in the sediment and the water column suggest that the flux of ammonium and phosphate will be from the sediment to the water

column, while the flux of nitrate will be from the water column to the sediment (Gíslason et al., 2004).

Study design

We conducted paired assays of pelagic N fixation and benthic nutrient flux during pelagic cyanobacteria (*Dolichospermum* spp.) blooms in the summers of 2022 (August) and 2023 (July and August). Our study focused on the South Basin of Mývatn and included four sites (E1, E3, E4, and E5) in 2022 and nine sites (E1, E2, E3, E4, E5, E6, MIK, ALF, and BTL) in 2023 (Fig. 1). Because we intended to investigate the effects of benthic primary producers on pelagic N fixation, we selected sites that represent variation in the abundances of the dominant benthic primary producers in the lake: mat-forming filamentous green algae (Cladophorales) and epipelagic diatoms. Additional sites were added in 2023 to increase the representation of both benthic primary producer community types. Because it was not always practical to conduct pelagic N fixation assays and benthic nutrient flux assays on the same date, pelagic N fixation assays and benthic nutrient flux assays were conducted at all sites over multiple dates and paired in both 2022 and 2023. We refer to sets of paired assays as events in this study. In the August 2022 event, pelagic N fixation assays were conducted on 2 August, 5 August, and 13 August and paired with a single benthic nutrient flux assay conducted on 7 August. In the July 2023 event, pelagic N fixation assays were conducted on 8 and 10 July and paired with benthic nutrient flux assays conducted on 9 and 11 July. In the August 2023 event, a pelagic N fixation assay was conducted on 4 August and paired with a benthic nutrient flux assay conducted on the same date.

The overall design of the measurements and analyses of the study are depicted in Figure 2. Briefly, three replicate benthic flux rates were measured for ammonium, nitrate, and phosphate for each site-event. The averages of the measurements from the three replicates were then used as the site-level estimates of benthic nutrient fluxes. Pelagic N fixation rates were similarly measured in three replicates for each site-event. Comparing these measurements of benthic nutrient fluxes and pelagic N fixation rates gives the site-level relationship between nutrient flux and N fixation (Fig. 2, Step i). We also investigated potential mechanisms that might control benthic nutrient fluxes at the core-level. Specifically, we first analyzed whether DO concentration within benthic nutrient flux assay cores was related to benthic nutrient fluxes while accounting for differences in interstitial nutrient concentrations among cores (Fig. 2, Step ii). We then analyzed whether the abundances of mat-forming Cladophorales and/or epipellic diatoms were related to DO concentration (Fig. 2, Step iii). In these analyses, we focus on DO as a measure not only of primary producer abundance, but also primary producer activity as gauged by the release of DO.

Pelagic N fixation assays

To measure pelagic N fixation, we collected an integrated sample of the water column at each site using a modified Schindler trap. Sites ranged in depth from 1.5 to 3.3m. At sites <2m in depth, we collected a sample from 0-1m. At sites >2m in depth, we collected a sample from 0-2m. All water column samples reached maximum depths >0.5m from the sediment surface to minimize sediment disturbance. Within 2 hours of collection, we gently stirred the unaltered integrated water column samples containing pelagic cyanobacteria from each site and added 125-

mL to three replicate gas-tight, septum-equipped 160-mL incubation bottles per site. Stocked incubation bottles were then immediately placed outdoors in clear plastic water baths. Incubation bottles were subject to ambient temperature and light intensity at the bottom of the water baths, with event means ranging from 9.3 to 15.4°C and 202 to 2118 $\mu\text{moles photons m}^{-2} \text{s}^{-1}$ photosynthetically active radiation (PAR) due to variation in weather conditions. Despite this variation in ambient PAR, all incubations were conducted at or above the observed saturating PAR for pelagic N fixation in Mývatn of 100 to 200 $\mu\text{moles photons m}^{-2} \text{s}^{-1}$ (Book et al. in review). Possible effects of variation in temperature were included in the analyses.

We used acetylene reduction assays (ARAs) to estimate the rate of pelagic N fixation in each incubation bottle. To initiate each ARA, we injected 15 mL of acetylene gas into each bottle. We produced acetylene gas immediately prior to injection by reacting ~5 g of calcium carbide with ~50 mL of water. Following acetylene injection, we gently inverted (60 s), vented, and again inverted (30 s) each bottle to homogenize the contents. We then took initial 3-mL gas samples from the headspace (35 mL) of each bottle and returned the bottles to water baths. After 2 h, we took final gas samples from the headspace of each bottle after gently inverting each bottle for 30 s. We determined the concentration of ethylene (produced via the reduction of acetylene by the nitrogenase enzyme) in initial and final gas samples within 48 h using a gas chromatograph (model 8610; SRI Instruments, Torrance, California, USA) equipped with a flame ionization detector (HayeSep® T, 80/100 mesh). Ethylene concentrations measured in the headspace were converted to total ethylene production (gas + aqueous phases) using the ideal gas law. We assumed equilibration between the headspace and aqueous phase at the incubation temperature, and used the temperature-specific Bunsen solubility coefficient for ethylene to estimate the

aqueous concentration (Breitbarth et al., 2004). We estimated the rate of N fixation ($\mu\text{g N h}^{-1}$) in each sample by converting the rate of ethylene production to the rate of N fixation using a 3:1 molar ratio (Capone, 2018).

We standardized pelagic N fixation rates by the abundance of pelagic cyanobacteria to account for variation in pelagic cyanobacterial density among events. Using abundance-standardized pelagic N fixation rates allowed us to focus on the effects of benthic nutrient fluxes on the capacity for N fixation within pelagic cyanobacteria after correcting for possible differences in cyanobacteria abundance. We estimated the cyanobacteria abundance in each incubation bottle as the concentration of phycocyanin ($\mu\text{g L}^{-1}$), the accessory pigment that is characteristic of cyanobacteria, using a fluorometric probe (FluoroSense, Turner Designs, San Jose, CA, USA) and multiplying this concentration by the incubated sample volume (mL). We recognize that pigment concentrations do not necessarily match biomass measurements (Wetzel 2001, Chapter 8), and our reported abundances are more accurately understood as scaled measures of phycocyanin concentration.

Benthic nutrient flux assays

To measure benthic nutrient fluxes, we collected three replicate intact sediment cores from each site using a Kajak corer (KC Denmark). Cores were transparent acrylic tubes (5 cm diameter x 33 cm height) with sediment and incubation headwater depths ranging from 11.5 to 27.0 cm and 16.0 to 29.5 cm (314 to 579 mL), respectively. Within 2 h of collection, we transferred cores to racks in outdoor clear plastic water baths.

To minimize variation in benthic nutrient fluxes driven by variation in incubation conditions, we performed benthic nutrient flux assays under controlled conditions. On the day following core collection, we standardized the initial headwater nutrient concentrations by replacing the headwater with 275 to 540 mL of local well water ($\text{PO}_4 = 0.015 \text{ mg L}^{-1}$, $\text{NO}_3 = 0.066 \text{ mg L}^{-1}$, and 0.0038 mg L^{-1}) leaving ~2 cm of lake water in each core to avoid disturbing the sediment surface. On sunny days, we used shade cloth to reduce ambient PAR to $<500 \text{ } \mu\text{moles photons m}^{-2} \text{ s}^{-1}$ to avoid photoinhibition. Due to variation in weather across events, mean temperature and PAR during flux assays ranged from 8.2 to 17.4°C and 154 to 357 $\mu\text{moles photons m}^{-2} \text{ s}^{-1}$. Because incubation conditions did not reflect in situ conditions at the sediment surface across sites and events, our measured benthic nutrient fluxes reflect potential rather than realized fluxes.

Following headwater replacement, we initiated benthic nutrient flux measurements by taking 5-mL initial headwater samples from the gently swirled headwater of each core. After 8 h, we gently swirled the headwater again and took 5-mL final headwater samples. Care was taken not to disturb the sediment surface during headwater sampling. All headwater samples were filtered (0.45 μm) and immediately frozen for subsequent analyses of nutrient concentrations.

Following benthic nutrient flux measurements, we characterized benthic primary producer abundances and benthic abiotic conditions in each core. First, we measured the concentration of DO (mg L^{-1}) in the headwater of each core using an optical DO probe (ProODO, YSI Inc., Yellow Springs, Ohio, USA). Second, we used a needle-fitted syringe to extract an interstitial water sample from 3 cm beneath the sediment surface in the center of each core, which we

immediately filtered (0.45 μm) and froze for subsequent analyses of nutrient concentrations. We then measured the thickness of Cladophorales mats (cm) above the sediment surface. After gently removing any visible present Cladophorales, we measured epipelagic diatom density ($\mu\text{g chl-a cm}^{-2}$) using a fluorometric BenthosTorch (bbe Moldaenke GmbH, Schwentinental, Germany).

We analyzed headwater and interstitial nutrient samples using ion chromatography (Dionex ICS-2100 and Dionex ICS-1100, ThermoFisher Scientific, Waltham, MA, USA) to determine the concentrations of dissolved inorganic nutrients including nitrate (NO_3^-), ammonium (NH_4^+), and phosphate (PO_4^{3-}). The lower detection limits for all nutrients were assessed using serial dilution assays and determined to be 0.0001 mg L^{-1} , which is very good relative to the standard for environmental samples (Agency for Toxic Substances and Disease Registry 2017).

We calculated benthic nutrient fluxes ($\text{mg m}^{-2} \text{ h}^{-1}$) for each core by standardizing changes in headwater nutrient concentration by sediment surface area, headwater volume, and flux assay duration. Positive fluxes reflect net release of nutrients into the headwater, while negative fluxes reflect net removal of nutrients from the headwater.

Analysis

We examined the relationship between site-level mean benthic nutrient fluxes and site-level mean pelagic N fixation rates using paired estimates of benthic nutrient fluxes and pelagic N fixation rates from multiple sites and events in 2022 and 2023 (Fig. 2, Step i). Measuring core-

level benthic primary producer abundances and core-level DO concentrations during our benthic nutrient flux assays also allowed us to investigate the potential role of benthic primary producers in mediating pelagic N fixation via their effects on benthic nutrient fluxes (Fig. 2, Steps ii and iii). We first (i) analyzed the effect of mean site-level benthic phosphate, ammonium, and nitrate fluxes on mean site-level pelagic N fixation. We then analyzed (ii) the effects of core-level DO concentrations on the benthic fluxes of phosphate, ammonium, and nitrate. Finally, we (iii) analyzed the effects of core-level benthic primary producer abundances (as determined by *Cladophorales* mat thickness and epipellic diatom density) on core-level DO concentrations.

The analyses (i) of the effects of benthic fluxes of phosphate, ammonium, or nitrate on pelagic N fixation rates used linear regression models, including event as a factor to account for variation in ambient conditions in the lake or during pelagic N fixation and benthic nutrient flux assays. For each event, we calculated the site-level mean flux rate of each nutrient (phosphate, ammonium, and nitrate) from the three flux cores and the site-level mean abundance-standardized pelagic N fixation rate from the three N fixation incubation bottles. We fit separate models for each nutrient including an interaction term between nutrient concentration and event; in no case was the interaction term significant ($P > 0.5$ for all three nutrients, so we present only the models without interactions. We also fit a multiple regression model that included all nutrient fluxes and event as predictors of the rate of abundance-standardized pelagic N fixation to explore whether there were interactions among nutrients in their effects on pelagic N fixation. A regression of phosphate flux on event, and temperature and PAR intensity during the incubation, showed that temperature affected phosphate flux ($P = 0.028$). This indicates that conditions during the assay affected our estimate of phosphate flux. To account for this measurement effect,

we repeated the analyses for the effects of phosphate flux on N fixation using the residuals from this regression to account for the dependence of flux on temperature, and all of the statistical conclusions remained the same; therefore, we report the model without temperature and PAR intensity.

To analyze (ii) the effect of DO concentration and interstitial nutrient concentrations on the flux rates of phosphate, ammonium, and nitrate from the sediment, we used core-level data from the benthic nutrient flux assays. We fit linear regression models for each nutrient, with flux rate as the response and DO concentration, interstitial nutrient concentration, and event as predictors. We log-transformed interstitial nutrient concentrations after adding 0.01 to reduce negative skew; decreasing 0.01 to 0 or increasing 0.01 to 1 did not change the statistical conclusions.

Finally, to analyze (iii) the effects of benthic primary producer abundances on DO concentration, we fit regression models to core-level data from the benthic nutrient flux assays, using DO concentration as the response, and Cladophorales mat thickness, epipellic diatom density, and event as predictors. Because the relationship between benthic primary producer abundances and DO concentration varied among events, we also included interactions between event and the abundance of each benthic primary producer. All analyses were conducted using R version 2024.12.0+467 (R Core Team 2016).

Results

Benthic nutrient fluxes and pelagic N fixation

Mean site-level pelagic N fixation rates ranged from 0.0061 to 0.14 $\mu\text{g N h}^{-1}$ (mean = 0.034 ± 0.036 SD), and mean abundances of cyanobacteria as measured by fluorometry ranged from 0.0011 to 0.018 mg phycocyanin (0.0075 ± 0.0045), resulting in mean abundance-standardized pelagic N fixation rates ranging from 1.47 to 11.76 $\mu\text{g N mg phycocyanin}^{-1}$ (4.40 ± 2.93). Mean site-level benthic phosphate flux ranged from -0.89 to 2.72 $\text{mg m}^{-2} \text{h}^{-1}$ (0.20 ± 0.55) and had a significantly positive effect on the rate of abundance-standardized pelagic N fixation (Table 1, Fig. 3a). In contrast, mean site-level benthic nitrate (-2.5 ± 4.0) and ammonium (0.025 ± 0.44) fluxes were not related to rate of abundance-standardized pelagic N fixation (Table 1, Fig. 3b,c) when considered alone. In the model that included all nutrients as predictors, benthic phosphate flux had a marginally significant positive effect on the rate of pelagic N fixation (Table 1). The apparent decrease in statistical support for the effect of benthic phosphate flux on pelagic N fixation in this model is the result of the small sample size ($n = 22$) relative to the number of predictor variables (four).

DO concentration, interstitial nutrient concentrations, and benthic nutrient fluxes

Core-level DO concentrations during the benthic nutrient flux assays ranged from 9.8 to 23.9 mg L^{-1} (15.4 ± 3.7) and had a significant negative effect on the fluxes of all nutrients (Table 2, Fig. 4a,c,e). Even though we anticipated that core-level interstitial nutrient concentrations would, in part, determine nutrient fluxes, this was the case only for nitrate which ranged from 0.00037 to 1.32 mg L^{-1} (0.24 ± 0.33) (Table 2, Fig. 4d). The lack of a relationship between interstitial nutrient concentrations of phosphate (3.62 ± 5.54) and ammonium (2.012 ± 2.40) and their fluxes

suggests an overwhelming effect of processes at the benthic surface in determining fluxes. The flux of nitrate also showed an effect of event, with lower fluxes in August 2022.

Benthic primary producer abundances and DO concentration

In the benthic nutrient flux assays, core-level Cladophorales mat thickness ranged from 0 to 8.0 cm (1.30 ± 1.76) and had a significant positive effect on DO concentration (Table 3, Fig. 5a).

The relationship between Cladophorales mat thickness and DO concentration varied among events (Fig. 5a). Core-level epipelagic diatom density ranged from 1.14 to 7.15 $\mu\text{g chl-a cm}^{-2}$ (3.6 ± 1.5) and had no significant effect on DO concentration (Table 3, Fig. 5b).

Discussion

Nutrient availability is an important determinant of N fixation rates in pelagic cyanobacteria (Howarth et al., 1988; Marcarelli et al., 2022; Smith, 1990; Vitousek et al., 2002). In shallow aquatic ecosystems, benthic primary producers can control nutrient release from the sediment to the pelagic zone (Hansson 1988; Carlton and Wetzel 1988; Dalsgaard 2003; Bartoli, Nizzoli, and Viaroli 2003; Thorbergsdóttir and Gíslason 2004; Spears et al. 2008), yet the relationship between benthic primary producers and pelagic N fixation is not well-understood. Using paired benthic nutrient flux and pelagic N fixation assays in Mývatn, Iceland, we showed that sites with increased benthic phosphate flux was associated with increased pelagic N fixation. Benthic phosphate flux was negatively associated with DO concentration at the sediment-water interface, which in turn was positively associated with benthic primary producer abundances, specifically

the mat thickness of Cladophorales. Together, these relationships suggest that benthic primary producers indirectly influence pelagic N fixation rates in Mývatn via their effects on benthic nutrient fluxes.

We observed that benthic phosphate flux had a positive effect on pelagic N fixation, while there were no effects of nitrate or ammonium fluxes. This suggests that phosphate availability, and not ammonium or nitrate availability, limited pelagic N fixation. This pattern of P limitation during pelagic cyanobacterial blooms is consistent with previous observations in Mývatn (Jónasson and Aðalsteinsson 1979; Ólafsson 1979b; Botsch and Book et al. in prep). Diazotrophic cyanobacteria such as *Dolichospermum* spp., which dominate pelagic cyanobacterial blooms in Mývatn, are able to acquire N from the atmosphere and, therefore, are expected to be limited by P (Howarth et al., 1988; Paerl, 2008; Smith, 1983, 1990). Thus, higher rates of phosphate release from the sediment may alleviate local P limitation of pelagic cyanobacteria and enable higher rates of N fixation. These findings also align with the growing body of research showing that internal loading of stored phosphate can trigger and/or sustain blooms of pelagic N fixing cyanobacteria (Orihel et al., 2015; Swann et al., 2024; Xu et al., 2021).

Benthic nutrient flux and pelagic N fixation assays were conducted under standardized conditions that did not necessarily reflect in situ conditions in Mývatn. Therefore, nutrient flux and N fixation rates represent the capacity for these processes under assay conditions rather than realized rates in the lake. In particular, flux assays were conducted under high light intensities that may have enabled high rates of benthic primary production and, consequently, oxygen production and/or nutrient uptake. This is especially possible given the timing of the study

during pelagic cyanobacterial blooms, which are known to attenuate light and suppress benthic primary production in Mývatn (McCormick et al., 2021). Nonetheless, the statistically significant positive relationship between benthic phosphate flux and abundance-standardized pelagic N fixation implies that the capacity for phosphate release from the sediment determines, in part, the physiological capacity of pelagic cyanobacteria to fix N. This thereby demonstrates that these two processes are ecologically linked in Mývatn. Moreover, because our assays were conducted under standardized conditions, measured rates reflect the outcome of accumulated variation in abiotic and/or biotic processes among sites rather than transient conditions in the lake. Therefore, the use of standardized assays improved our ability to identify mechanisms underlying the relationships between benthic primary producers, nutrient fluxes, and pelagic N fixation.

In our benthic nutrient flux assays, DO concentration had a statistically significant negative effect on the flux rates of all nutrients. DO concentrations were, in turn, positively affected by *Cladophorales* mat thickness. Therefore, high DO concentrations can reasonably be interpreted as indicative of elevated rates of benthic primary production. Many studies have previously shown that high rates of benthic primary production are associated with high rates of nutrient assimilation at the sediment-water interface and thereby reduce nutrient release via diffusive flux to the overlying water column (Hansson 1988; Dalsgaard 2003; Bartoli, Nizzoli, and Viaroli 2003; Spears et al. 2008). Thus, the negative effect of DO concentration on nutrient fluxes may have been due to nutrient uptake by benthic primary producers. In a prior study on internal loading in Mývatn, Thorbergsdóttir and Gíslason (2004) observed a similar negative effect of benthic algal production on ammonium and phosphate fluxes. It is unlikely that the negative effect of DO concentration on nutrient flux rates was due to variation in redox-mediated

processes (i.e. phosphate binding/release, nitrification) at the sediment-water interface, because DO concentrations in all of our flux assay cores ($>9 \text{ mg L}^{-1}$) are indicative of oxic conditions (Dadi et al., 2023) at the sediment surface. However, we cannot exclude the possibility of increased sediment-surface DO changing redox potentials deeper in the sediment through DO diffusion.

We expected interstitial nutrient concentrations to be positively related to nutrient fluxes due to diffusive flux. However, only interstitial nitrate concentrations positively affected nitrate fluxes. The lack of an effect of interstitial phosphate and ammonium concentrations on the respective fluxes of those nutrients suggests that abiotic and/or biotic processes at the sediment-water interface were the primary determinants of ammonium and phosphate release in our study (Levine and Schindler 1992; Bartoli, Nizzoli, and Viaroli 2003; Spears et al. 2008). Specifically, our finding that high DO concentrations reduced the fluxes of all nutrients, and that nutrient uptake by benthic primary producers likely explained this reduction, suggest that nutrient uptake by benthic primary producers prevented the release of diffused nutrients to the overlying water column. Despite also having a negative effect on nitrate flux, nutrient uptake by benthic primary producers did not decouple interstitial nitrate concentrations from nitrate fluxes. This suggests that nitrate may have been assimilated less efficiently/preferentially by benthic primary producers, as has been observed in Mývatn in the past (Thorbergdóttir & Gíslason, 2004).

High phosphate availability in the pelagic zone of Mývatn sustains high N fixation rates; when standardized by volume, mean pelagic N fixation rates from our measurements in Mývatn ($19.79 \pm 21.19 \text{ } \mu\text{mol N}_2\text{-N m}^{-3} \text{ h}^{-1}$) lie between the median (4) and top quartile (30) of measurements

from among 534 lakes worldwide (Fulweiller et al. 2025). In part, this is explained by the high P concentrations in the groundwater springs that feed the lake ($0.05 \text{ mg L}^{-1} \text{ P}$) (Ólafsson 1979b). Our results also suggest that internal loading of P is important. Internal P loading is generated from the incorporation of P into the benthos, either by the uptake of inorganic P by benthic primary producers or the incorporation of pelagically derived organic matter into the benthos which is subsequently remineralized and released back to the pelagic zone. A dynamic N budget for Mývatn (Book et al. in prep) shows that 2/3 of organic N in the pelagic zone is incorporated into the benthos. We suspect that roughly the same proportion of pelagic organic P is incorporated into the benthos, implying that more than half of the P needed to sustain pelagic N fixation is obtained via internal loading. This magnitude of internal P loading, and the sensitivity of P flux to benthic primary producers at the benthic/pelagic interface, underscores the potential importance of benthic primary producers in governing the location, timing, and magnitude of pelagic N fixation.

Our results show that variation in benthic primary producer abundances is linked to variation in pelagic cyanobacterial N fixation rates across space and time in Mývatn. This effect was indirect and likely mediated by phosphate uptake by benthic primary producers at the sediment-water interface. Similar effects of benthic primary producers on pelagic primary production have been documented at Mývatn (McCormick et al., 2021) and elsewhere (Carlton & Wetzel, 1987; Hansson, 1988; Jäger & Diehl, 2014). We show that benthic primary producers can similarly reduce pelagic cyanobacterial N fixation, likely via the same mechanism of reducing nutrient flux from the benthos. Because cyanobacterial N fixation can respond rapidly to variation in nutrient availability (Aubriot, 2019; Kolzau et al., 2018; Oliver et al., 2012), spatial variation in

nutrient release from the benthos may drive spatial variation in cyanobacterial N fixation in the pelagic zone even in well-mixed lakes like Mývatn. It is this responsiveness of N fixation to nutrient availability that likely made it possible for us to detect the effect of benthic nutrient fluxes on pelagic N fixation. Thus, N fixation served as a sensitive biological signal of nutrient availability driven by internal loading.

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Figures and Tables

Table 1. Regression models for the effects of site-level mean nutrient fluxes on site-level mean abundance-standardized pelagic N fixation rates. Analyses were conducted for each nutrient separately, and also for all nutrients together. The P-values for event July 2023 and event August 2023 give the significance of the difference from event August 2022.

nutrient	parameter	estimate	SE	P
PO ₄	PO ₄ flux	2.68	1.14	0.030
	event July 2023	-3.72	1.25	0.008
	event August 2023	-3.44	1.35	0.020
NO ₃	NO ₃ flux	0.11	0.18	0.538
	event July 2023	-5.17	1.50	0.003

	event August 2023	-5.52	1.56	0.002
NH ₄	NH ₄ flux	1.77	2.02	0.393
	event July 2023	-5.82	1.80	0.005
	event August 2023	-5.55	1.45	0.001
PO ₄ + NO ₃ + NH ₄	PO ₄ flux	2.809	1.372	0.057
	NO ₃ flux	-0.068	0.267	0.802
	NH ₄ flux	0.243	3.079	0.938
	event July 2023	-3.563	2.069	0.104
	event August 2023	-3.135	1.865	0.112

Table 2. Regression models for the effects of core-level DO concentration on core-level benthic nutrient flux rates during benthic nutrient flux assays. The P-values for event July 2023 and event August 2023 give the significance of the difference from event August 2022.

	parameter	estimate	SE	P
PO ₄ flux	DO concentration	-0.05	0.02	0.023
	log(interstitial PO ₄ + 0.01)	0.06	0.05	0.201
	event July 2023	-0.11	0.18	0.545
	event August 2023	-0.36	0.25	0.158
NO ₃ flux	DO concentration	-0.65	0.09	<0.001
	log(interstitial NO ₃ + 0.01)	1.19	0.22	<0.001
	event July 2023	5.31	0.75	<0.001
	event August 2023	2.21	0.80	0.008
NH ₄ flux	DO concentration	-0.04	0.01	0.003
	log(interstitial NH ₄ + 0.01))	0.01	0.02	0.553
	event July 2023	0.63	0.11	<0.001
	event August 2023	0.17	0.14	0.216

Table 3. Regression model for the effects of core-level Cladophorales mat thickness and epipellic diatom density on core-level DO concentrations during benthic nutrient flux assays. The P-values for event July 2023 and event August 2023 give the significance of the difference from event August 2022. Event * benthic primary producer abundances show the interactions between the event and abundance of each benthic primary producer type.

parameter	estimate	SE	P
mat thickness	2.65	0.29	<0.001
diatom density	0.60	0.33	0.079
event July 2023	1.70	2.13	0.427
event August 2023	-1.97	2.16	0.365
(event July 2023) by (mat thickness)	-1.11	0.34	0.002
(event August 2023) by (mat thickness)	-1.25	0.33	<0.001
(event July 2023) by (diatom density)	-0.21	0.48	0.671
(event August 2023) by (diatom density)	-0.24	0.43	0.584

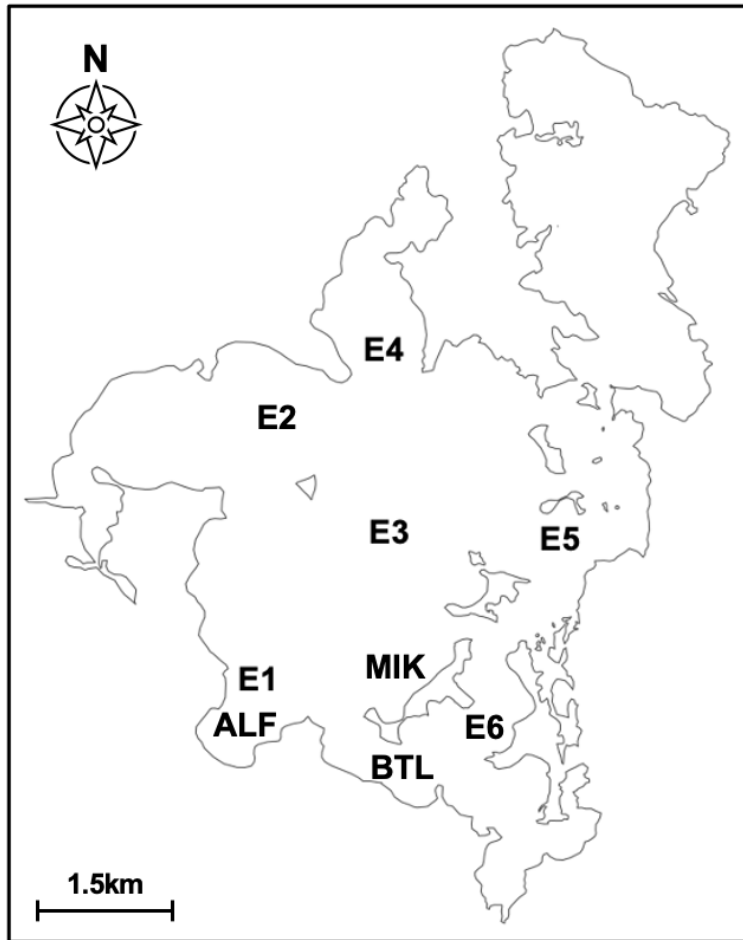


Figure 1. Map of site locations in Lake Mývatn

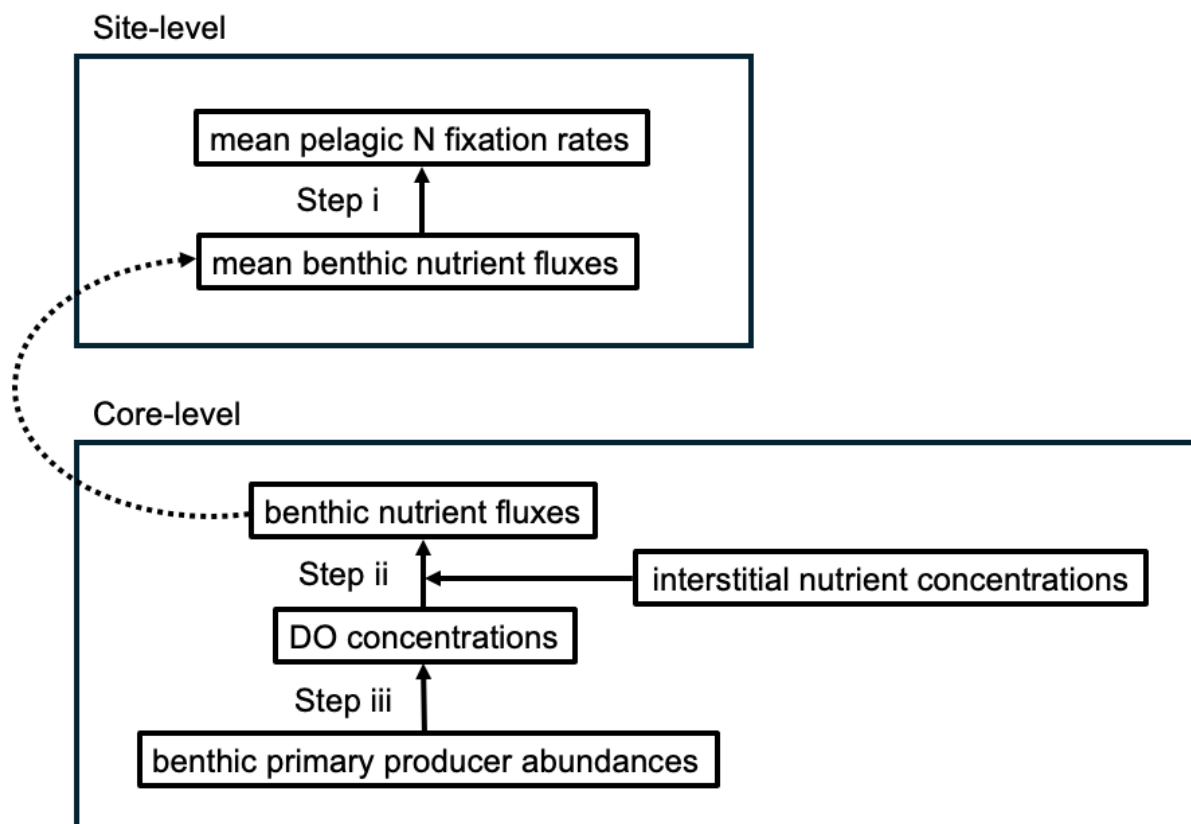


Figure 2. A conceptual diagram of the relationships analyzed in our study.

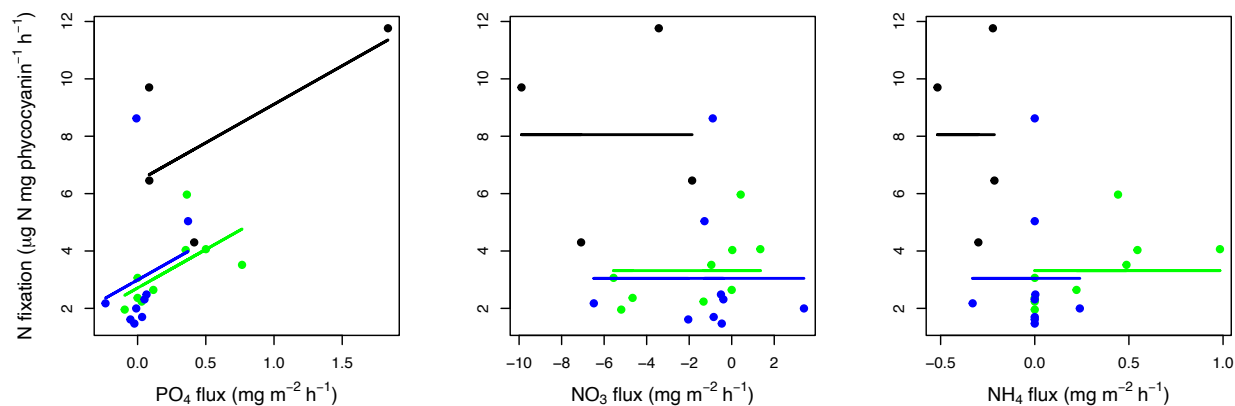


Figure 3. Relationship between site-level mean benthic nutrient flux rates of phosphate (a), nitrate (b), and ammonium (c) and site-level mean abundance-standardized pelagic N fixation rates across sites ($n= 4$ to 9) and events (color). Event is significant in all panels, but there is a significant slope only in panel (a) for phosphate.

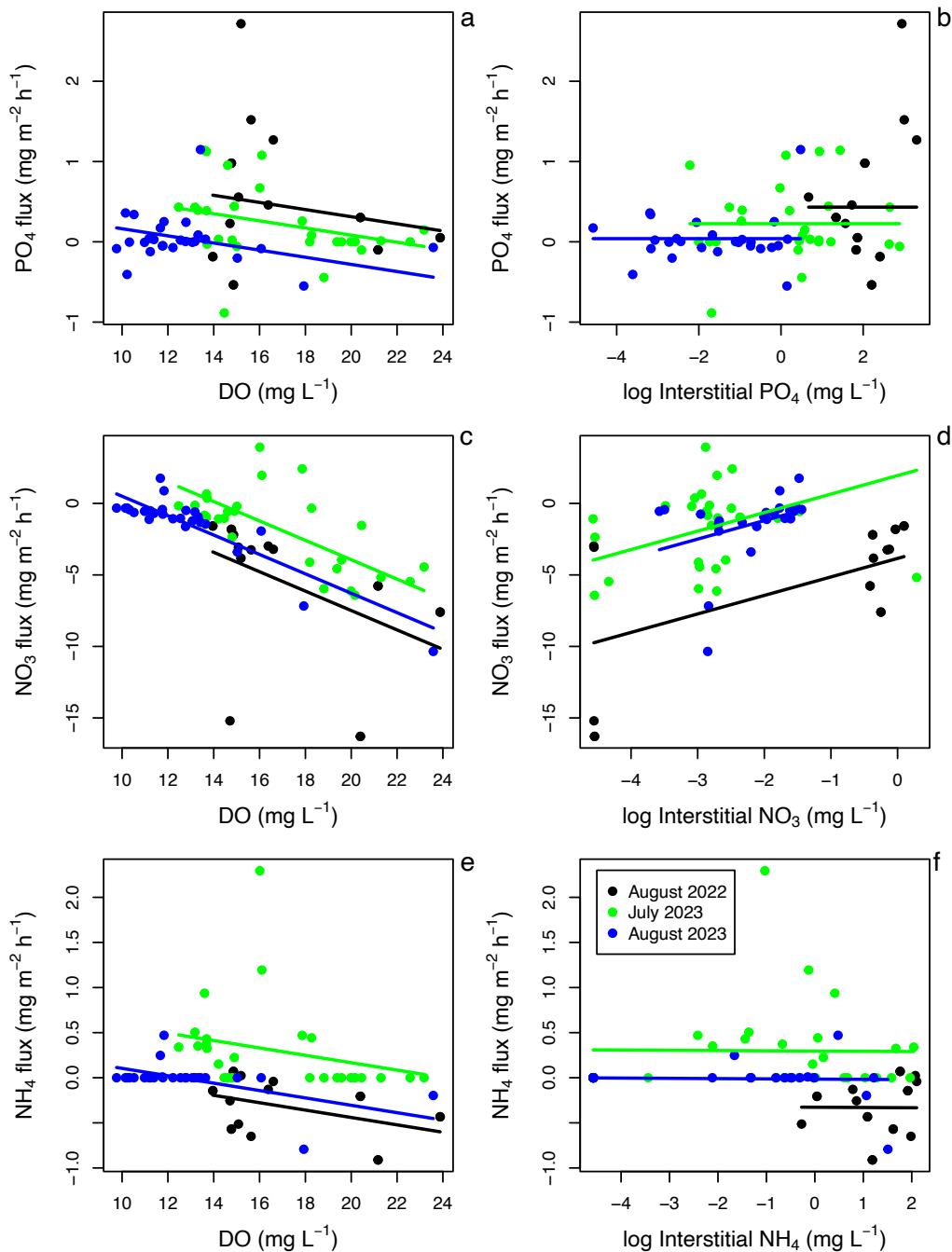


Figure 4. Relationship between core-level DO concentrations and core-level benthic nutrient flux rates (a,c,e) and interstitial nutrient concentrations ($\log(x + 0.01)$) and benthic flux rates (b,d,f) across sites and events (color). Relationships that are non-significant ($P > 0.05$) are depicted as lines with slope zero.

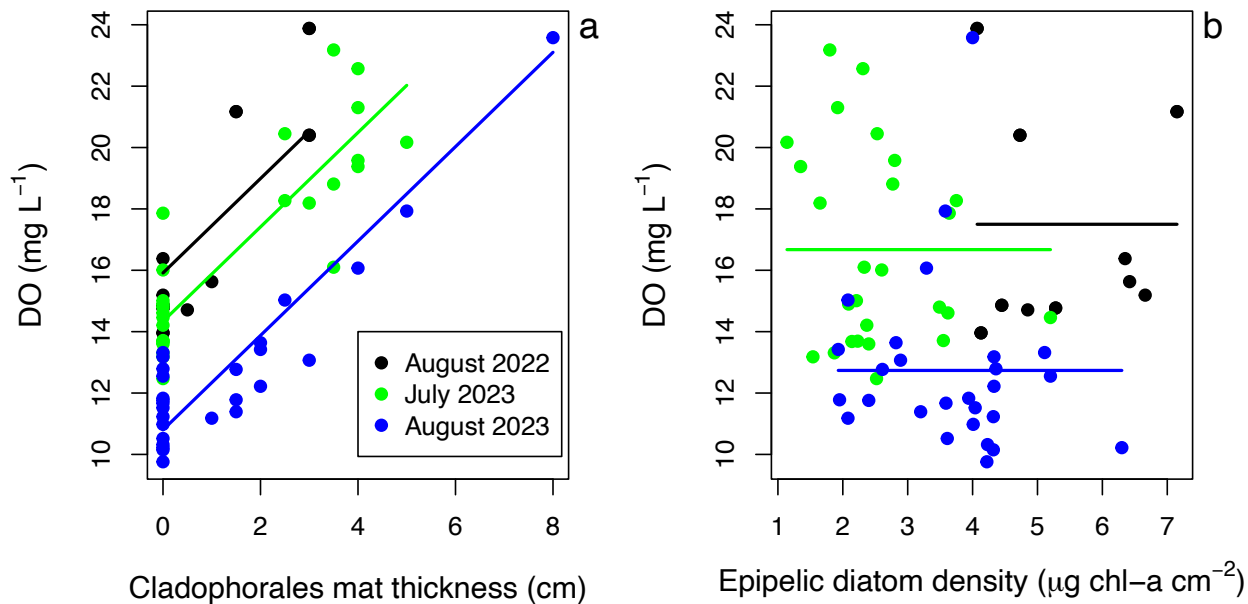


Figure 5. Relationship between core-level (a) Cladophorales mat thickness and (b) epipellic diatom density on core-level DO concentration across sites and events. Relationships that are non-significant ($P > 0.05$) are depicted as lines with slope zero.

CHAPTER 3

Patterns of benthic and pelagic nitrogen fixation and retention in Lake Mývatn*

Abstract

In nitrogen-limited lakes, cyanobacterial nitrogen (N) fixation rates can be high. However, the effect of cyanobacterial N fixation on ecosystem N availability depends, in part, on the retention of fixed N. Here, we explore patterns of benthic and pelagic N fixation and fixed N retention in Lake Mývatn, Iceland. Our updated N budget showed that the annual rate of lake-scale N fixation needed to account for the deficit between external N supply and N loss varies greatly from year to year, primarily due to variation in the magnitude of N loss via outflow and midge emergences. Extrapolations from recent direct measurements of pelagic and benthic N fixation under scenarios of different benthic and pelagic N fixer abundances showed that the capacity for N fixation is much higher in the pelagic than the benthic zone. We then explored the degree to which N fixed in the pelagic zone is retained and recycled within the lake using a dynamic N budget that leveraged data on patterns of N loss via the outlet river Laxá. This revealed that approximately two-thirds of organic N in the pelagic zone must be incorporated into the benthos to explain patterns of N loss via outflow from the river Laxá. Together, these results suggest N fixation in pelagic cyanobacterial blooms may supply most of the N for benthic primary production in Mývatn.

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Introduction

In lakes with low external nitrogen (N) supply relative to phosphorus (P) (i.e. N-limited lakes), rates of N fixation in diazotrophic primary producers (cyanobacteria) can be high (Schindler, 1977; Flett et al., 1980; Howarth et al., 1988; Scott & Grantz, 2013; Marcarelli et al., 2022).

However, the extent to which cyanobacterial N fixation leads to increased N availability for non-diazotrophic primary producers (N-limited primary producers) depends on the retention of fixed N (Scott & McCarthy, 2010; Grantz et al., 2014; Scott et al., 2019). Furthermore, the location where fixed N is retained and the timing of fixed N recycling matters (Scott and Grantz 2013), as non-diazotrophic primary producers are often distributed and active unevenly across space and time within lakes (Elser et al., 2007). Therefore, determining not only the rate of N fixation but also the distribution and timing of fixed N cycling is necessary for understanding the role of N fixation in supporting ecosystem-level primary production.

In recent years, studies documenting the limited capacity of N fixation to increase N availability in lakes have challenged the P limitation paradigm (Scott & McCarthy, 2010; Andersen et al., 2025), which posits that N fixation in N-limited lakes will alleviate N limitation and lead to functional P limitation (Schindler 1977). However, most of these studies have focused on the relationship between N fixation and the alleviation of N limitation within a single habitat of a lake (Scott et al., 2007; Scott & McCarthy, 2010; Scott & Grantz, 2013; Andersen et al., 2025). The effect of N fixation in one habitat on N limitation in another (e.g., pelagic N fixation and N limitation in benthic habitats) has not been well-studied (*but see* McCarthy et al. 2016). This issue is particularly relevant for shallow lakes, where essential ecosystem processes performed

by primary producers can occur in both benthic and pelagic habitats (Vadeboncoeur et al., 2001; Vadeboncoeur & Steinman, 2002; Vadeboncoeur et al., 2003, 2008; McCormick et al., 2021).

Lake Mývatn is a shallow, eutrophic lake in northeastern Iceland which supports high annual primary production (Einarsson et al., 2004). Due to the high availability of light and, relative to the water column, nutrients at the sediment-water interface, non-diazotrophic benthic algae dominate annual primary production in the lake (Einarsson et al., 2004; McCormick et al., 2021). However, primary production in the non-diazotrophic benthic algae is expected to be N-limited due to the low availability of N relative to P in the sediments (Ólafsson, 1979b), which is the primary source of nutrients for benthic algae in the lake (Thorbergsdóttir & Gíslason, 2004). Therefore, N fixation is thought to play a key role in sustaining benthic primary productivity in Mývatn (Ólafsson 1979b; Einarsson et al. 2004). The most conspicuous N fixers are pelagic cyanobacteria of the genus *Dolichospermum*, which form dense blooms in some summers, and the N fixing epiphytes of mat-forming green algae (Cladophorales) in the benthos (Einarsson et al., 2004).

Past studies have shown that, on an areal basis, N fixation in pelagic *Dolichospermum* exceeds N fixation in benthic Cladophorales mats during *Dolichospermum* blooms in Mývatn (Book et al. in review). The contrast between temporal patterns of benthic-dominated primary production and pelagic-dominated N fixation, with N fixation occurring over much shorter periods when there are *Dolichospermum* blooms, raises the possibility that N fixed in the pelagic zone is retained and recycled by benthic primary producers. However, on the scale of the lake, the importance of pelagic N fixation may vary from year to year. This is because the relative abundances of pelagic

Dolichospermum (Einarsson et al. 2004; McCormick et al. 2021) and benthic Cladophorales mats (along with their N fixing epiphytes) (Einarsson et al., 2004) vary annually, and this will lead to annual variation in benthic and pelagic N fixation rates. Furthermore, the retention rate of N fixed in the pelagic zone is unknown. Given that high benthic primary production forms the base of the Mývatn food web (Einarsson et al., 2004), understanding how fixed N becomes available to non-diazotrophic benthic algae in the lake is crucial.

Here, we investigate patterns of annual lake-scale benthic and pelagic N fixation and retention in Mývatn. To do this, we first update the existing N budget for Mývatn to estimate how much annual lake-scale N fixation is needed to account for the deficit between annual external N supply and N loss. Then, we estimate annual lake-scale benthic and pelagic N fixation using directly measured areal N fixation rates (Book et al. in review). To determine the effect of variation in the abundances of conspicuous pelagic (*Dolichospermum*) and benthic (epiphytes of Cladophorales mats) N fixers, we consider four scenarios reflecting extreme but plausible variation in these N fixer abundances: high/low pelagic N fixer abundance crossed with high/low benthic N fixer abundance. For each scenario, we adjust the areal rate, extent, and duration of N fixation in both zones based on our understanding of benthic and pelagic N fixers and their interactions in the lake. Finally, we build a dynamic N budget to explore the degree to which N fixed in the pelagic zone is incorporated into the benthos. To calibrate the dynamic N budget, we compare the results to published data on temporal patterns of N outflow from the lake (Ólafsson, 1979b; Eiríksdóttir et al. 2018).

Methods

Study site

Lake Mývatn is a large (37 km²), shallow (mean depth = 2.3 m) lake in northeast Iceland (65°40'N, 17°00'W) (Einarsson et al. 2004). It consists of two basins, North and South, connected by a narrow strait. Owing to its shallow depth, the lake does not stratify, and the water residence time is only about 27 days (Ólafsson, 1979a). The lake is eutrophic due to high nutrient concentrations in groundwater springs (mean N = 0.08 mg L⁻¹, mean P = 0.05 mg L⁻¹) along its eastern shoreline, which account for the majority of the annual external nutrient supply to the lake (N = 1.4 g m⁻² year⁻¹, P = 1.5 g m⁻² year⁻¹) (Ólafsson, 1979b; Jónsson 2016). However, the mean molar N:P ratio of these springs (2:1) is lower than the Redfield ratio of 16:1 (Redfield, 1934), making non-diazotrophic primary production in the lake potentially N-limited (Ólafsson 1979b). The majority of annual primary production in the lake occurs in non-diazotrophic epipelagic microalgae, primarily diatoms (Einarsson et al., 2004; McCormick et al., 2021). Pelagic primary production is dominated by bloom-forming pelagic cyanobacteria belonging to the genus *Dolichospermum* (Jónasson & Aðalsteinsson, 1979; McCormick et al., 2021). In addition to being the dominant primary producers in the lake, epipelagic microalgae are an important food source for larval midges (Chironomidae) which range in annual density in the lake over 4 orders of magnitude (Gardarsson et al., 2004; Ives et al., 2008; Einarsson et al. in prep).

Internal processes, including internal loading of recycled N from the sediment and N fixation, are understood to supply N to primary producers in Mývatn based on annual N budgets for the lake (Gíslason et al., 2004; Ólafsson, 1979b; Jónsson 2016). The internal loading rate of N has been

estimated to range from -15.8 to $12.1 \text{ mg N m}^{-2} \text{ d}^{-1}$ (Thorbergisdóttir & Gíslason, 2004). N fixation occurs in pelagic and benthic primary producers in the lake (Book et al. in review). *Dolichospermum*, which often form blooms during the summertime, are the most common N fixers in the pelagic zone (Jónasson and Aðalsteinsson 1979). Cyanobacterial epiphytes of the mat-forming green algae *Cladophora glomerata* and *Aegagropila linnaei* (Cladophorales), including cyanobacterial endosymbiont-containing *Epithemia* spp. and *Calothrix* spp., are the most common N fixers in the benthic zone. The abundances of pelagic *Dolichospermum* and benthic Cladophorales mats vary dramatically over space and time in the lake (Einarsson et al. 2004).

Updating the static N budget

Sources and sinks of N for Mývatn have previously been identified and quantified in N budgets by Ólafsson (1979b) and Jónsson (2016) (Table S2). We refer to these N budgets as static, because they address only the balance of inputs and outputs of N on a yearly basis, as opposed to investigating the N dynamics within and among years. Both authors estimated the annual lake-scale rate of N fixation as the difference between annual external N supply and annual N loss (Table S2). Ólafsson (1979b) estimated N supply via groundwater springs and N loss via surface water, sedimentation, and denitrification (Table S2). The estimated rate of N fixation was 301 tons N yr^{-1} . Jónsson (2016) estimated annual external N supply and N loss via the same sources as Ólafsson (1979b) using a combination of Ólafsson's data and more recent measurements of the biogeochemistry and hydrology of groundwater and surface water inflows and outflows. Estimates of external N supply via atmospheric deposition and human activity in the Mývatn

region were also added (Jónsson 2016). N fixation was estimated to be 199 tons N yr⁻¹ (Table S2). The difference between N fixation estimates in these two studies could be explained by the difference in *Dolichospermum* abundances in the lake during the years when the studies were conducted (Jónsson 2016). Ólafsson (1979b) estimated N loss in a year with high *Dolichospermum* abundance, while Jónsson (2016) estimated N loss in a year with low abundance.

We updated the static N budget for Mývatn to include the loss of N caused by adult midge emergences, which vary in magnitude from year to year due to variation in midge densities. We also partitioned the supply of N via groundwater and surface water using data from Ólafsson (1979b). Finally, we provide a new estimate of N loss via permanent sedimentation using a sediment core from the Höfði inlet (Hauptfleisch & Einarsson, 2012). Additional details on N budget calculations are given in *Supplementary Methods: Updating the N budget for Lake Mývatn*.

Estimating annual lake-scale benthic and pelagic N fixation

The estimates of N fixation derived from the static N budgets assume that any unexplained N deficit (N loss not matched by N supply) is due to N fixation. However, these estimates do not account for annual variation in N fixer abundances in Mývatn or provide information regarding the distribution of N fixation among benthic and pelagic zones. We estimated annual benthic and pelagic N fixation by extrapolating recently measured areal N fixation rates in benthic Cladophorales mats, which host N fixing epiphytes, and pelagic *Dolichospermum* (Book et al. in

review) to the lake-scale. We accounted for annual variation in benthic and pelagic N fixer abundances by estimating N fixation rates under multiple hypothetical scenarios that reflect extreme but plausible variation in N fixer abundances in Mývatn. The scenarios include combinations of high and low abundances of pelagic *Dolichospermum* and benthic Cladophorales mats, specifically: high pelagic + high benthic (HH), high pelagic + low benthic (HL), low pelagic + high benthic (LH), and low pelagic + low benthic (LL). To estimate annual pelagic, benthic, and total lake-scale N fixation under each scenario, we set the values of three variables for each zone: the areal rate of N fixation ($\text{g N m}^{-2} \text{d}^{-1}$), the areal extent of N fixers (m^2), and the annual duration of N fixation (d) (Table 1). We based our values of these variables on past studies and observations of *Dolichospermum* and Cladophorales mats in Mývatn.

Areal N fixation rates in pelagic *Dolichospermum* and benthic Cladophorales mats were measured at four sites in the South Basin during a *Dolichospermum* bloom between July and August 2022 (Book et al., in review). Areal pelagic N fixation rates, integrated over the water column, ranged from 0.0076 to 0.04838 $\text{g N m}^{-2} \text{d}^{-1}$ and increased with *Dolichospermum* density, despite self-shading. Areal pelagic N fixation rates at sites where Cladophorales mats were present were roughly 50% lower than at sites without mats, and we retained this negative association in the scenarios. Because pelagic N fixation rates were measured when *Dolichospermum* was present, minimum areal pelagic N fixation rates in this study do not reflect a year without a *Dolichospermum* bloom. However, separate assays of pelagic N fixation during years with no *Dolichospermum* bloom have documented negligible or zero fixation. Benthic N fixation rates ranged from 0 to 0.00116 $\text{g N m}^{-2} \text{d}^{-1}$ and decreased with increasing *Dolichospermum* density.

The areal extent of N fixation is determined by the spatial distribution of *Dolichospermum* blooms in the pelagic zone and Cladophorales mats in the benthic zone. Based on satellite imagery (Einarsson and Eiríksdóttir 2024, Fig. 12), *Dolichospermum* blooms can cover up to 90% of the lake in years with large blooms. The extent of *Dolichospermum* is assumed to be zero in low bloom years, although *Dolichospermum* may be present at low densities (Jónasson & Aðalsteinsson, 1979). Past studies (Einarsson et al. 2004) and satellite imagery (unpublished) show that the extent of Cladophorales mats ranges from 5 to 65% of the lake area (1.65 to 21.5 km²), in some years covering the majority of the benthic surface in the South basin (Einarsson et al. 2004).

The annual duration of N fixation in each zone was estimated based on the typical seasonality of environmental conditions that favor N fixation in the lake. In the pelagic zone, *Dolichospermum* blooms can occur between early July and mid-September (Jónasson and Aðalsteinsson 1979), and thus pelagic N fixation was assumed to occur during this period in high bloom years. In the benthic zone, Cladophorales mats are present and environmental conditions may support N fixation from ice-off (typically ~15 May) through mid-September, although shifts in benthic light and nutrient availability during *Dolichospermum* blooms tend to disfavor benthic N fixation (McCormick et al., 2021; Ólafsson, 1979b; Book et al. in review; Botsch and Book et al. in prep). Therefore, we adjust the duration of benthic N fixation based on whether *Dolichospermum* blooms were assumed to be present.

HH scenario – This scenario reflects a year with a large *Dolichospermum* bloom and extensive Cladophorales mats (Table 1). We assume the maximum areal extent of *Dolichospermum* (90% of lake area = $2.97 \times 10^7 \text{ m}^2$) and Cladophorales mats (65% of the lake area = $2.15 \times 10^7 \text{ m}^2$). We assume that benthic N fixation occurs at the maximum observed areal rate ($0.00116 \text{ g N m}^{-2} \text{ d}^{-1}$) from ice-off (15 May) until the onset of the *Dolichospermum* bloom (1 July) (47 d). We assume that pelagic N fixation occurs at 50% of the maximum observed areal rate ($0.02419 \text{ g N m}^{-2} \text{ d}^{-1}$), reduced due to reflect observed suppression by Cladophorales mats, over the maximum bloom duration (1 July to 15 September, 76 d).

HL scenario – This scenario reflects a year with a large *Dolichospermum* bloom and limited Cladophorales mats (Table 1). As in the HH scenario, we assume that benthic N fixation in Cladophorales mats occurs at the maximum areal rate from ice-off until the onset of the bloom, but over the minimum areal extent of Cladophorales mats (5% of lake area = $1.65 \times 10^6 \text{ m}^2$). Due to the limited extent of Cladophorales mats, we assume that pelagic N fixation is not suppressed and occurs at the at the full maximum observed areal rate ($0.04838 \text{ g N m}^{-2} \text{ d}^{-1}$) over the maximum bloom duration and areal extent.

LH scenario – This scenario reflects a year with no *Dolichospermum* bloom and extensive Cladophorales mats (Table 1). We assume no pelagic N fixation and no negative effect of a *Dolichospermum* bloom on benthic N fixation. We assume that benthic N fixation in Cladophorales mats occurs at the maximum observed areal rate and extent over the full growing season (15 May to 15 September, 123 d).

LL scenario – This scenario reflects a year with no *Dolichospermum* bloom and limited Cladophorales mats (Table 1). As in the LH scenario, we assume no pelagic N fixation and favorable conditions for benthic N fixation over the growing season. However, we assume that benthic N fixation occurs over the minimum areal extent of Cladophorales mats.

Dynamic N budget

Our whole-lake extrapolations of pelagic and benthic N fixation give information not only about where N fixation occurs but also when, since pelagic N fixation is largely confined to years with short-duration *Dolichospermum* blooms. The extrapolations present a question: since most primary production occurs in the benthos, is benthic N fixation enough to supply the N necessary for this production, or does N fixed in the pelagic zone have to be made available to the benthos? To answer this question, we built a dynamic N budget to explore the degree to which N fixed in the pelagic zone is retained and recycled in the lake. The dynamic N budget resembles static N budgets in the sense that it consists of mass-balance equations. However, these equations are iterated on a daily time scale over the course of a year to account for seasonal variation in N fixation rates, and they include multiple years to account for annual variation in pelagic cyanobacterial abundances. This approach makes it possible to leverage information about seasonal patterns of N loss via the outlet river Laxá, for which there are two detailed data sets (Ólafsson, 1979b; Eiríksdóttir et al. 2018). These seasonal patterns of N loss contain information about N retention, because if N is fixed over a short period of time in the summer but lost via the river Laxá year round, then there must be retention of N in the lake. Because the water residence

time in the lake is only 27 days (Ólafsson, 1979b), any retention beyond this time frame would have to be benthic.

The dynamic N budget is given by three differential equations for changes in the organic N pool (both particulate and dissolved) in the pelagic zone at time t , $P(t)$; the inorganic N pool in the pelagic zone that is available to both pelagic and benthic primary producers, $I(t)$; and the benthic organic and inorganic N, $B(t)$. Because seasonal patterns of organic and inorganic N loss via the Laxá differ, we consider pelagic organic and inorganic N pools separately. In contrast, we use "benthic N" broadly to include the inorganic N within interstitial water, the organic N contained within the photosynthetically active surface of the sediment, and the organic N contained in deeper sediments where remineralization occurs; although we pool organic and inorganic N in $B(t)$, we assume only inorganic N is released to the pelagic zone. The equations are

$$\frac{dP}{dt} = Nfix_P(t) + Nu_{uptake_P}(t) I(t) - s_P P(t) - flow_P P(t)$$

$$\frac{dI}{dt} = I_{in} + Nflux_B(t) B(t) - s_I I(t) - Nu_{uptake_P}(t) I(t) - flow_P I(t)$$

$$\frac{dB}{dt} = Nfix_B(t) + s_P P(t) + s_I I(t) - Nflux_B(t) B(t) - s_B B(t)$$

Here, in the first equation $Nfix_P(t)$ is the N fixation rate in the pelagic zone that can depend on time to allow seasonal variation in pelagic N fixation; to account for years with different pelagic cyanobacterial abundances, $Nfix_P(t)$ can also differ across years. N fixed in the pelagic zone is incorporated into the pool of pelagic organic N, $P(t)$. There is also seasonal variation in the

uptake rate of pelagic inorganic N, $N_{uptake_P}(t)$, that corresponds to the growing season when pelagic autotrophs and heterotrophs assimilate nutrients. Pelagic organic N is potentially lost by transport to the benthic zone at rate s_P and is also lost via the outlet river Laxá where the flow rate is $flow_P$. Because the water discharge rate from Mývatn changes little over the course of a year (Ólafsson 1979a), we assume $flow_P$ is constant.

In the second equation, inorganic N, $I(t)$, is supplied by external sources (e.g., groundwater, aerial deposition) at a rate of I_{in} . It is also increased by the remineralization and release of benthic N, and decreased through uptake by benthic organisms. We summarize both of these processes by assuming there is a seasonally dependent flux rate of inorganic N between the benthic and pelagic zones, $Nflux_B(t)$, that depends on the availability of organic and inorganic N in the benthos, $B(t)$, and decreases in the summer when remineralized N from the sediment is assimilation by benthic autotrophs and heterotrophs at the sediment-water interface. Benthic organisms also remove pelagic inorganic N, $I(t)$, at a constant rate s_I . Finally, $I(t)$ is lost via the outlet river Laxá at rate $flow_P$. The rates of supply and loss of $B(t)$ are the converse of the rates already described for $P(t)$ and $I(t)$, since these are mass-balance equations. In addition, there is loss of N through permanent sedimentation at rate s_B of N that is not remineralized.

The dynamic N budget has a simple structure, and the parameter values are estimated roughly. Nonetheless, because it incorporates seasonal variation in N dynamics and separates pelagic and benthic zones, we can use it to understand the nature of fixed N retention and recycling in Mývatn. In particular, it allows us to determine the likely proportion of N fixed in the pelagic zone that is incorporated into the benthic zone. If observed N dynamics in Mývatn (e.g., patterns

of N loss via the outlet river Laxá) can only be explained when a certain proportion of N fixed in the pelagic zone is incorporated as organic N into the benthos, this suggests the degree of pelagic incorporation that must occur in the lake. Thus, the key parameter in the dynamic N budget is s_P : if $s_P = 0$, then no organic N from the pelagic zone enters the benthic zone. We obtained estimates for all other parameters from the static N budget (Table S2) and other sources, leaving only s_P to be determined. Values of the other parameters and sources are given in Table 2, and a diagram of the dynamic N budget is given in Figure 1.

To determine the ecologically plausible range of values of s_P , we used two data sets giving the outflow of organic and inorganic N from Mývatn from the outlet river Laxá (Fig. 2). The data set from Ólafsson (1979b) represents a year with high *Dolichospermum* abundance, while the data set from Eiríksdóttir et al. (2018) represents a year with low *Dolichospermum* abundance. Nonetheless, the concentration of N in the outlet river (particularly the organic N fraction) is similar in both data sets.

To account for annual variation in pelagic N fixer abundances, we simulated alternating years reflecting the HH and LH scenarios that we investigated for the static N budget (Table 1). This alternation of years is not designed to mimic any specific observations of Mývatn but instead is to account for the roughly equal frequency of years with high vs. low pelagic cyanobacteria abundances (McCormick et al., 2021). For the HH scenario, we assumed that pelagic N fixation starts on day-of-year 182 (1 July) and continues for 76 days; for the LH scenario, pelagic N fixation was zero (Fig. S1). Benthic N fixation starts on day-of-year 135 (15 May) and lasts 123 days. In the HH scenario, however, benthic N fixation is zero for the 76 days of high pelagic N

fixation. Rather than use the experimentally determined rates of pelagic and benthic N fixation (Table 1), we changed pelagic N fixation from 0.02419 to 0.115 g N m⁻² d⁻¹, and benthic N fixation from 0.00116 to 0.00059 g N m⁻² d⁻¹; these N fixation rates give 3.43 and 0.119 tons N d⁻¹ for the lake, respectively (Table 2). These changes preserve the relative rates of benthic and pelagic N fixation used in the HH and LH scenarios (Table 1), but increase the total N fixation in the HH scenario to match our estimate from the static N budget of an average of 296 tons; this value is the midpoint of the range of values in our static N budget (Table S2) under the assumption that the sedimentation rate is 242 tons N y⁻¹. We calculated the permanent N sedimentation rate of 242 tons N y⁻¹ from a sediment core (see *Supplementary Methods: Updating the N budget for Lake Mývatn*) that we think more accurately reflects the current sedimentation rate in Mývatn than the value of 74 tons N y⁻¹ from Ólafsson (1979b).

Our greatest uncertainties are the estimates of $Nuptake_P(t)$ and $Nflux_B(t)$. However, these affect the exchange between inorganic and organic N in the pelagic zone, and between pelagic and benthic zones, and therefore they have small effects on the overall N budget for the lake and on the effects of incorporation of organic pelagic N into the benthos (i.e., the focal parameter s_P). We assume that both $Nuptake_P(t)$ and $Nflux_B(t)$ have a 120-day "summer" in which $Nuptake_P(t)$ takes its maximum value and $Nflux_B(t)$ takes its minimum value, with a 60-day "ramp" period on either end of the summer (Fig. S1). The maximum value of $Nuptake_P(t)$ is taken to be $100 flow_P$, implying that inorganic N in the pelagic zone will almost certainly be taken up before it flows from the lake, while the minimum value is $flow_P$, giving the case in which half of the input inorganic N flows through the lake without being taken up. We scaled $Nflux_B(t)$ so that the mean time that N stays within the benthos is 7 years, which is the remineralization rate that we

calculated from a sediment core (see *Supplementary Methods: Updating the N budget for Lake Mývatn*). Direct measurements of the flux of inorganic N from the benthos to the pelagic zone show that the flux is dynamic, changing through time and space (Thorbergsdóttir & Gíslason, 2004; Book and Adler et al. in prep). However, in the context of the overall, long-term N budget for the lake, we use the remineralization rate in the sediment to measure the net rate of flux from the benthos. The minimum value of $Nflux_B(t)$ occurs in summer to account for the uptake of N by growing benthic algae, and the winter maximum is set at ten times higher. Finally, the permanent sedimentation rate of N is set at half the mean value of $Nflux_B(t)$ so that one-third of the N in the benthos is lost; this permanent loss rate matches our analyses of a sediment core.

Results

Updated N budget

Our updated N budget reflected the two previous N budgets by Ólafsson (1979b) and Jónsson (2016), although we emphasize a greater possible range of values for sources and losses (Table 3). Our partitioned estimates of N supply via groundwater and surface water were 42 to 66 and 0.88 to 1.13 metric tons N yr⁻¹, respectively (Table S1). We estimated total external N supply to range from 56 to 80 metric tons N yr⁻¹ by summing these partitioned estimates of groundwater and surface water with other external N sources previously identified and estimated in Ólafsson (1979b) and/or Jónsson (2016) (Table S2).

We estimated N loss via midge emergence to range from 0 to 50 metric tons N yr⁻¹ (Table S2). Our estimated rate of N loss via permanent sedimentation was 242 metric tons N yr⁻¹ (Table S2). We tentatively estimated a rate of N loss via the microbial N transformation annamox of 0 metric tons N yr⁻¹ (Table S2). By summing these estimates with previously identified and calculated estimates of N loss from Ólafsson (1979b) and/or Jónsson (2016), we estimated total N loss to range from 281 to 573 metric tons N yr⁻¹ (Table S2, Table 3). The deficit between total external N supply and total N loss in our updated N budget ranged from 201 and 517 metric tons N yr⁻¹. As in Ólafsson (1979b) and Jónsson (2016), we assume that N fixation accounts for this deficit (Table S2, Table 3).

Estimates of annual lake-scale benthic and pelagic N fixation

The estimated annual lake-scale N fixation rates under the four scenarios of high/low benthic and pelagic N fixer abundances showed that pelagic N fixation dominated in years of high pelagic N fixer abundances (HH and HL scenarios), while benthic N fixation dominated in years of low pelagic N fixer abundances (LH and LL scenarios) (Table 4). However, the annual rate of lake-scale N fixation was much higher in years of high pelagic N fixer abundances than in years of low pelagic N fixer abundances due to the low maximum rate of annual lake-scale benthic N fixation. Even under the LH scenario, which reflected optimal conditions for benthic N fixation, total annual benthic N fixation contributed only about 3 metric tons N yr⁻¹ (Table 4). In contrast, total annual lake-scale N fixation under the optimal scenario for pelagic N fixation was 109 metric tons N yr⁻¹ (Table 4). Notably, this is only about 50% of the minimum rate of N fixation that we estimated in our updated static N budget for the lake (Table S2). Despite the discrepancy

between estimates of annual lake-scale N fixation in our static N budget (Table 3) and scenarios (Table 4), the scenarios reveal the much higher potential for N fixation in the pelagic than benthic zone.

Dynamic N budget

The dynamic N budget was designed to estimate the proportion of pelagic organic N that must enter the benthos to reconcile two observations: first, our observation that areal N fixation was higher in the pelagic zone than in the benthos and therefore occurs as a short-duration pulse during episodic summer blooms of N fixing *Dolichospermum* (Table 1); and second, the observation that the outflow of N through the outlet river Laxá was distributed fairly evenly annually and among years (Fig. 2). Reconciling these two observations involves considering the incorporation of N fixed in the pelagic zone into the benthos, because the water residence time in the lake is only 27 days and, therefore, N cannot be retained for long in the pelagic zone.

For illustration, we considered cases in which 10%, 66%, and 90% ($s_P = 0.11 * flow_P$, $1.94 * flow_P$, and $9 * flow_P$) of the pelagic organic N is incorporated into the benthos for the case when scenarios HH and LH alternate among years (Fig. 3, Table 5). For all cases we assumed that the external supply of N was the midpoint of our range of estimates (Table S2) and used the total N fixation rate of 296 metric tons N yr⁻¹ which is the value we calculated under the assumption of a sedimentation rate of 242 metric tons N yr⁻¹ and no midge emergence. For 66% incorporation the peak discharge concentration was 415 ug L⁻¹, which closely matched 421 ug L⁻¹ that was reported by both Ólafsson (1979b) and Eiríksdóttir et al. (2018) (coincidentally the same value).

This parameterization for s_P gave a permanent sedimentation rate of 225 tons N yr⁻¹ and an outflow of 208 tons N yr⁻¹, which roughly match the estimates of 242 and 243 tons N yr⁻¹ from our static budget, respectively. If only 10% of the pelagic organic N is incorporated into the benthos, then in years of high pelagic N fixation, there is a large peak in the loss of N from the outlet of 1034 ug L⁻¹, and a higher average annual outflow of 371 tons N yr⁻¹. In contrast, when there is a 90% incorporation rate, there is a small peak in the loss of N from the outlet of 124 ug L⁻¹, and a lower annual outflow of 103 tons N yr⁻¹. This argues that roughly two-thirds (66%) of the pelagic organic N is incorporated into the benthic.

Discussion

In N-limited lakes, cyanobacterial N fixation rates can be high (Schindler, 1977; Flett et al., 1980; Howarth et al., 1988; Scott & Grantz, 2013; Marcarelli et al., 2022). However, the effect of cyanobacterial N fixation on ecosystem N availability depends, in part, on the retention of fixed N (Scott & McCarthy, 2010; Grantz et al., 2014; Scott et al., 2019). We explored the dynamics of benthic and pelagic N fixation and fixed N retention in Lake Mývatn, Iceland. Our updated static N budget showed that the annual rate of lake-scale N fixation needed to account for the deficit between external N supply and N loss varies greatly from year to year, primarily due to variation in the magnitude of N loss via outflow and midge emergences. Extrapolations from recent direct measurements of pelagic and benthic N fixation under scenarios of different benthic and pelagic N fixer abundances showed that the capacity for N fixation is much higher in the pelagic than the benthic zone. We then explored the degree to which N fixed in the pelagic zone is retained within the lake using a dynamic N budget that leveraged data on patterns of N

loss from the lake. This revealed that approximately two-thirds of organic N in the pelagic zone is likely incorporated into the benthos. Together, these results suggest that pelagic N fixation is an important source of N for benthic primary producers in Mývatn.

As in previously published N budgets for Mývatn (Ólafsson, 1979b; Jónsson 2016), external N supply was lower than N loss in our updated static N budget, indicating the existence of internal N supply. Our updated N budget expands on previous N budgets by including minimum and maximum estimates of external N supply and N loss, revealing the potential for the N deficit to vary widely from year to year. In previously published N budgets for Mývatn (Ólafsson, 1979b; Jónsson 2016), internal N supply via N fixation was assumed to account for the N deficit.

Variation in the N deficit was driven by variation in N loss, which has been attributed to variation pelagic cyanobacterial abundance in Mývatn (Jónsson 2016). Because pelagic cyanobacteria in Mývatn are N fixers (Jónasson & Aðalsteinsson, 1979), variation in the N deficit driven by variation in pelagic cyanobacterial abundance may accurately reflect variation in N fixation rates. However, variation in the N deficit could also be driven by N loss not attributable to N fixation that occurred within the year. For example, previously stored N can be released via internal loading and lost if not re-assimilated. Therefore, N fixation may not match the N deficit in a given year, leading to static N budgets with a wide range of values to account for variation among years.

In addition to estimating lake-scale N fixation as the deficit between external N supply and N loss, we estimated annual pelagic and benthic N fixation using measured areal N fixation rates. The main conclusion from these estimates is that the potential for pelagic N fixation is much

greater than benthic N fixation. While we are confident in this conclusion, the maximum annual N fixation rate estimated from observed areal N fixation rates was about 50% of the minimum N deficit from our static N budget. The empirically estimated N fixation rate could be lower than the N fixation rate estimated as the N budget deficit for three general reasons. First, there is high uncertainty in some of the supply and loss rates of N. For example, Ólafsson (1979b) estimate a sedimentation rate of 74 tons N yr⁻¹ based on a centuries-long sediment core, whereas we estimated 242 tons N yr⁻¹ using a sediment core from a different location focusing on 30 years of deposition. Given the potential variation in N dynamics both spatially and temporally, better estimates of N supplies and deficits would require extensive further measurements. Second, the measurements of N fixation may be biased low. These N fixation rates from Book et al. (in review) were measured using the acetylene reduction method (Capone, 2018), and Kunsza and Hall (2023) have recently shown that this method underestimates N fixation at lower temperatures. Extrapolating the results of Kunsza and Hall (2023) to 12°C, the average temperature at which N fixation was measured by Book et al. (in review), the measured values of N fixation from the acetylene reduction method would be 50% underestimates. Third, there could be sources of external N supply that have not been adequately accounted for in static N budgets for the lake including run-off of sheep manure or fertilizer from surrounding farms. It is also possible that N fixation by other cyanobacteria or heterotrophs is much higher than expected based on our observations, and that there are sources of N loss that we have not adequately accounted for. Specifically, denitrification, which contributes substantially to N loss and the perpetuation of N limitation in many lakes (Andersen et al., 2025; McCarthy et al., 2016; Scott et al., 2019b; Scott & Grantz, 2013; Scott & McCarthy, 2010), may be non-negligible despite being assumed to be zero in static N budgets by Ólafsson (1979b), Jónsson (2016), and us. While we

do not think that these other possible N supplies and losses contribute substantially to the N budget for Mývatn, we cannot exclude them.

Although pelagic N fixation appears to have the most potential to supply N to the lake, most primary production is carried out by benthic algae in most years (McCormick et al., 2021). Observations of the annual pattern of N outflow via the outlet river Laxá (Ólafsson, 1979b; Jónsson, 2016) allowed us to explore the likely retention of fixed N using our dynamic N budget. We found that estimates of annual and peak outflow in our dynamic N budget matched observed patterns in the lake when the incorporation of pelagic organic N into the sediment N pool was about two-thirds. This suggests that the majority of N fixed by pelagic cyanobacteria is retained via sedimentation and, at least potentially, recycled by benthic primary producers in Mývatn.

There is ongoing discussion around two related questions regarding N fixation in N-limited lakes. First, what is the relationship between ecosystem-level N fixation and ecosystem-level N limitation of primary production (Vitousek et al., 2002; Scott & McCarthy, 2010; Scott & Grantz, 2013; Andersen et al., 2025)? This is especially pertinent to shallow lakes such as Mývatn wherein primary production can be dominated by benthic algae while N fixation can be dominated by pelagic cyanobacteria. Second, what is the fate of fixed N within a lake (Howarth et al., 1988; Vitousek et al., 2002; Scott & McCarthy, 2010; Scott & Grantz, 2013; McCarthy et al., 2016; Marcarelli et al., 2022; Wu et al., 2022; Andersen et al., 2025)? Again, this question is particularly important for lakes such as Mývatn wherein pelagic cyanobacteria account for the majority of N fixation but advection of pelagic cyanobacteria is likely high due to short water residence time. Our dynamic N budget addresses these specific questions in Mývatn, and a

similar approach could be employed for other lake studies. Several characteristics of the Mývatn N budget made this exercise possible including the availability of N loss data via the outlet river Laxá and the presence of relatively few (including many constant) external N sources and sinks. However, the exercise may be more complicated in other lakes with many, diffuse, and/or highly variable sources of external N supply and/or N loss. Implementing a dynamic N budget would ideally include within and among-year variation in all supplies and losses. Nonetheless, in the tradition of using N budgets to give a broad-brush depiction of N supply and loss from a lake, the dynamic N budget takes a step towards greater realism, making it possible to give at least provisional answers to these two general questions about N fixation in lakes.

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Figures and Tables

Table 1. Summary of assumed areal rates, extents, and durations of N fixation in benthic and pelagic N fixers under scenarios of high (H) and low (L) N fixer abundances.

Scenario	Areal rate of N fixation (g N m ⁻² d ⁻¹)		Areal extent of N fixers (m ²)		Annual duration of N fixation (d)	
	Benthic	Pelagic	Benthic	Pelagic	Benthic	Pelagic
HH	0.00116	0.02419	2.15 x 10 ⁷	2.97 x 10 ⁷	47	76
HL	0.00116	0.04838	1.65 × 10 ⁶	2.97 x 10 ⁷	47	76
LH	0.00116	0	2.15 x 10 ⁷	0	123	0
LL	0.00116	0	1.65 × 10 ⁶	0	123	0

Table 2. Parameter descriptions and values used in the dynamic N budget for Mývatn. Values for $Nfix_P$ are given for two scenarios corresponding to years of high (H) and low (L) pelagic N fixer abundances (Table 4).

Parameter	Units	Value	Description
$flow_P$	d^{-1}	1/27	water flow rate
I_{in}	tons N d^{-1}	0.186	inorganic N in the inflow
$max(Nfix_P)$	tons N d^{-1}	L: 0 H: 3.43	maximum daily pelagic N fixation
$max(Nfix_B)$	tons N d^{-1}	0.119	maximum daily benthic N fixation
$max(Nuptake_P)$	d^{-1}	$100 * flow_P$	maximum daily pelagic uptake rate of inorganic N
$min(Nuptake_P)$	d^{-1}	$flow_P$	minimum daily pelagic uptake rate of inorganic N
$mean(Nflux_B)$	d^{-1}	$1/(7*365)$	mean release rate of N from the sediment
$max(Nflux_B)/min(Nflux_B)$	d^{-1}	10	$max(Nflux_B)/min(Nflux_B)$
s_B	d^{-1}	$mean(Nflux_B)/2$	permanent sedimentation of 1/3 of benthic organic N
s_I	d^{-1}	$10 * flow_P$	uptake rate of pelagic inorganic N by benthic algae
s_P	d^{-1}	variable	incorporation rate of pelagic organic N in the sediment

Table 3. Summary of N supply, N loss, and N deficit estimates (metric tons N yr⁻¹) from static N budgets for Mývatn. More detailed estimates are provided in Table S2.

Study	External N supply	N loss	N deficit
Ólafsson (1979b)	54	355	301
Jónsson (2016)	80	279	199
This study	56 – 80	281 – 573	201 – 517

Table 4. Estimated rates of benthic, pelagic, and total annual lake-scale N fixation (metric tons N yr⁻¹) in Mývatn under scenarios of high (H) and low (L) benthic and pelagic N fixer abundances.

Scenario	Benthic N fixation	Pelagic N fixation	Total N fixation
HH	1.17	54.6	55.8
HL	0.0903	109	109
LH	3.07	0	3.07
LL	0.236	0	0.236

Table 5. Comparison of dynamic N budget estimates using different values of assumed incorporation of pelagic organic N into the benthos, s_p . All units are metric tons N yr⁻¹ except Peak Outflow which is the concentration of organic and inorganic N in the outflow in $\mu\text{g N L}^{-1}$ (Figs. 2 and 3). The row labeled "static budget" gives the best estimate appropriate for the case simulated as well as the range of values from our static N budget in parentheses (Table S2); the two values under Peak Outflow in this row are from the Ólafsson (1979b) and Eiríksdóttir et al. (2018) data sets (Fig. 2) which both equal 421 after anomalous values are removed from Ólafsson (1979b).

Incorporation assumption	External supply	N fixation	Permanent Sedimentation	Annual Outflow	Peak Outflow ($\mu\text{g L}^{-1}$)
10%	68	296	61	371	1034
66%	68	296	225	208	415
90%	68	296	330	103	124
static budget	68 (56 – 80)	296	242 (74 – 242)	243 (207 – 281)	421, 421

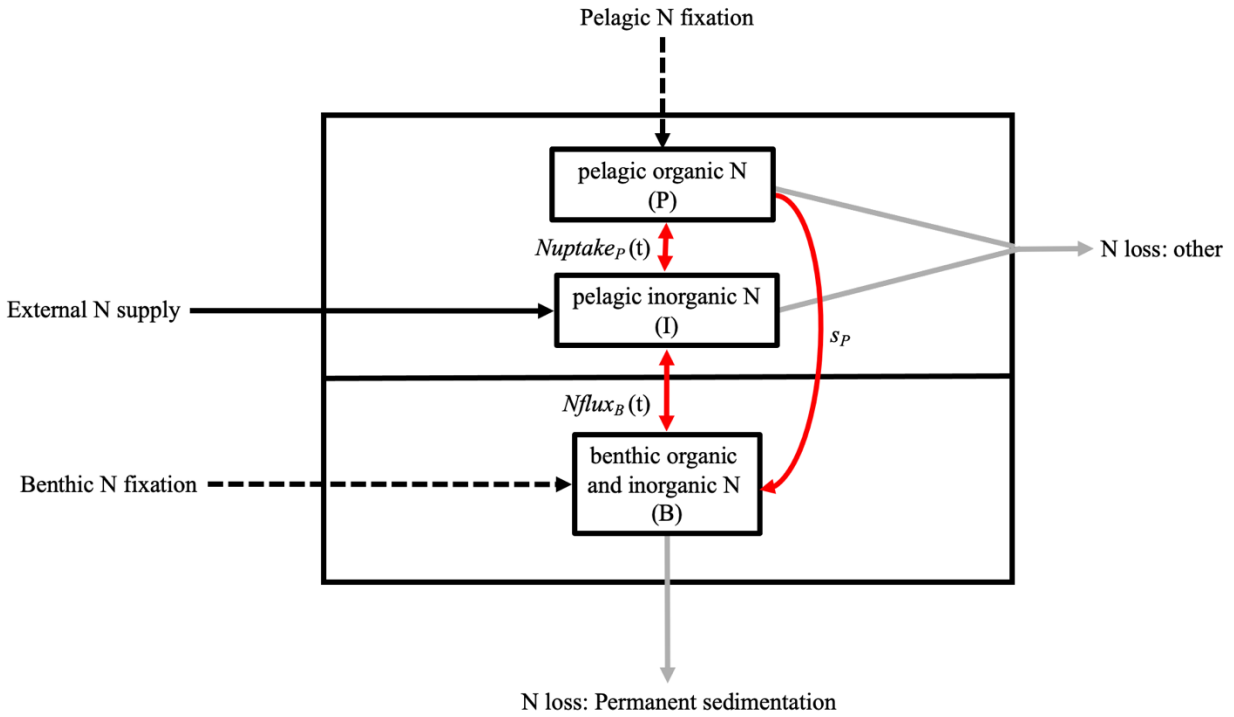


Figure 1. Diagram of the dynamic N budget for Mývatn.

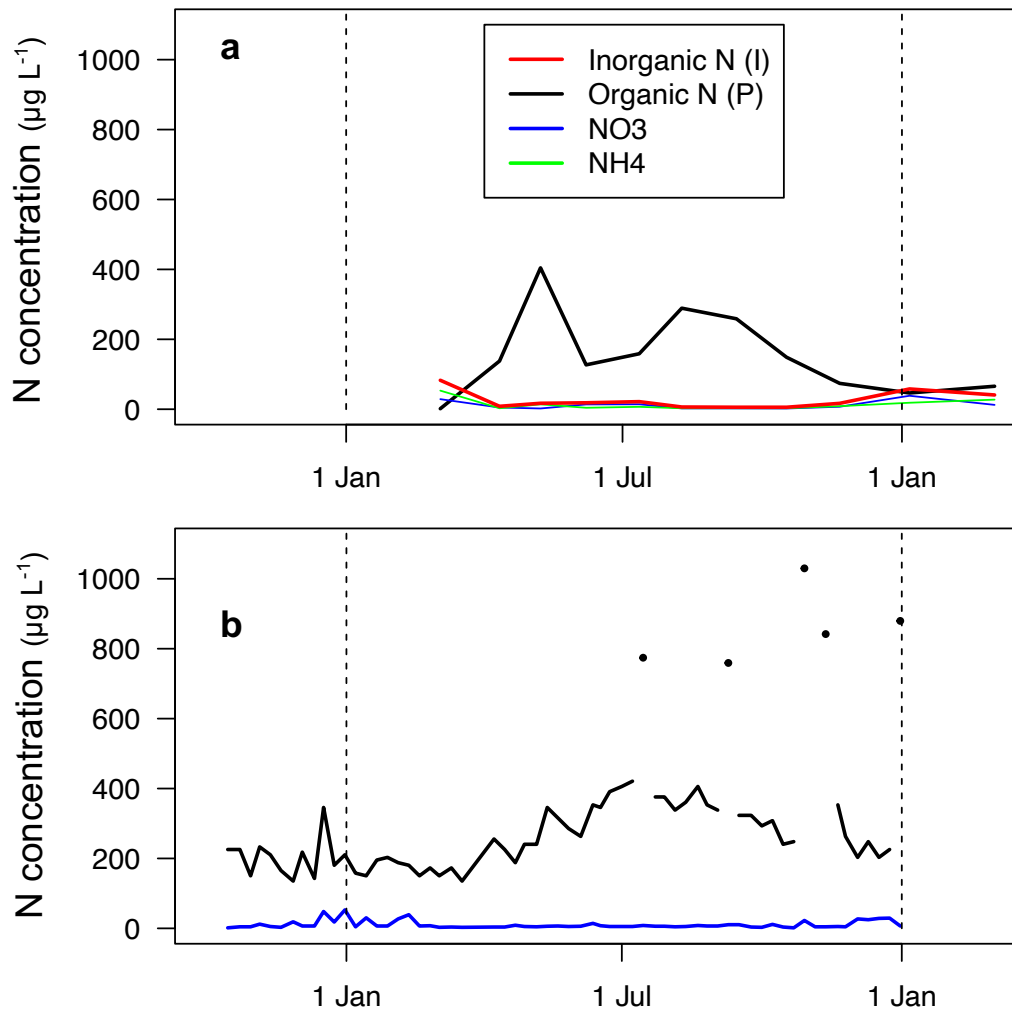


Figure 2. N concentrations measured in the outlet river Laxá at Geirðistaðir from (a) Eiríksdóttir et al. (2018) and (b) Ólafsson (1979b). Measurements from Eiríksdóttir et al. (2018) are from March 2000 to March 2001 and show concentrations of organic (black) and inorganic (red) N, with inorganic N partitioned into nitrate (blue) and ammonium (green). Measurements from Ólafsson (1979b) are from October 1973 to January 1974 and show concentrations of organic N (black) and nitrate (blue). Anomalous values attributed to inhomogeneity of water samples from Ólafsson (1979b) are represented as dots.

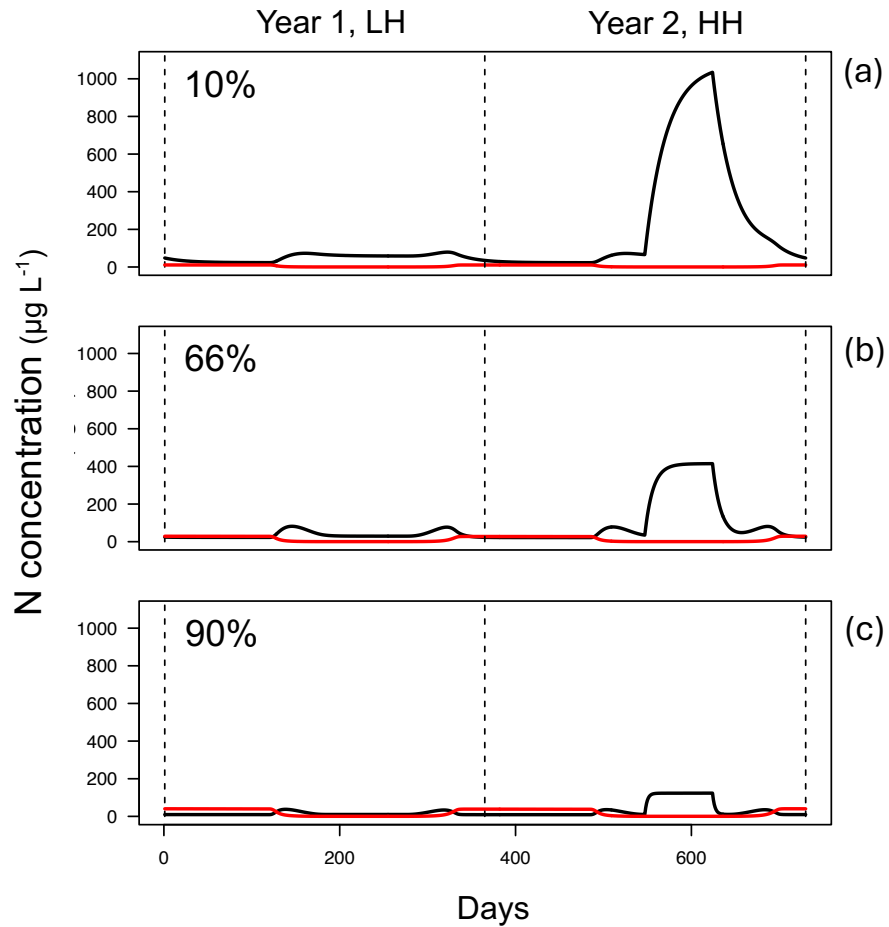


Figure 3. Dynamic N budget estimates of organic (black) and inorganic (red) N concentrations in the outlet river Laxá assuming that years alternate between LH and HH scenarios of pelagic/benthic N fixer abundances. Panels represent estimates when 10% (a), 66% (b), and 90 (c) of pelagic organic N is assumed to be incorporated into the benthos. The black line gives the concentration of organic N and the red line inorganic N.

Appendix B: Supplementary Materials for Chapter 2

Supplementary methods

Updating the N budget for Lake Mývatn

Here, we explain how we constructed an updated static N budget for Mývatn. We do not provide new estimates of N supply and loss via the majority of inputs and outputs and instead use values from previously published N budgets by Ólafsson (1979b) and Jónsson (2016), both of which are outlined in Table S1. However, we do make a few additions. To the existing N budgets, we add a potentially important source of N loss from the system: midge emergences. We also use data provided in Ólafsson (1979b) and Jónsson (2016) to directly partition N supply via groundwater springs and surface water, which was not done explicitly in either of the previous N budgets. Finally, we add a newly estimated permanent sedimentation rate. We briefly summarize how we derived estimates of all inputs (external N supply) and outputs (N losses)- either from previously published N budgets found in Ólafsson (1979b) and Jónsson (2016) or new calculations- below.

External N supply

Surface water

N supply to Mývatn via surface water is relatively low due to the high porosity of the lake's drainage basin (Ólafsson 1979b). Surface run-off is estimated to be zero (Ólafsson 1979b, p. 89;

Jónsson 2016, Table 1). The river Grænilækur, which flows from the upstream, spring-fed lake Grænavatn into the South Basin of Mývatn, provides the only surface water N supply to the lake (Ólafsson 1979b, p. 89). In previously published N budgets, surface water N supply via the Grænilækur has not been explicitly partitioned from groundwater N supply to the South Basin (Table S2). Here, we estimate annual surface water N supply to the South Basin via the Grænilækur separately by multiplying the mean annual flow rate of the river by its mean N concentration (Table S1). Because Ólafsson (1979b) and Jónsson (2016) reported different mean annual flow rates for the river of 5 (Ólafsson 1979b, p. 88) and $6.4 \text{ m}^3 \text{ s}^{-1}$ (Jónsson 2016, Table 3), respectively, we calculated a range of surface water N supply rates using both values (Table S1). Our estimate of the mean N concentration of the river is derived from Ólafsson (1979b, Fig. 4). The estimated range of external N supply via surface water is 0.88 to 1.13 metric tons N yr^{-1} (Table S1, S2).

Groundwater

Groundwater springs account for the majority of external N supply to Mývatn (Ólafsson 1979b; Jónsson 2016). The mean annual flow rates (Ólafsson 1979b, p. 88; Jónsson 2016, Table 3) and mean N concentrations (Ólafsson 1979b, Fig. 14; Jónsson 2016, Table 10) of groundwater springs feeding the lake differ between North to South Basins and over time. Here, we estimate a range of groundwater N supply rates to each basin by multiplying the range of mean annual flow rates by the range of mean N concentrations as reported by Ólafsson (1979b) and Jónsson (2016). We estimated the mean N concentration of groundwater springs feeding the North and South Basins by summing and averaging the $\text{NO}_3\text{-N}$ concentrations of individual springs (and

excluding the river Grænilækur) provided by Ólafsson (1979b, Fig. 14). We summed the range of estimates of groundwater N supply to each basin to estimate a range of total groundwater N supply of 41.8 to and 66.3 metric tons N yr⁻¹ (Table S1, S2).

Other

We include estimates of external N supply via two additional categories- aerial deposition and human activity- directly from Jónsson (2016, Table 1) (Table S2).

N loss

Permanent sedimentation

We used a sediment core taken in the sheltered bay at Höfði to estimate the rate of permanent sedimentation of N from the lake and the rate at which N is remineralized and released from the sediment. This sediment core is described in detail by Hauptfleisch et al. (2012) and Einarsson et al. (2016). The sediment core allowed an accurate and high-resolution depth-age model, and the time period covered (roughly 30 years) encompassed the time frame of most remineralization and recycling of N in the sediment. The concentration of N was determined during stable isotope analysis at 0.5-cm increments of the core (Fig. S2). We analyzed these data to obtain the retention rate of N, that is, the amount of N per cm² of a given age in years. We estimated that 1/3 of the N is permanently sedimented as the asymptote of the retention rate at older ages, and the remineralization rate of 7 years from the half-life of the N that is not permanently

sedimented. The sedimentation rate given by Ólafsson (1979b) is based on the concentration of N in the sediment around a depth dated to 1477. For the dynamic N budget model, we have preferred the higher sedimentation rate calculated from the last 30 years given by the Höfði core to better reflect the more-recent lake processes.

Denitrification

Ólafsson (1979b, p. 109) speculatively assumed that the rate of denitrification in Mývatn was zero, and Jónsson (2016, Table 1) adopted this value (Table S2). This assumption was based upon the established relationships between the rate of denitrification and environmental factors such availability of nitrate, oxygen, dissolved organic carbon, and temperature, which all tend to limit denitrification in Mývatn (Ólafsson 1979b). However, denitrification rates in shallow, eutrophic lakes can be substantial (Jensen et al. 1992; Ahlgren et al. 1994), which suggests that the process may occur in Mývatn particularly during the summer when environmental factors shift to favor its occurrence. Here, we continue to assume a denitrification rate of zero (Table S2) but recognize that denitrification rates need to be measured to accurately determine the effect of the process on the N budget of Mývatn.

Annamox

Anaerobic ammonium oxidation (annamox) to N₂ has not been studied in Mývatn nor included in any existing N budgets of the lake. In freshwater lakes, the occurrence and rate of annamox in the sediment has been shown to be influenced by environmental factors including oxygen,

dissolved inorganic ammonium and nitrate availability, dissolved organic carbon, and temperature (Zhang et al. 2023). The levels of these factors in Mývatn tend to disfavor annamox. However, it is possible that annamox occurs at varying rates over space and time due to the high variability of some of these environmental factors in Mývatn. We assume an annamox rate of zero (Table S2), but the process needs to be measured directly to determine its importance for the N budget of Mývatn.

Surface water

N loss via the lake's only surface water outlet river Laxá has been estimated to range from 207 (Jónsson 2016, Table 1) to 281 (Ólafsson 1979b, p. 109) metric tons yr⁻¹ (Table S2). We include both estimates in our N budget to reflect the range of potential N loss via surface water, which varies from year to year. The low and high estimates of N loss reported here reflect years of low and high *Dolichospermum* spp. abundance, respectively (Jónsson 2016). Variation in other processes such as the rate of internal loading may also contribute to variation in N loss via the Laxá, as N released from the sediment may flow out of the lake if not re-assimilated.

Midge emergence

Larval midges (Chironomidae) feed on algae and detritus in and on the sediment surface of Mývatn before emerging as adults to mate over land (Einarsson et al. 2004). These emergences, which vary in size by over 5 orders of magnitude (Ives et al. 2008), export N from Mývatn to the surrounding terrestrial landscape and represent a loss of N from the aquatic ecosystem (Dreyer et

al. 2015). Loss of N via midge emergences was not included in N budgets by Ólafsson (1979b) or Jónsson (2016). Here, we incorporate N loss via midge emergences as an additional variable in the N budget.

We estimated the range of potential N loss via midge emergences across the range of plausible emergence magnitudes (Table S2). To determine the maximum plausible rate of N loss via midges, we first multiplied the maximum observed density of fourth instar larval midges (i.e. the density of midges in the final instar before pupation which are likely to emerge that year) from long-term monitoring of the lake by the area of the lake (37 km²) to estimate the number of midges likely to emerge from the lake. We then multiplied the number of midges by the mean fourth instar dry weight of 0.232 mg (Botsch 2023) to determine the total amount of mass lost. Because midges are 10% N by mass (Gratton et al. 2008), we reduced the total amount of mass loss to 10% of the total. Finally, we reduced the mass of N leaving the lake by 50% because female midges, which account for roughly 50% of the population, return to the lake to lay eggs and die after mating (Armitage 1995). In years with low midge abundance, the N loss from emergence is essentially zero.

Supplementary Tables and Figures

Table S1. Summary of values used to estimate external N supply via surface water and groundwater in our updated static N budget for Mývatn. Values derived from Ólafsson (1979b) are marked with ¹ and values derived from Jónsson (2016) are marked with ².

	Basin	Flow rate (m ³ s ⁻¹)	N concentration (g N m ⁻³)	Total N supply (metric tons N yr ⁻¹)
Surface water	North	0	-	0
	South	5 ¹ – 6.4 ²	0.0056 ¹	0.88 – 1.13
	Total	5 ¹ – 6.4 ²	-	0.88 – 1.13
Groundwater	North	7.1 ¹ – 8.3 ²	0.11 ¹ – 0.15 ²	24.6 – 39.3
	South	16.6 ² – 20.9 ¹	0.033 ¹ – 0.041 ²	17.2 – 27.0
	Total	-	-	41.8 – 66.3

Table S2. Detailed summary of external N supply, N loss, and N deficit estimates (metric tons N yr⁻¹) from static N budgets for Mývatn.

Study	External N supply	N loss	N deficit
Ólafsson (1979b)	Spring water: 54 Total: 54	Surface water: 281 Sedimentation: 74 Microbial N transformations Denitrification: 0 Total: 355	Total: 301
Jónsson (2016)	Spring water: 67 Atmospheric deposition Precipitation: 3.7 Dry matter: 0.3 Human activity Human settlement: 1.2 Agriculture: 6.0 Tourism: 1.8 Total: 80	Surface water: 207 Sedimentation: 72 Total: 279	Total: 199
Current study	Groundwater: 41.8 – 66.3 Surface water: 0.88 – 1.13 Atmospheric deposition Precipitation: 3.7 Dry matter: 0.3 Human activity Human settlement: 1.2 Agriculture: 6.0 Tourism: 1.8 Total: 56 – 80	Surface water: 207 – 281 Sedimentation: 74 – 242 Microbial N transformations Denitrification: 0 Annamox: 0 Midge emergence: 0 – 50.0 Total: 281 – 573	Total: 201 – 517

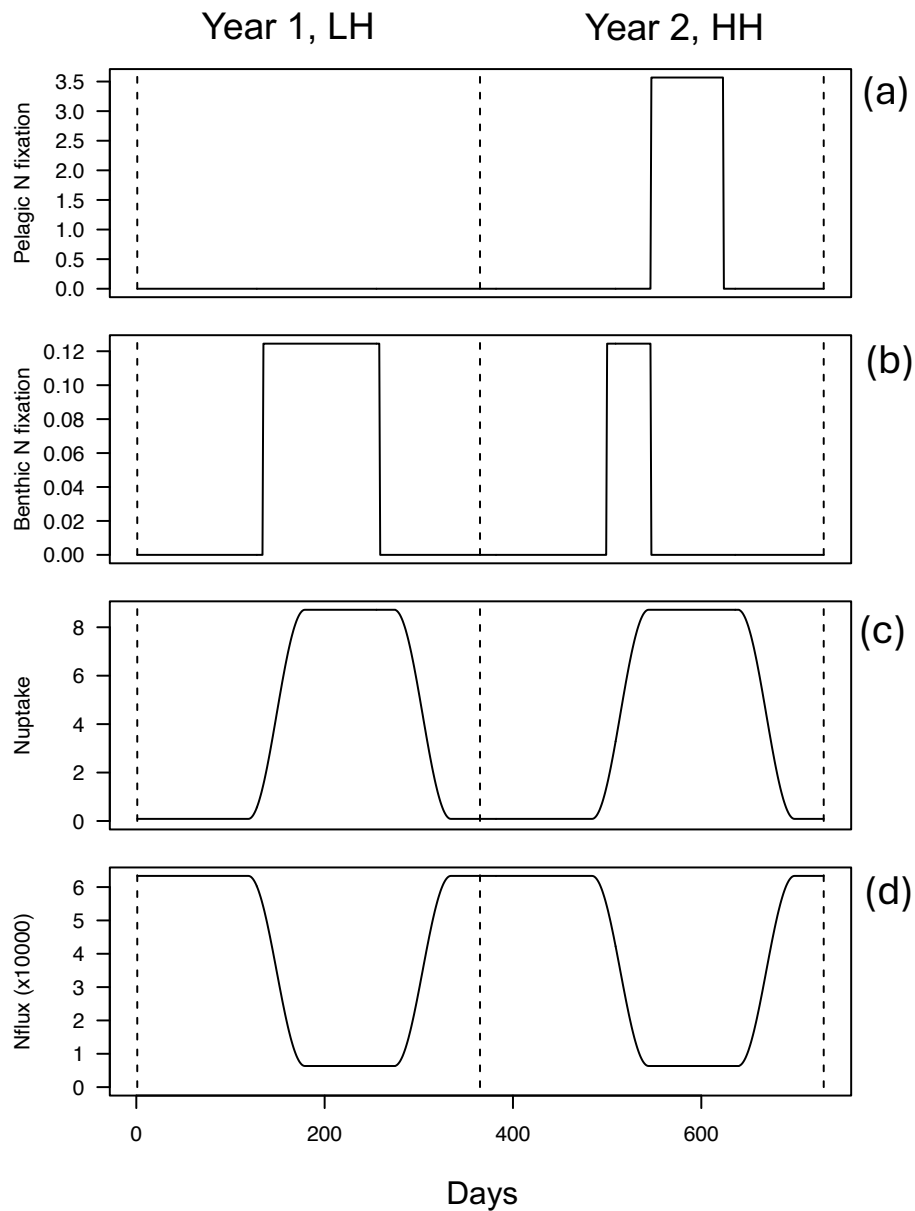


Figure S1. Dynamic N budget assumptions about annual patterns of (a) N fixation in pelagic ($Nfix_P$) and (b) benthic zones ($Nfix_B$), (c) uptake of inorganic N in the pelagic zone ($Nuptake_P$), and (d) flux rate of N from benthic to pelagic zones ($Nflux_B$). Years alternate between LH and HH scenarios of pelagic/benthic N fixer abundances.

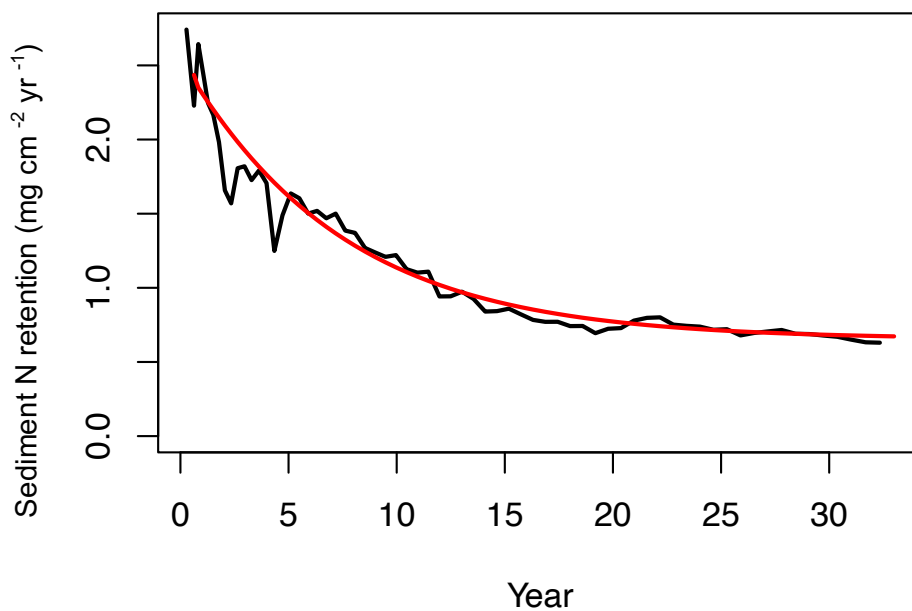


Figure S2. Sediment N retention as a function of sediment age in a sediment core taken from Höfði bay in Mývatn. The decrease in sediment N retention with age is due to remineralization and release of N from the sediment.

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