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Benthic cyanobacteria of the genus *Nostoc* are a source of microcystins in Greenlandic lakes and ponds

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Abstract

1. Benthic primary producers are recognised for their important role in contributing to ecosystem productivity and nutrient cycling in lake and stream ecosystems, particularly in polar environments. In Arctic lakes, benthic producers often comprise mats or colonies of cyanobacteria capable of producing cyanotoxins. However, the extent to which benthic communities contribute cyanotoxins in polar regions remains poorly described.
2. We evaluated the potential for benthic colonies of the cyanobacterium *Nostoc pruniforme* from lakes in Kangerlussuaq, Greenland, to contribute microcystins (MCs) to lake water using three approaches. First, we dissected field-collected *Nostoc* colonies and measured MCs within multiple layers of fresh colony tissue. Second, we conducted a laboratory experiment to evaluate the temporal dynamics of MC release by incubated, intact colonies. Finally, we quantified whether MC concentrations in water and sediment samples in the field were higher in and above dense bands of benthic *Nostoc* as compared to bare sediment.
3. Field-collected *Nostoc* colonies contained MCs throughout the colony tissue, suggesting that damage to colonies from grazers or physical disturbance could facilitate the release of toxins into the water. Undamaged *Nostoc* colonies incubated in high-nutrient conditions in the laboratory leaked MCs into the surrounding water at a steady mass-specific rate over the course of 7 days.
4. Microcystin concentrations in water and sediment from two Greenlandic lakes were highly variable, but slightly higher in lake water immediately above dense bands of *Nostoc* than in water immediately above bare sediments, suggesting that benthic *Nostoc* colonies contribute cyanotoxins to lake water and that MCs vary at very fine, 1–2 m spatial scales.
5. Benthic cyanobacteria may be important in releasing MCs into aquatic ecosystems, especially in systems where benthic producers dominate, such as polar environments.

KEYWORDS

Arctic, cyanotoxins, *Nostoc pruniforme*, spatial distribution, toxin release

1 | INTRODUCTION

Cyanotoxins are a growing health concern worldwide due to their adverse effects on diverse biota, including toxicity to humans, terrestrial animals, and aquatic organisms such as zooplankton and fish (Buratti et al., 2017; Ferrão-Filho & Kozlowsky-Suzuki, 2011). While our understanding of cyanotoxins in freshwater ecosystems is derived largely from pelagic cyanobacterial taxa in temperate or tropical regions, there is a growing body of literature demonstrating that benthic cyanobacteria produce cyanotoxins in diverse habitats worldwide (Bouma-Gregson, Kudela, & Power, 2018; Cantoral Uriza et al., 2017; Fetscher et al., 2015; Kelly et al., 2019; Quiblier et al., 2013). Accounting for cyanotoxin production and movement through both pelagic and benthic pathways is an important next step in ecology, similar to advances in understanding the roles of benthic organisms in ecosystem productivity and nutrient cycling (Mariash et al., 2014; Vadeboncoeur et al., 2002).

Partitioning cyanotoxin production via benthic versus pelagic pathways may be particularly important in polar aquatic ecosystems where cyanobacteria often thrive as mats or multicellular colonies that reside in the benthos and dominate ecosystem productivity (Quesada et al., 2008; Vincent & Hobbie, 2000; Vincent & Laybourn-Parry, 2008). Benthic mats can be important nutrient subsidies for pelagic consumers (Cazzanelli et al., 2012; Mariash et al., 2014; Rautio & Vincent, 2006), and there is a growing body of literature demonstrating cyanotoxin production in either benthic mats or colonies from polar ecosystems (reviewed by Cantoral Uriza et al., 2017; Quiblier et al., 2013; see individual citations in the next paragraph).

Cyanotoxins have been detected in association with multiple genera of benthic-dwelling cyanobacteria from lakes and streams worldwide (Quiblier et al., 2013). These genera include *Microcoleous* (formally *Phormidium*) (Borges et al., 2015; Bouma-Gregson et al., 2018; McAllister et al., 2016; Wood et al., 2006), *Oscillatoria* (Aboal & Puig, 2005; Izaguirre et al., 2007; Mez et al., 1997,1998), *Anabaena* (Aboal & Puig, 2005; Bouma-Gregson et al., 2017; Mohamed et al., 2006; but see Kelly et al. 2019), *Lyngbya* (Aboal & Puig, 2005; Hitzfeld et al., 2000; Jungblut et al., 2006), and *Nostoc* (Hitzfeld et al., 2000; Kust et al., 2018; Mohamed et al., 2006; Oudra et al., 2009; Wood et al., 2008). A handful of studies have reported detectable cyanotoxins in Antarctic microbial mats (Hitzfeld et al., 2000; Jungblut et al., 2006; Kleinteich et al., 2014; Puddick et al., 2015; Wood et al., 2008), but fewer studies report cyanotoxins in Arctic environments, particularly in benthic habitats (Chrapusta et al., 2015; Kleinteich et al., 2012,2013; Trout-Haney, 2017).

Many lakes and ponds surrounding Kangerlussuaq, Greenland, contain large colonies of benthic cyanobacteria of the genus *Nostoc*, referred to colloquially as *sea tomatoes* by local Greenlanders (Figure 1). *Nostoc* is a genus of filamentous cyanobacteria inhabiting a wide range of aquatic and terrestrial ecosystems worldwide (Dodds et al., 1995). In oligo- and mesotrophic freshwater bodies in temperate and Arctic regions, *Nostoc* often take the form of spherical benthic colonies, with heterocytes enabling them to fix nitrogen and a thick gelatinous matrix protecting them against harsh conditions such as prolonged periods of freezing and drought (Sand-Jensen, 2014). In Kangerlussuaq lakes, the most commonly observed species is the smooth, spherical colonies of *Nostoc pruniforme* (hereafter referred to as *Nostoc*), ranging in circumference from <1 cm to >30 cm (Figure 1). The density of *Nostoc* varies considerably among lakes in this region, ranging from no visible colonies to >9,000 colonies/m^2 benthos (J.V.Trout-Haney, personal observation). *Nostoc* colonies are often found congregated in dense bands at *c*. 1–3 m depth, resulting in large areas of the benthos covered in layers of *Nostoc* colonies (Figure 1).

Our previous work has shown that Kangerlussuaq-area lakes contain measurable concentrations of the cyanotoxin microcystin (MC) in water (Trout-Haney et al., 2016), *Nostoc* colonies, and multiple aquatic organisms, including benthic snails (*Lymnaea* sp.) and chironomid larvae, which are commonly found on or inside *Nostoc* colonies. We hypothesised that *Nostoc* contribute to lake water MC concentrations, given the high density of *Nostoc* colonies, and the known presence of MC in *Nostoc* tissue from these lakes. It is perhaps not surprising that colonial *N. pruniforme* are MC producers, as *Nostoc* benthic mats have been shown to produce MCs across a wide geographic range, including in temperate Australia (Gaget et al., 2017), streams in California (Fetscher et al., 2015), an alpine river in Morocco (Oudra et al., 2009), segments of the Nile River in Egypt (Mohamed et al., 2006), the Baltic Sea (Surakka et al., 2005), riverine systems in New Zealand (Wood et al., 2006), Antarctic ponds in the McMurdo Dry Valleys (Wood et al., 2008), and meltwater ponds on the McMurdo Ice Shelf (Hitzfeld et al., 2000; Jungblut et al., 2006; Wood et al., 2008).

However, it is not yet known how these toxins are distributed within a colony. If toxins are stored disproportionately in certain layers of the colony, this may indicate more active toxin-producing or storage areas and have implications for toxin leakage into surrounding water. Moreover, when snails and benthic larvae graze epiphytic matter from inside or on the surface of colonies, they may both ingest MCs from trace amounts of *Nostoc* tissue and release MCs into the water via damage to the colony.

To begin filling in these gaps, we dissected colonies of *Nostoc* collected from a Kangerlussuaq lake to examine the distribution of MCs within the colony matrix. We then conducted a laboratory experiment to evaluate the potential for incubated colonies to release MCs into the surrounding water. Finally, we measured MCs in water and sediment from two lakes in Kangerlussuaq, Greenland, to determine whether there were spatial differences in the concentration of MCs given the presence of a *Nostoc* band (Figure 1).

2 | METHODS

2.1 | Collection of *Nostoc* colonies

We collected live *Nostoc* colonies from Sea Tomato Lake and Little Sugarloaf Lake, near Kangerlussuaq, Greenland, in July 2014 and 2015 (Figure S1). We used colonies from both lakes in our toxin leakage

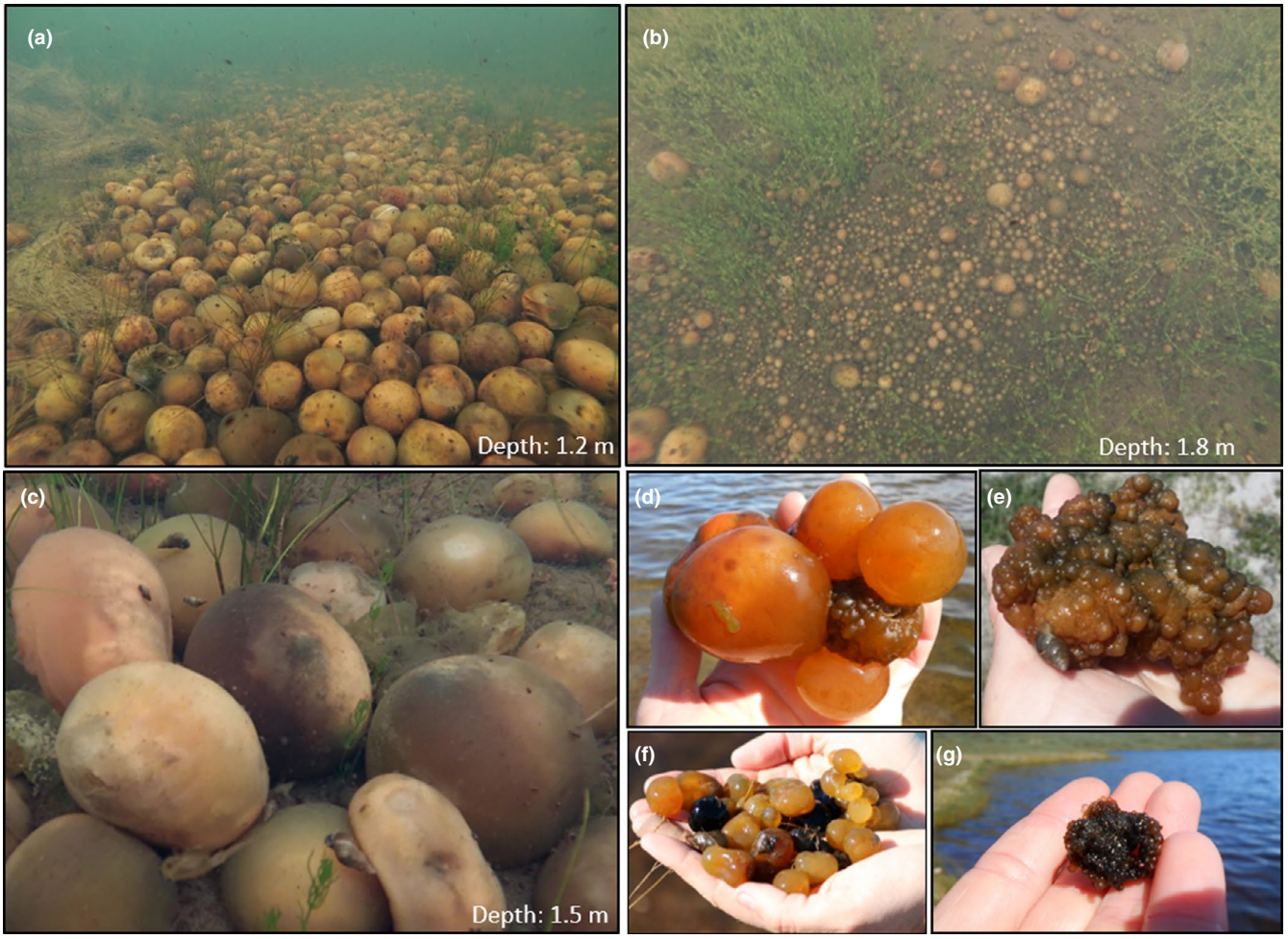

FIGURE 1 Photographs highlighting variation in sizes, colours, and morphologies of *Nostoc* among lakes in Kangerlussuaq. Photographs include: (a) a dense band of large *Nostoc pruniforme*; (b) the edge of a *Nostoc* band containing mixed small and large *N. pruniforme*; (c) snails (*Lymnaea* spp.) grazing on *Nostoc*; and (d–g) a variety of colony morphologies, including (e) *Nostoc zetterstedtii*, which are found in several Kangerlussuaq lakes at considerably lower densities than *N. pruniforme*

experiment, while colonies from only Sea Tomato Lake were used in colony dissections since Sea Tomato Lake contained an extremely high density of colonies and thus we expected minimal effects of collection on the *Nostoc* population. From each lake, we collected live, intact colonies with clearly undamaged outer sheaths (i.e. no holes or tears) from a depth of *c*. 0.25 m at three locations roughly evenly spaced around the lake. We placed colonies directly from the lake into 0.5-L bags and filled the bags with filtered (<50 μm) water from their lake of origin until the colony was submerged. Within 24 hr, we returned to the laboratory at Dartmouth College in Hanover, NH, transferred live colonies to clean 500-ml beakers (keeping them in water from their lake of origin), and placed the beakers in a Percival incubator (Percival Scientific, Inc.) at 17°C and 115 μmol m^{-2} s^{-1} irradiance on a 16:8 hr light:dark cycle until analysis or experimentation 1 week later.

2.2 | Dissection of *Nostoc* colonies

Before dissection, we rinsed each of the 10 *Nostoc* colonies thoroughly with distilled water and patted the colony dry with a clean Kimwipe to remove surface moisture. We photographed the colony, recorded its circumference and mass, and then sliced it into three even cross-sections before dissecting the layers using a combination of scalpels, tweezers, and a small ruler. Each cross-section was divided into four layers: the outer sheath (outer *c*. 1 mm of tissue); two mid-layers (the mid-outer layer extends from 1 mm of the outer sheath to halfway to the core, and the mid-inner layer extends from that halfway point to 1 mm from the core); and the core (extending from the centre of the colony to approximately 0.15 times the length of radius of the cross-section). In very small colonies, it was not possible to obtain an accurate dissection of the mid-layer and only the outer and core layers were collected. We proceeded by removing duplicate samples of 5–12 g tissue from each layer, measuring the (wet) mass of each sample, and placing each subsample into a 2.0 ml centrifuge tube. We then added 0.5 ml distilled water to each tube and froze each sample at −80°C. To prepare for MC extraction, we released cell-bound MC in *Nostoc* tissue by thoroughly macerating thawed samples using glass rods and dissection blades. We then reconstituted the sample with a known volume of distilled water before MC measurement (see Section 2.5).

2.3 | Microcystin leakage from *Nostoc* colonies

To assess whether MCs are released from live *Nostoc* colonies, we incubated live colonies collected in July 2015 in laboratory incubators and measured MC release over a 1-week period. All colonies used in the experiment were large (mean ± 1 *SD* wet mass, 30.4 ± 8.7 g), intact, and never frozen. Colonies with tears, gashes, or otherwise visibly weakened tissue on the outer sheath were not included to avoid possible leakage of toxins from damaged tissue. We weighed colonies after thoroughly rinsing them with distilled water and patting them dry with a clean paper towel until the surface was no longer moist. We then placed colonies into acid-washed glass Mason jars (Ball) filled with 250 ml fresh Modified Bold 3N media (MB-3N, recipe from the UTEX Culture Collection), which was used successfully to culture *Nostoc* colonies in preliminary studies from 2013–2014. This culture medium represents a highly enriched nutrient environment (e.g. 749 mg/L $NaNO_3$, 175 mg/L KH_2PO_4, and 75 mg/L K_2HPO_4 plus additional macro- and micronutrients), relative to water from the source lake (mean ± 1 *SD* total phosphorus and total nitrogen in the source lake were 14 ± 2.5 μg/L and 1,890 ± 35.5 μg/L, respectively, in 2013–2014). Each jar was closed with a custom-made lid with a built-in air tube for bubbling (sealed around the opening with parafilm), and a second tube that was immersed 3–5 cm into the media, extending up through the lid (sealed with parafilm) and attached to a syringe specific for sampling that jar. This setup allowed us to sample the media without opening jars and risking environmental contamination.

The experiment included four density treatments, run in triplicate for a total of 12 jars. The density treatments were as follows: Control = no colonies, Low = one colony, Medium = two colonies, and High = five colonies. We included a mix of colony sizes per treatment to ensure the total *Nostoc* mass was similar among replicates (Table S1). Colonies in the high-density treatment were packed snugly to simulate conditions within a *Nostoc* band, but all colonies were still able to fully rotate and remain submerged during bubbling. Treatments were distributed haphazardly in a Percival incubator (Percival Scientific, Inc.) set at 17°C and 115 μmol m^{-2} s^{-1} irradiance on a 16:8 hr light:dark cycle. We bubbled all jars lightly for 6–8 hr each day to simulate natural turbulence in the lake water (J.V.T.H., personal observation).

We sampled the media from each jar immediately after introducing colonies, and again approximately once each day for 7 days (15–22 October 2015). At each sampling, we lightly stirred the jar to ensure the water was mixed, collected 8 ml of media (3.2% of total volume), transferred it to a clean, pre-labelled plastic scintillation vial, and then used a new syringe to replace the sampled volume with fresh media. Samples were frozen at −20°C until MC extraction (see Section 2.5).

2.4 | In-situ MC measurements inside and outside of *Nostoc* bands

In July 2015, we collected water and sediment samples from three locations within two distinct zones—inside the *Nostoc* band and outside of the band (defined as bare sediment at least 1 m from the edge of the band)— from Sea Tomato Lake and Little Sugarloaf Lake. At each location, we collected three samples: surface water, water just above the benthos, and lake sediment. Samples inside *Nostoc* bands were taken at lake depths between 1 and 1.5 m (roughly 15 m from the shoreline) and samples outside *Nostoc* bands were taken at depths between 2 and 2.5 m (roughly 20 m from the shoreline). Locations were chosen haphazardly along the largest visible *Nostoc* band or the bare lake bottom adjacent to it. A swimmer placed an underwater flag at each site to enable a kayaker to take a surface sample directly above the underwater location; this sample was taken by rinsing an acid-washed 20 ml plastic scintillation vial three times with lake water then skimming the upper 0–2 cm of the surface with the bottle to collect surface water. The swimmer then used a disposable syringe to carefully collect 20 ml of water from (1) inside the *Nostoc* band, with the tip of the syringe drawing water from directly above the top layer of colonies in the *Nostoc* band, and (2) outside the band, with the syringe drawing water from 1 cm above bare sediment (sediments were not disturbed during this process). The syringes were passed up to the kayaker and transferred to acid-washed 20-ml plastic scintillation vials that had also been rinsed three times with surface water. After the three water samples had been collected, the swimmer collected sediment (comprised primarily of silty clay loam) to a depth of 5 cm (i.e. from below the *Nostoc* band or the bare sediment outside a band) by boring a 120-ml plastic specimen container (Therapak, LLC) into the sediment, sealing the opening with a thick plastic square, and handing it to the kayaker to store upside-down until processing.

In the laboratory, we filtered the water samples through a 2-μm isopore membrane filter (TTTP, Millipore Sigma) and froze them at −20°C. We transferred the sediment into an aluminium dish and placed the dish into a drying oven at 60°C for 48 hr. We stored the dried sediment samples in acid-washed 20-ml plastic scintillation vials until toxin analysis. To prepare dry sediment for MC extraction, we weighed and thoroughly macerated the sample using glass rods and dissection blades. We then reconstituted the sample with a known volume of distilled water before MC measurement (see Section 2.5).

2.5 | Microcystin extraction and analysis

To extract MCs from water samples and reconstitute tissue or sediment samples, we used triplicate freeze-thaw cycles (−80°C), interspersed with a sonic water bath incubation (10 min intervals) and 5–10 s vortex, following existing protocols (Banack et al., 2015). We then centrifuged samples at 9,660 ***g*** for 2 min and used the supernatant for analysis. We analysed duplicates of each post-extraction homogenate and included further replication for quality control analyses (Appendix S1).

We detected MC using the high sensitivity protocol for an enzyme-linked immunosorbent assay (ELISA) with a method limit of detection of 0.071 ng/ml (Envirologix, Inc). The ELISA does not distinguish between microcystin variants and as such we use the term MC to refer

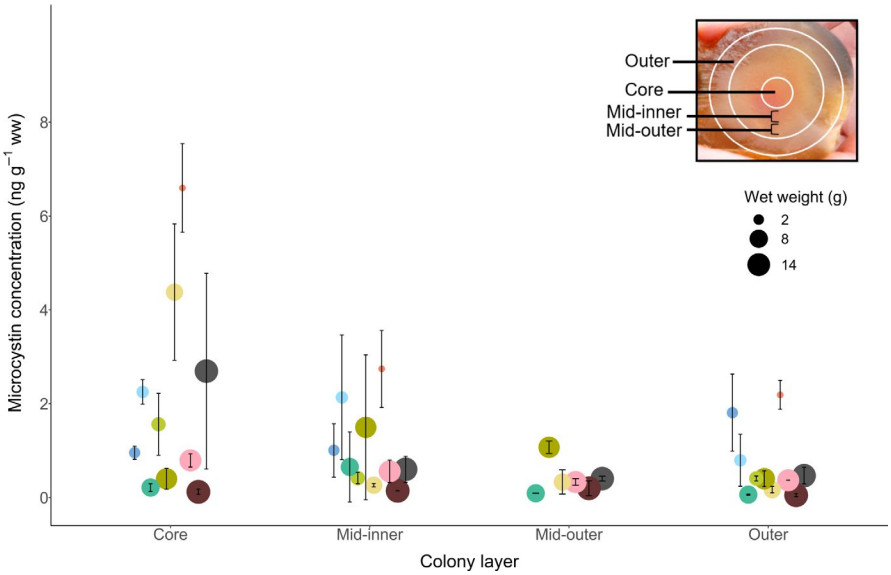

FIGURE 2 Mean microcystin concentrations in four dissected layers of *Nostoc pruniforme* colonies ± 1 *SD* for two replicate subsamples. Point sizes correspond to colony mass (wet weight in grams) and colours correspond to individual colonies. The overlayed image depicts the cross-section of a colony with layers labelled

to four possible microcystin toxin variants (MC-LR, MC-LA, MC-RR, MC-YR) and the structurally similar nodularin toxin (Envirologix ELISA kit EP022-HS). When extracted samples were below the kit detection limit, which occurred in about 25% of our total samples, we transferred a known volume of sample fluid to borosilicate serum bottles, refroze the fluid, and lyophilised in a freeze-dry system (Labconco) under vacuum (*c.* 30 × 10^3 mbar) at −50°C for 18–24 hr to dryness. We then rehydrated samples to achieve a 10-fold concentration and bring them into the range of sensitivity of the ELISA.

2.6 | Statistical analyses

We conducted all analyses using R 3.6.0 (R Core Team, 2019), including the package *lme4* (Bates et al., 2015). For the colony dissections, we fit a general linear model to test for the effect of colony layer (core, mid-outer, mid-inner, outer) on natural log-transformed MC concentrations (log-transformation needed to meet model assumptions of normality), controlling for individual colony as a random effect. We used Tukey's HSD to identify differences. To test for the effect of *Nostoc* density through time on MC concentrations in the leakage experiment, we first square-root transformed MC concentrations to normalise residuals and homogenise variance across treatments, since the presence of many zeros precluded log-transformation. We then fit a general linear model for the fixed effects of treatment, time, and their interaction, controlling for the jar as a random effect. To estimate the mass-specific rates of toxin leakage per hour, we calculated the slope of the regression lines for each density treatment at the time points of interest. We then divided each rate by the average total *Nostoc* mass per jar to estimate the concentration of MC released per gram of *Nostoc* per unit time (ng MC (g of *Nostoc*)$^{-1}$ hr^{-1}). To determine whether there were differences in MC concentrations in the water samples from the two different water depths (surface and benthic water) and two zones (inside vs. outside *Nostoc* bands) at each of the three sites within each lake, we used a general linear model with fixed effects for water depth, zone, and their interaction, controlling for lake and replicate nested within zone (i.e. paired samples of benthic and surface water) as random effects. We analysed sediment separately as a general linear model with a fixed effect for zone, controlling for lake and replicate nested within zone as random effects. Where there were significant effects, we used Tukey's post hoc HSD test to identify the differences.

3 | RESULTS

3.1 | Dissected *Nostoc* colonies

All four layers of *Nostoc* colonies contained MCs (Figure 2). Mean MCs varied significantly by colony layer ($F_{3,59} = 7.6$, $p < 0.001$), with significantly higher concentrations in the core than in the outer sheath (Tukey's HSD; $t_{59} = 4.7$, $p < 0.001$). Mean MCs did not differ significantly between any other colony layers. To test for the influence of the three colonies with the highest core MC concentrations on our results, we reran the ANOVA with those concentrations removed and found that MCs still varied significantly by layer ($F_{3,42} = 2.98$, $p = 0.04$), with the core remaining higher than the outer layer (Tukey's HSD; $t_{42} = 2.8$, $p = 0.04$).

3.2 | MC leakage from *Nostoc* colonies

Incubated *Nostoc* colonies released MCs into the surrounding media throughout the 7-day experiment (Figure 3). Concentrations of MCs were higher in jars with higher colony densities (Figure 3). There was a significant interaction between density and time ($F_{15,41} = 3.1$, $p = 0.0018$) on square-root transformed MC, with high-density colonies producing more MCs relative to medium- and low-density colonies through time. There were also significant main effects of both time ($F_{5,41} = 12.1$, $p < 0.0001$) and colony density ($F_{3,41} = 117.6$,

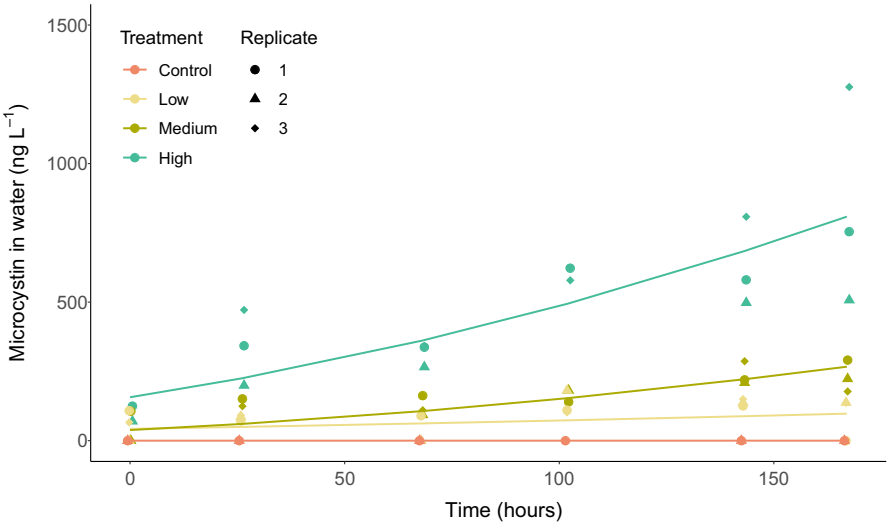

FIGURE 3 Changes in microcystin concentration in water incubated with four densities of *Nostoc* in a laboratory experiment over the course of 1 week. Treatments correspond to *Nostoc* colony density as follows: Control = 0 colonies; Low = 1 colony, Medium = 2 colonies, High = 5 colonies. All treatments were sampled at the same time points and symbols are jittered to show replicates. Regression lines represent back-transformed predicted values from the general linear model predicting square-root transformed microcystin from density, time, and their interaction, with replicate as a random effect

$p < 0.0001$). MCs in the control jars were below the detection limit of the ELISA, even after 10× concentration by freeze-drying, demonstrating no MC contamination.

Based on our modeled rates of MC release for each density treatment (Figure 3), we estimated that after the first 24 hr, colonies at low and high densities would have similar mass-specific MC release rates (0.01 vs. 0.02 ng MC (g of *Nostoc*)$^{-1}$ hr^{-1}). After 7 days, low- and high-density colonies continued to have similar mass-specific MC release rates (0.01 vs. 0.03 ng MC (g of *Nostoc*)$^{-1}$ hr^{-1}, respectively).

3.3 | In-situ MC measurements inside and outside of *Nostoc* bands

There was substantial variability in MC concentrations in water samples from different depths and zones within the two sampled lakes (Figure 4): MC concentrations ranged from 12.1 ± 2.3 ng/L (mean ± 1 *SD*) in benthic water outside the *Nostoc* band to 195.6 ± 252.2 ng/L in benthic water inside the band. Mean MC concentrations depended on the interaction between the presence of a *Nostoc* band (zone) and the water depth (surface vs. benthos; $F_{1,15} = 6.2$, $p = 0.03$). Water from the benthos contained higher mean MCs when it came from within a *Nostoc* band rather than bare sediment (Tukey's HSD: $t_{15} = 2.1$, $p = 0.03$). For the sediment samples, there were no significant differences in mean MCs from inside versus outside *Nostoc* bands ($F_{1,4} = 1.5$, $p = 0.28$). Notably, however, the significant interactive effect of zone (*Nostoc* band) and water depth (surface vs. benthos) on MC concentrations is largely influenced by two samples with the highest MC concentrations, both from benthic water inside *Nostoc* bands (one in Sea Tomato Lake and one in Little Sugarloaf Lake, Figure 4a,c). These two samples also represent the locations with the largest differences in MC concentrations between benthic water and surface water (Sea Tomato Lake: benthic water = 475.1 ng/L higher than surface water; Little Sugarloaf Lake: benthic water = 243.4 ng/L higher than surface water; Figure 4a,c). When we repeated our statistical analyses with the largest outlier removed (benthic water from inside a *Nostoc* band in Sea Tomato Lake, Figure 4a), the interaction between zone and depth was weaker but remained statistically significant ($F_{1,14} = 5.2$, $p = 0.039$). However, when we also removed the second highest sample (benthic water from inside a *Nostoc* band in Little Sugarloaf Lake, Figure 4c), there were no significant effects of zone, depth, or their interaction on MC concentrations. Sediment samples from the two lakes showed no differences in MCs inside and outside of *Nostoc* bands, probably due to the high level of variability among replicates (Figure 2b).

4 | DISCUSSION

This study demonstrates that benthic colonial *Nostoc* produce and have the potential to contribute MCs to Greenlandic lakes and ponds. First, field-collected *Nostoc* colonies contained MCs throughout the entire colony matrix, indicating that damage to any part of the colony (e.g. through outer sheath damage or internal burrowing by aquatic invertebrates) would probably facilitate toxin release. Second, laboratory-incubated, intact *Nostoc* colonies leaked MCs into surrounding water, demonstrating that undamaged colonies are capable of releasing MC at rates associated with their density. Finally, the concentration of MCs in water near the bottom varied with the presence of a *Nostoc* band, suggesting that field variation in MCs due to *Nostoc* is detectable at spatial scales of 1–2 m, at least at some sites and lakes.

Our study presents the first evidence of within-colony variability in tissue-bound MC content and temporal variability in MC release rates among *Nostoc* colonies. Freshly collected colonies of *Nostoc* contained MCs throughout the colony matrix, with highest average concentrations and higher variability in the core. We do not yet know whether this pattern stems from higher production of MCs in the core. Studies on colonies of *Nostoc parmeliodes* (Dodds, 1989) and *Nostoc sphaeroides* (Deng et al., 2008) have found higher filament densities in outer layers compared to inner layers (Deng

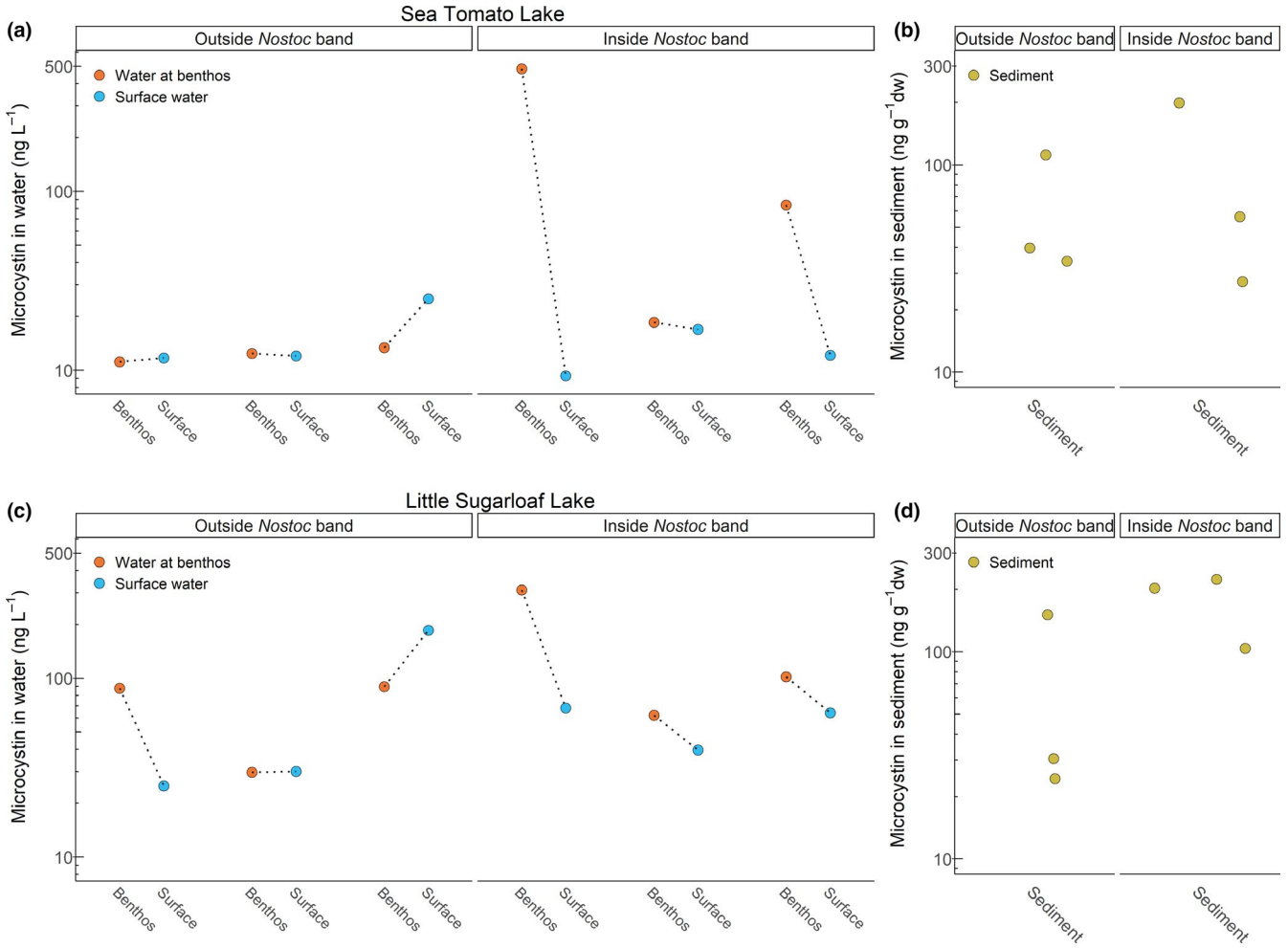

FIGURE 4 Microcystin concentrations in (A, C) water and (B, D) sediment samples from inside and outside dense *Nostoc* bands within two lakes in Kangerlussuaq, Greenland, Sea Tomato Lake (upper panels) and Little Sugarloaf Lake (lower panels). (A, C) Comparison of the two depths at which water was sampled: surface water (blue points) and water at benthos (orange points, water from 1 cm above the *Nostoc* band or the bare sediments) at each of three locations. (B, D) Sediment samples collected to a depth of 5 cm. Each point is a single water or sediment sample. Outside *Nostoc* band refers to areas of benthos with few or no *Nostoc* colonies (1–1.5 m from the edge of a *Nostoc* band, Figure 1a–c). Inside *Nostoc* band refers to areas of the benthos covered in *Nostoc* colonies (Figure 1a–c). Note the logarithmic scale of the *y*-axes

et al., 2008; Dodds, 1989, but see also Kviderova, 2018). Our results could indicate that MCs are produced more readily in the core, or that MCs are leaked more readily from the outer layers, resulting in lower outer layer concentrations. Further, the filament microstructure may vary by colony. For instance, Deng et al. (2008) found that the light intensity under which colonies are grown can influence the filament distribution, with low-light conditions resulting in roughly homogenously distributed filaments and high-light resulting in fewer core filaments with a higher proportion of gelatinous or liquid matrix. Likewise, it is possible that some of the variability we observed in the distribution of MCs reflects variability in the distribution of filaments that have developed as a result of environmental factors such as light availability during growth. In the context of lake cyanotoxins, the presence of MCs throughout the colony matrix indicates that damage to the sheath could liberate toxins into the surrounding water. We frequently observed snails, chironomid larvae and dytiscid larvae both on the surface and inside *Nostoc* colonies (J.V.T.H., personal observation). This suggests that, in addition to releasing toxins through damage, benthic invertebrate grazing may facilitate the transfer of toxins into the aquatic food web through incidental consumption of *Nostoc* tissue or epiphytes (Trout-Haney, 2017).

Even without damage by grazers, our results suggest that *Nostoc* are capable of releasing MCs in laboratory incubators. If *Nostoc* under natural conditions release MCs at rates comparable to our laboratory conditions, the ambient concentrations of MCs available for uptake by aquatic organisms may be higher in the presence of *Nostoc* colonies. Further, the high-density treatments resulted in higher MC concentrations than low and medium densities, suggesting that large *Nostoc* bands might act as hot-spots for MC release into lake water. The significant interaction between density and time indicates that through time, high-density colonies released increasingly more MCs than low and medium densities of *Nostoc*. If this trend continued at a broader temporal scale, it would suggest that *Nostoc* bands that have been stable and

established for longer periods of time have higher MC production relative to newly established bands. However, we do not yet know at what point in time concentrations saturate or decrease, which environmental factors might affect the rates of MC production, or the mechanism by which the MCs measured in our experiment entered the water (e.g. whether they were exported out of the cell or intracellular and lysed during the extraction process).

Whether the release rates measured in the laboratory reflect field conditions is also a matter for consideration. On the one hand, the release rates measured in the laboratory could be underestimates of MC release under natural conditions. Our experiment used only colonies with intact sheaths, whereas colonies growing in natural conditions often undergo damage to the sheath from crowding (e.g. high-density *Nostoc* lakes often have colonies stacked in layers on the benthos), turbulence, and intense seasonal freeze–thaw cycles (Wood, 2015). We expect that damage to the sheath could increase the level of toxin leakage, both as MCs bound to tissue and as unbound, extracellular MCs when tissue is degraded. Alternatively, by providing colonies in the laboratory with regular light, oxygenation, and well-balanced growth medium, it is also possible we created a low-stress environment in which *Nostoc* produced more toxins than in natural ponds in the tundra. While the culture media represent higher-nutrient conditions, the two lakes in the present study are mesotrophic (mean phosphorus and nitrogen ± 1 *SD* in 2013–2014 were 16 ± 2.7 μg/L and 3,115 ± 623.2 μg/L, respectively), with the relatively high levels of nitrogen potentially reflecting the nitrogen-fixation ability of *Nostoc* colonies. Although the complex combination of environmental factors affecting cyanotoxin production has not been fully resolved, light, temperature, nutrients (Banerji et al., 2019; Chaffin et al., 2018,2019; Cirés & Ballot, 2016; Dai et al., 2016; Jiang & Zheng, 2018; Peng et al., 2017), and oxidative stress (Chen et al., 2016; Kurmayer, 2011; Zilliges et al., 2011) appear to be important drivers of MC production in other taxa. To fully reconcile laboratory and field estimates of MC release, further experimentation is required over longer periods of time, particularly given the slow growth and long life spans typical of *N. pruniforme* (Dodds & Castenholz, 1988; Møller et al., 2014).

The in-situ measurements of water inside and outside of *Nostoc* bands provide an example of within-lake spatial MC variability. The higher MC levels in water above *Nostoc* bands are consistent with our laboratory incubation experiment, in which more densely packed *Nostoc* released MCs at higher rates than low-density or solitary colonies. However, while we removed a large fraction of potential toxin-producers from the water samples (>2 μm), we cannot rule out potential cyanotoxin contributions from picocyanobacteria. We carefully inspected sediment samples to ensure there were no visible *Nostoc* colonies; however, if there were *Nostoc* microcolonies (<0.2 μm, Deng et al. 2008) present, they could have contributed to the sediment MC concentrations. Additionally, given the high degree of variability in water and sediment MC concentrations across the scale of a few metres, these in-situ results suggest that larger sample sizes are necessary for future studies to confidently identify patterns in the distribution of MCs within a single lake. Further, while it is possible that additional sampling would reveal a significant effect of *Nostoc* bands on lake MCs, it is also likely that processes such as wind-driven lake mixing, settling of dead plankton, and movement of pelagic and benthic invertebrates are capable of homogenising MCs within the lake water and sediments (Cirés et al., 2013; Song et al., 2015; Verspagen et al., 2005). Given that the density of zooplankton aggregations has been identified as an important factor in the degree of lake biomixing (Simoncelli et al., 2017), it is likely that the dense populations of large zooplankton in these lakes, due to an absence of fish, have at least a small-scale effect on lake mixing, potentially further enhanced by the long daylight hours during which these organisms can be active in the Arctic summer.

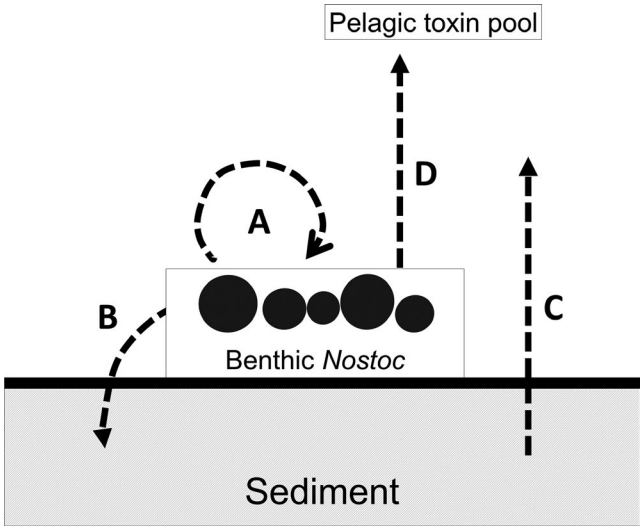

FIGURE 5 Schematic illustrating multiple routes through which benthic *Nostoc* colonies may contribute microcystins (MCs) into lake water. Once released from colonies, cyanotoxins could (A) remain close to the benthos (Cantoral Uriza et al., 2017; Chen & Xie, 2005; Jonasson et al., 2010; Ozawa et al., 2003), (B) settle into the sediment (C) become resuspended in the water column following disturbance to the sediment (Bolotaolo et al., 2020; Song et al., 2015; Verspagen et al., 2005), and/or (D) enter the pelagic toxin pool (Ferrão-Filho, Herrera, & Echeverri, 2014; Ibelings & Chorus, 2007; Kotak et al., 1996; Smith & Haney, 2006; Zamora-Barrios et al., 2019) as either tissue-bound MCs or extracellular MCs when tissue is degraded

Based on these results, we propose a conceptual model of potential pathways through which *Nostoc* could contribute MCs to the aquatic environment (Figure 5). For example, toxins produced by a *Nostoc* colony could: (1) remain close to the benthos, primarily available to benthic organisms; (2) settle into the sediment and either become locked away or resuspended (3) back into the water column following disturbance; and/or (4) enter the pelagic toxin pool where they can be ingested by pelagic organisms, sink back to the benthos, or be aerosolised from the water surface (Banack et al., 2015).

The degree to which benthic cyanotoxins are transferred through the food web may depend on whether whole cells or lysed cell contents are released, and whether toxins move into the water column or are trapped in the sediment. Additionally, while the MC concentrations in these Greenlandic lakes were all below the

threshold for safe human consumption set by the World Health Organisation (1,000 ng/L), it remains unknown at what threshold MC level aquatic invertebrates begin experiencing effects. Our previous work has demonstrated that even at these low MC concentrations, aquatic invertebrates from multiple functional groups contain MCs in their body tissues, indicating that the food web may be an important conduit for MC transfer to higher trophic levels.

Our understanding of the role that benthic communities play in aquatic ecosystems is continually advancing as we recognise the importance of benthic organisms in processes such as primary production (Althouse et al., 2014; Vadeboncoeur et al., 2014; Vadeboncoeur & Power, 2017) and energy transfer to food webs (Marcus & Boero, 1998; Mariash et al., 2014; Schindler & Scheuerell, 2002; Vadeboncoeur et al., 2002). This may be particularly true in polar environments, where abiotic conditions such as cold temperatures and short growing seasons limit pelagic primary producers and promote the productivity of benthic communities that take advantage of nutrient-rich sediments (Quesada et al., 2008; Rautio & Vincent, 2006; Vadeboncoeur et al., 2008). Arctic benthic communities can provide critical subsidies for the rest of the food web (Mariash et al., 2014; Siehoff et al., 2009)—although cyanotoxins may represent the *dark* side of subsidies (Walters et al., 2008). While we are still developing an understanding of the relative contributions of benthic and pelagic communities to toxin production in these lakes, we now know that *Nostoc* colonies produce MCs throughout their tissues, colonies are capable of leaking MCs at rates that increase with colony density, and that variation in MCs due to a single species of *Nostoc* may be detectable at a spatial scale of 1–2 m.

Our results reinforce a growing body of literature documenting a wide range of *Nostoc spp*. capable of producing MCs (Bajpai et al., 2009; Beattie et al., 1998; Kust et al., 2018; Sivonen et al., 1990,1992; Teneva et al., 2012). Taken together, these results suggest that benthic cyanobacterial species may be important in the production and release of MCs into aquatic ecosystems, particularly in polar ecosystems where benthic cyanobacteria often dominate primary productivity. Future studies are needed to determine the extent to which benthic communities play a role in the production and bioavailability of cyanotoxins in water bodies across a broader range of ecosystems.

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DATA AVAILABILITY STATEMENT

Data supporting the findings of this study are available through Dryad Digital Repository: https://doi.org/10.5061/dryad.p5hqbzkn1.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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