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Widespread occurrence of filamentous *Thioploca* bacteria in low-sulfate Great Lakes sediments with implications for sulfur and nitrogen cycling

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ABSTRACT

Thioploca spp. are large sulfur oxidizing bacteria that form centimeter-scale filaments in aquatic sediments. Marine *Thioploca* can form dense mats that strongly affect biogeochemical cycling in sediments. While freshwater and brackish *Thioploca* can also achieve high abundances, their ecology is poorly understood and their metabolisms in low-sulfate environments are enigmatic. We quantified freshwater *Thioploca* biomass and its links to environmental characteristics at 33 sites in the Great Lakes in the Apostle Islands region (Lake Superior) and Green Bay (Lake Michigan). Vertical distributions of *Thioploca* and the associated sediment chemistries were analyzed in sediment cores from eight sites. Despite low sulfur availability, *Thioploca* attained biomasses comparable to those in marine sediments, up to 250 g m^{-2} wet weight. Abundances were highest in the top 5 cm of sediment and were greater in fine-grained than coarse-grained sediments. *Thioploca* was common at both eutrophic and oligotrophic locations, suggesting that sediment carbon availability was not a limiting factor. Porewater profiles indicated possible ammonia oxidation in the anoxic sediment layers occupied by *Thioploca*, which would argue against the dissimilatory nitrate reduction to ammonium (DNRA) route of nitrate reduction common in marine *Thioploca*. With known metabolic capabilities for coupling oxidation of sulfide or thiosulfate to denitrification, freshwater *Thioploca* may play a quantitatively important role in the benthic cycling of nitrogen and sulfur, though in ways that differ from those of marine *Thioploca* species. Our results indicate that *Thioploca* may be widespread in depositional zones of the Great Lakes, with yet underappreciated effects on local elemental cycling.

Introduction

Large sulfur oxidizing bacteria of the family *Beggiatoaceae* (Gammaproteobacteria) are visually conspicuous sediment bacteria that can play an important role in biogeochemical cycling of aquatic systems (Teske and Salman, 2014; Jørgensen, 2021). The bacteria owe their size to a large accumulation of nitrate, frequently stored in vacuoles, which may be used as an electron acceptor for the oxidation of sulfide to sulfate, with elemental sulfur (S^0) generated as an intermediate storage product (reviewed in Schulz and Jørgensen, 2001). Many members of the family are chemolithotrophic, coupling sulfide oxidation with reduction of oxygen or nitrate, and some freshwater members are mixotrophic or heterotrophic, additionally capable of utilizing organic carbon substrates (Teske and Salman, 2014). Members of this group include thioplocas (herein used to describe the genus *Thioploca sensu stricto* and *Ca. Marithioploca*; Salman et al., 2013), which are widely distributed in surficial sediments of marine and freshwater systems (Teske and Salman 2014). Thioplocas are distinguished by their filamentous morphology with multiple trichomes bundled together in a common mucilaginous sheath, 0.5–15 cm in length, which functions as a tunnel through which the filaments migrate with gliding motility (Maier, 1984; Jørgensen and Gallardo, 1999). Thioplocas use motility to adjust their position in sediments relative to their substrate requirements, gaining a competitive advantage where electron donors (e.g., sulfide) and electron acceptors (e.g., nitrate) are spatially separated by centimeter-scale distances (Huettel et al., 1996, Jørgensen and Nelson, 2004, Teske and Salman, 2014).

Among the best studied thioplocas are a marine group recently re-classified to the candidate genus *Marithioploca* (Salman et al., 2013). Mixed assemblages of *Ca. Marithioploca* spp. form expansive microbial mats covering an estimated 10,000 km^2 along the Pacific continental shelf of Chile and Peru below the eastern south tropical oxygen minimum zone (Gallardo, 1977; Schulz et al., 1996).

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Marine thioplocas have also been observed in other oxygen restricted zones or basins (Schmaljohann et al., 2001; Prokopenko et al., 2011). The *Ca. Marithioploca* mats can attain wet weights of up to 1,000 g m^{-2} and have a significant influence on biogeochemical dynamics in marine sediments (Schulz et al., 1996). These thioplocas couple the oxidation of sulfide to dissimilatory nitrate reduction to ammonia (DNRA; Høgslund et al., 2009). As a consequence, sediments occupied by abundant *Ca. Marithioploca* are major centers of reactive N recycling rather than nitrogen loss by denitrification (Thamdrup and Canfield, 1996; Otte et al., 1999; Teske and Salman, 2014). However, the relationship between the presence of marine thioplocas and sediment nitrogen cycling is complex, with net nitrogen loss by anammox occurring in some marine regions due to close interactions between thioplocas and anammox bacteria (Prokopenko et al., 2011, 2013), and with DNRA and denitrification co-occurring in mixed assemblages of thioplocas and *Beggiatoa* spp. in a hydrothermal vent region (Schutte et al., 2018). In marine sediments, *Ca. Marithioploca* also contribute significantly to sulfide oxidation, enabling production of sulfate in deeper sediment layers (i.e., above concentrations observed in the water column; Suits and Arthur, 2000), and significantly decreasing sulfide flux into the water column (Dale et al., 2016; Sommer et al., 2016).

Although marine thioplocas have received much attention, the earliest descriptions of thioplocas come from freshwater sediments (Lake Constance, Germany, Lauterborn, 1907; and the Neva River, Russia, Wislouch, 1912). There are two recognized *Thioploca* species reported from fresh and brackish waters (*T. ingrica* and *T. schmidlei*), currently differentiated only by morphology (Maier, 1984). Of these, *T. ingrica* is more commonly occurring and the only freshwater *Thioploca* that has so far been characterized molecularly (Teske and Salman, 2014). *Thioploca ingrica*, which is closely related to both *Ca. Marithioploca* spp. and the freshwater species *Beggiatoa alba* (Kojima et al., 2015), occurs in brackish to freshwater sediments (Høgslund et al., 2010). Smaller than marine thioplocas, freshwater *Thioploca* spp. still have sizable trichomes (2.0–9.0 μm diameter; Maier 1984) and form visually conspicuous sheathed bundles with bundle diameters reported between 30 and 126 μm (Dermott and Legner, 2002). Although large vacuoles are not visible, *T. ingrica* may store nitrate intracellularly at high concentrations (Høgslund et al., 2010). Recent genomic and proteomic evidence showed that *T. ingrica* possesses the genetic repertoire to perform complete denitrification (i.e., conversion of NO_3^- to N_2 gas), but not DNRA (Kojima et al., 2015). The metabolic activity of freshwater *Thioploca* in lake sediments may therefore have different effects on N cycling than thioplocas in marine settings. *Thioploca ingrica* also has the full genetic repertoire for sulfide oxidation characteristic of the family *Beggiatoaceae* (Kojima et al., 2015) and has been observed to store S^0 internally in freshwater lacustrine systems (Maier, 1984; Dermott and Legner, 2002), demonstrating involvement in sulfur cycling in these environments. Yet, inorganic sulfur concentrations are typically 3 orders of magnitude lower in freshwaters than in modern marine systems, and sulfide produced in lacustrine sediments may be rapidly scavenged by iron (Fakhraee et al., 2017). How *Thioploca* makes a living in freshwater systems with such a low supply of sulfide is therefore perplexing and remains an open question (Kojima et al. 2007; Zemskaya et al. 2009; Nemoto et al., 2011; Fakhraee, 2018). Investigating their biogeochemical strategies and their evolutionary links to marine thioplocas may ultimately provide insights not only into their functioning in modern lacustrine systems but also into the pathways of microbial evolution in other environments, including Earth's early oceans, which had much lower sulfate concentrations for most of their geologic history (Fakhraee et al., 2019).

In marine settings, the distribution patterns and biogeochemical role of thioplocas suggest that their niche is sediments that are rich in organic matter. Such sediments have low oxygen concentrations, high rates of sulfate reduction and correspondingly high upward fluxes of sulfide. (Jørgensen and Gallardo, 1999; Teske and Salman, 2014). Combined with nitrate presence, such conditions are favorable for sulfide oxidizers, which have access to both catabolic energy and carbon substrates. By contrast, the niche for *Thioploca* in freshwater habitats is far less constrained. In freshwater Lake Biwa (Japan), for example, densities of *T. ingrica* were observed to increase in tandem with increasing lake eutrophication, hypolimnetic oxygen depletion, and potentially with sediment sulfate reduction rates (Nishino et al., 1998), suggesting an ecological niche similar to marine thioplocas in the oxygen minimum zones. However, *T. ingrica* has also been found in oligotrophic systems, like Lake Baikal (Zemskaya et al., 2009) and in both oligotrophic and eutrophic regions of the Laurentian Great Lakes (Dermott et al., 2005). The drivers of freshwater *Thioploca* abundance and their biogeochemical roles thus remain poorly understood.

To address uncertainties about the distribution, abundance, and biogeochemical function of freshwater *Thioploca*, we investigated sediments spanning a wide range of environmental conditions at 33 sites in the Apostle Islands area of Lake Superior and in Green Bay, Lake Michigan. We address the following questions: (1) How abundant is *Thioploca* in the Apostle Islands and Green Bay? (2) What drives *Thioploca* distribution in these regions? (3) How do *Thioploca* interact with sediment chemistry, especially with regard to N and S cycling? We hypothesized that *Thioploca* biomass would be positively correlated with site trophic status and sediment organic matter content. Current evidence indicates *T. ingrica* is capable of coupling sulfur oxidation with both aerobic respiration and with nitrate reduction to dinitrogen gas (Høgslund et al., 2010, Kojima et al., 2015). We examined the porewater distribution of ammonia to assess if there was evidence for quantitative impacts of *T. ingrica* on nitrogen cycling in these sediments. Finally, we examined *Thioploca* cells with microscopy to assess evidence of sulfur accumulation.

Methods

Study sites

Twenty sites in the Apostle Islands region (Lake Superior) and 13 sites in Green Bay (Lake Michigan) were sampled between June 17 and June 28, 2019 aboard the *R/V Kingfisher*. Sites were chosen to span a range of depths – from 9 to 85 m. Sites also spanned a gradient of trophic status – from highly eutrophic sites in southern Green Bay to oligotrophic sites in the Apostle Islands (Fig. 1, Electronic Supplementary Material (ESM) Table S1). At each site, discrete water column samples were collected approximately 0.5 m below the surface and frozen for total phosphorus (TP) analysis. Secchi depth was measured as an indicator of water clarity, and a CTD (EXO2 Multiparameter Sonde, YSI, Yellow Springs, OH) profile was taken to measure temperature, oxygen, and chlorophyll fluorescence throughout the water column.

Ponar sampling

At each site, a petite ponar sampler (sampling area 0.0231 m^2) was used to collect triplicate sediment samples for *Thioploca* biomass quantification, surface sediment elemental analysis for organic carbon, nitrogen, and sulfur, and visual assessment of sediment coarseness. The sediment texture in the ponar samples was

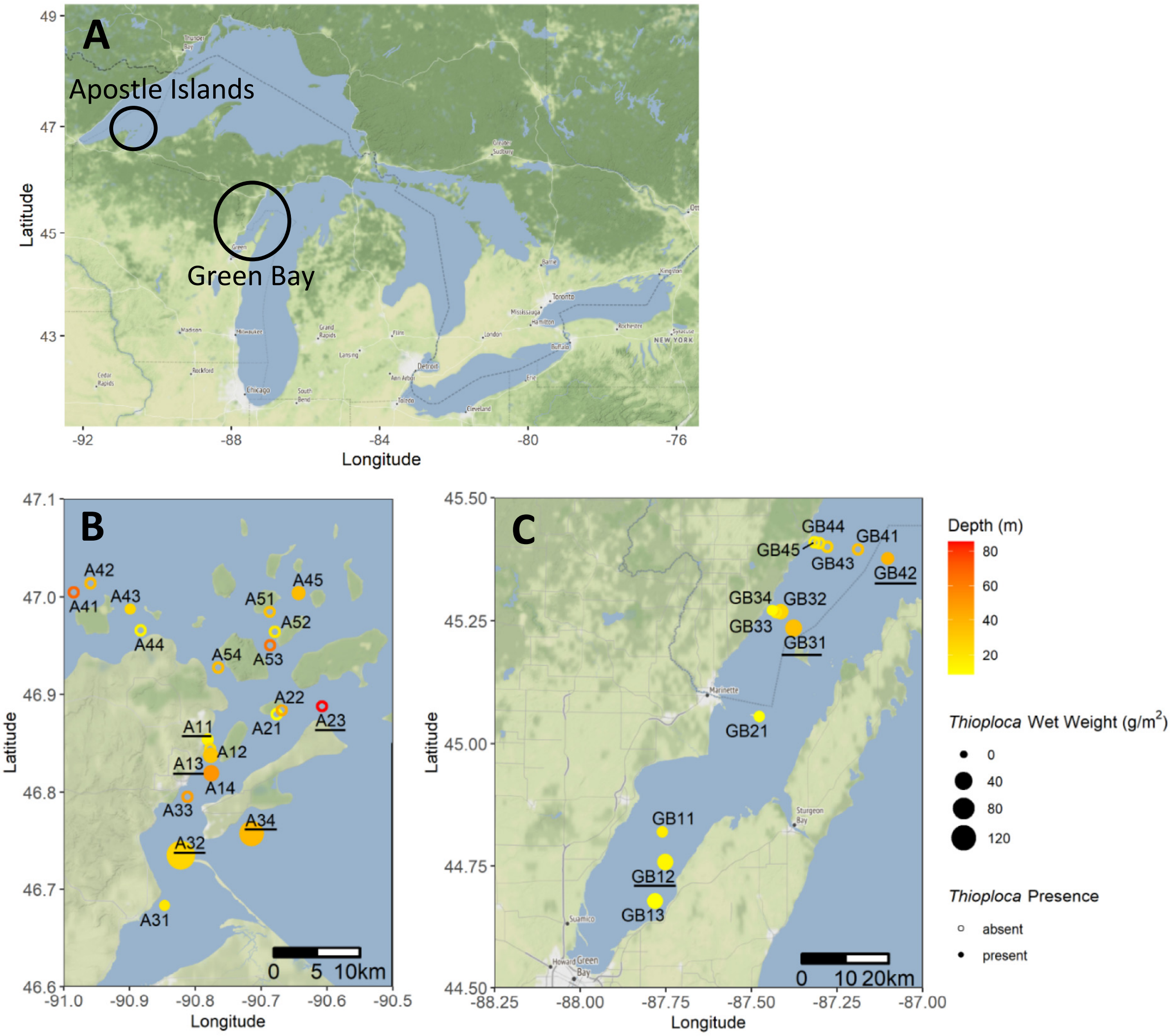

Fig. 1. (A) The circles represent a zoomed-out view of the areas in panels B and C. (B and C) Water column depth (m), *Thioploca* presence and wet weight (g/m^{-2}) in the Apostle Islands (B) and Green Bay (C) during June 2019, based on ponar grab samples. Sediment cores were collected at underlined sites. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

examined visually and broadly categorized as predominantly fine-grained organic-rich muds or coarse-grained mineral sands. A small sample (1–2 cm^3) for sediment CNS analysis was also collected from each site by carefully scooping off a spoonful of sediment from the top centimeter of one ponar sample and freezing the sample in a Whirl-Pak bag until analysis. Sediment samples were rinsed through a 600 µm sieve, and retained material was preserved in buffered 7% formalin for quantification of *Thioploca* biomass.

Sediment core collection and processing

At a subset of sites (5 in the Apostle Islands, 3 in Green Bay; Fig. 1), triplicate sediment cores with intact sediment–water interfaces were collected with a gravity corer (80 cm long, 7 cm diameter polycarbonate core liners). Sediment cores were capped and stored upright in core liners in a dark cooler with lake water until their return to the lab for *Thioploca* biovolume quantification and sediment and porewater chemical analyses. Sediment cores were processed within 12 h of collection.

In the lab, one core from each site was extruded and sliced at 0.5 to 1 cm intervals down to 10–20 cm for *Thioploca* biovolume quantification. The sediment slices were rinsed through a 600 µm sieve and retained material was preserved in buffered 7% formalin. A second replicate core from each site was extruded and sliced at 0.5 to 1 cm intervals in a glove bag under a N_2 atmosphere for extraction of porewater for ammonia and sediment CNS analysis. Porewater was extracted by centrifuging the samples at 4000 rpm for 10 min in 50 mL centrifuge tubes. The supernatant was decanted, filtered through a 0.22-µm membrane syringe filter, and frozen at −20 °C. The remaining sediment was also frozen (−20 °C) for sediment CNS analysis. The final replicate core from each site was used for estimating dissolved oxygen flux by microsensor depth profiling. Oxygen concentrations were measured at 1 to 5 mm increments using a Unisense OX-N O_2 microsensor (1.1 mm sensor tip diameter; Unisense A/S, Aarhus, Denmark) mounted on a micromanipulator. The oxygen sensor was calibrated using a 2-point calibration with 100% saturation measured in air saturated water and 0% saturation measured in N_2 saturated water.

Water column nutrient analysis

Total phosphorus (TP), as a proxy for productivity, was measured in epilimnetic water samples following persulfate digestion

(APHA method 4500-P, APHA, 2012b). Samples (triplicate analytical replicates) were digested with a potassium persulfate solution and analyzed with a nutrient autoanalyser (AQ400 Discrete Analyzer; Seal Analytical, Mequon, WI; MDL = 0.003 mg P/L). Calibration was performed using standard stock phosphorus solution with a 10 point calibration curve. Depth profiles of chlorophyll *a* concentration were estimated fluorometrically in-situ with a EXO2 Multiparameter Sonde (YSI, Yellow Springs, Ohio) and values were averaged over the epilimnion (ESM Table S1).

Ponar sample processing

Thioploca wet weight was determined in all ponar samples using the method described in detail in McKay (2021). Briefly, formalin-preserved material that was retained following sieving through a 600-µm mesh was rinsed with de-ionized (DI) water. *Thioploca* filaments were then sorted from debris and other material using fine forceps under a dissecting microscope at 30–70× magnification. The wet weight of the cleaned *Thioploca* tufts was measured after dabbing off excess water on Kimwipes and weighing tufts to the nearest 0.001 g.

Sediment core processing

Vertical distribution of *Thioploca* biovolume was determined in the sediment core slices by taking photomicrographs of preserved *Thioploca* at 75× magnification under a dissecting microscope using the method described in detail in McKay (2021). Briefly, the formalin-preserved material from each core slice was rinsed with DI water and transferred to a Sedgewick Rafter counting slide. Each sample was carefully scanned through under a dissecting microscope, and a photomicrograph was taken of any view that included *Thioploca*. The length of the *Thioploca* bundles in each image was traced using ImageJ software (Schneider et al., 2012), measuring the length of the traced lines.

In addition to length (l, mm), average bundle diameter (d, mm), for each core slice was determined by measuring 20 random bundles from each core slice at 250× magnification in order to calculate *Thioploca* bundle biovolume. *Thioploca* biovolume (V_{bio}, mm^3 *Thioploca* cm^{-3} sediment) was calculated assuming the bundles to be cylindrical: $V_{bio} = \pi\left(\frac{d}{2}\right)^2 l$. The volume of sediment was simply the volume of the core slice.

Sediment porewater analysis

Ammonia in sediment porewater samples was measured using a benchtop phenate method (APHA, 2012a). Porewater aliquots (500 µL) were mixed with a phenol solution, a sodium nitroprusside solution, and an oxidizing solution to produce indophenol upon reacting with the ammonia in the porewater. Absorbance at 640 nm was measured in a spectrophotometer (Genesys 30 Visible Spectrophotometer, Waltham, MA). Standard solutions were also prepared using the same method with a stock ammonium chloride solution. Duplicates were included every ten samples. We submitted porewater samples for nitrate analysis by ion chromatography at an external lab, but all results were below detection, likely due to analytical error.

Sediment CNS content analysis

Bulk sediment from cores was freeze dried for 72 h and homogenized with a mortar and pestle. Homogenized sediment (25–60 mg) was weighed with a microbalance into silver capsules (10 × 10 mm Elemental Microanalysis silver capsules) with approximately 50 µL of MilliQ water, and the samples were placed in a fumigator with a beaker of 12 M HCl for 6 to 8 h to remove inorganic C. The samples were then dried completely on a hot plate at approximately 50 °C for 6 to 8 h. The silver capsules were carefully folded shut with blunt forceps and then encased in tin capsules (9 × 10 mm Costech tin capsules) and again folded shut with blunt forceps. Standards were prepared from Costech BBOT and Leco Proximate + Coal Reference Material Part No. 502-442. The standards did not require fumigation and were measured directly into tin capsules (5 × 9 mm Costech tin capsules). The sediment samples were analyzed for organic C, N, and S (as % element by weight) with an Elementar Vario EL Cube CHNS elemental analyzer (Ronkonkoma, NY). Duplicates were included every ten samples.

Data analysis and statistical methods

All statistical analyses were conducted using R (version 3.6.3) and were based on site-averages for each variable. To explore a potential relationship between *Thioploca* occurrence and environmental parameters, a principal component analysis (PCA) was performed which included surface water TP, Secchi depth, and surface sediment percent organic C, N, and S. The PCA was based on a correlation matrix to standardize the data because the predictor variable units are not comparable. A logistic regression was performed to explore the probability of *Thioploca* presence/absence at different values of PCA component 1 using the *glm* function in R with a binomial distribution. A Welch's *t*-test was performed to test whether average *Thioploca* wet weight differed between coarse- and fine-grained sediments.

Diffusive oxygen uptake and ammonia flux were calculated from concentration depth profiles using Fick's first law of diffusion, as previously described (Malkin et al., 2017) using the oxygen and ammonia concentration depth profiles from the sediment cores. Oxygen and ammonia diffusion coefficients were obtained from the *marelac* package in R (version 2.1.10; Soetaert et al., 2012).

Results

Thioploca abundance and environmental conditions

The 33 sites sampled in the Apostle Islands and Green Bay spanned a range of trophic status. Surface water total phosphorus ranged from below detection at many sites in the Apostle Islands to 16.01 µg/L in southern Green Bay, surface water chlorophyll *a* ranged from 0.40 to 8.13 µg/L, and Secchi depth ranged from 1.9 to 10.4 m (ESM Fig. S1). Surface sediment organic carbon, nitrogen, and sulfur content also spanned one to two orders of magnitude with values of 0.08–8.48% C, 0.06–1.19% N, and 0.01–0.23% S. Hypolimnetic conditions at all sites were oxic, with near-bottom water oxygen levels ranging 77–100% air saturation, with the lowest values occurring in southern Green Bay.

Thioploca filaments, tentatively ascribed to *T. ingrica* based on morphological characteristics (Maier, 1984), were observed at 8 of the 20 sites in the Apostle Islands (Superior) and at 8 of the 13 sites in Green Bay (Michigan). Trichomes were of uniform diameter along their observed length, with diameters of 4.6 ± 0.1 µm (mean ± standard deviation; n = 10 filaments). Bundles of trichomes shared a common sheath and were observed to have independent gliding motility (Fig. 2A). Refractive globules identified as sulfur inclusions were observed in all filaments (Fig. 2B). The terminal cells were tapered, which is typical, though not strictly diagnostic of *T. ingrica* (Maier, 1984). At the sites where it was present, average *Thioploca* wet weight ranged from 0.79 to 157 g m^{-2} *Thioploca* in sediment in the Apostle Islands and from 1.2 to 28 g m^{-2} *Thioploca* in sediment in Green Bay (Fig. 1). Within sites, *Thioploca*

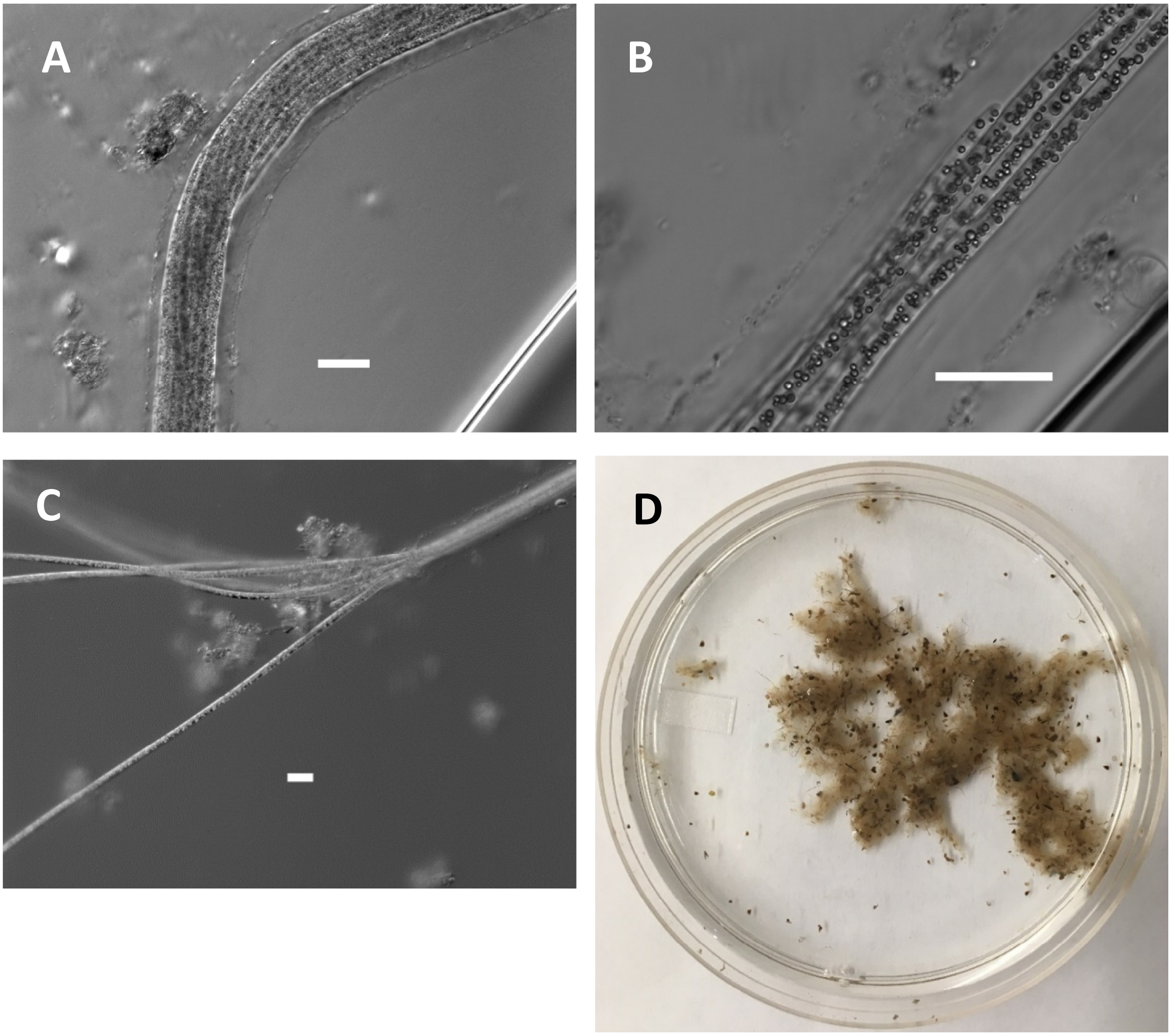

Fig. 2. Photomicrographs of *Thioploca* from the Apostle Islands region of Lake Superior. All scale bars are 20 µm. (A) 40× magnification, multiple braided trichomes visible. (B) 100× magnification, visible sulfur globules. (C) 20× magnification, trichomes emerging from sheath. (D) Unmagnified tufts of *Thioploca* in a petri plate of 10 cm diameter.

density was highly variable among replicate ponar samples (average coefficient of variation across sites = 70%), with the highest variability occurring at the highest density sites (ESM Table S1).

Principal component analysis on site water quality and sediment parameters (Secchi depth, surface water total phosphorus, and sediment organic carbon, nitrogen, and sulfur) efficiently summarized site environmental characteristics with 65.6% and 18.9% of the variation explained by components 1 and 2, respectively (Fig. 3). Lower values of PCA components 1 and 2 correspond to higher TP, higher sediment CNS, and lower Secchi depths, all indicators of more eutrophic water column conditions. The PCA showed some evidence of separation between sites with and without *Thioploca* along a eutrophication gradient, with more of the *Thioploca*-present sites falling at lower values of PCA components 1 and 2, and more of the *Thioploca*-absent sites occurring at higher values of PCA components 1 and 2. A logistic regression on *Thioploca* presence and PCA component 1 showed a borderline significant relationship, with *Thioploca* more likely to be present under more eutrophic conditions (Fig. 3; Predicted logit of *Thioploca* presence = −0.41 * PC1 − 0.90, odds ratio = 0.66, z = −1.92, p = 0.056). However, the pattern was not very strong.

Thioploca was present at 12 of the 13 sites that had fine-grained sediment and at only 4 of the 20 sites that had coarse-grained sediment. The mean *Thioploca* wet weight was significantly higher in fine-grained sediment (Welch's *t*-test: t = −2.33, df = 12.04, p = 0.038). *Thioploca* wet weight was 30.5 ± 46.0 g m^{-2} among sites with fine-grained sediment (mean ± standard deviation) and 0.7 ± 2.2 among sites with coarse-grained sediment (Fig. 4).

Thioploca vertical distribution and sediment chemistry

Thioploca was present in 7 of the 8 sediment cores that were analyzed for *Thioploca* biovolume and porewater and sediment chemistry, and was found primarily in micro-oxic and anoxic sediment layers. *Thioploca* was most abundant in the top 5 cm of sediment in all the cores, except at the GB12 site (the most eutrophic site cored) where the biovolume was similar between 1.5 and 9.5 cm deep in the sediment (Fig. 5). In sediments from the Apostle Islands, *Thioploca* was detectable to maximum depths of 2–3 cm, while in sediments from Green Bay, *Thioploca* was found to maximum depths of 5–10 cm. In sediment cores with *Thioploca*, oxygen penetrated to 11 ± 4.9 mm (mean ± SD) depth in the sediment. The

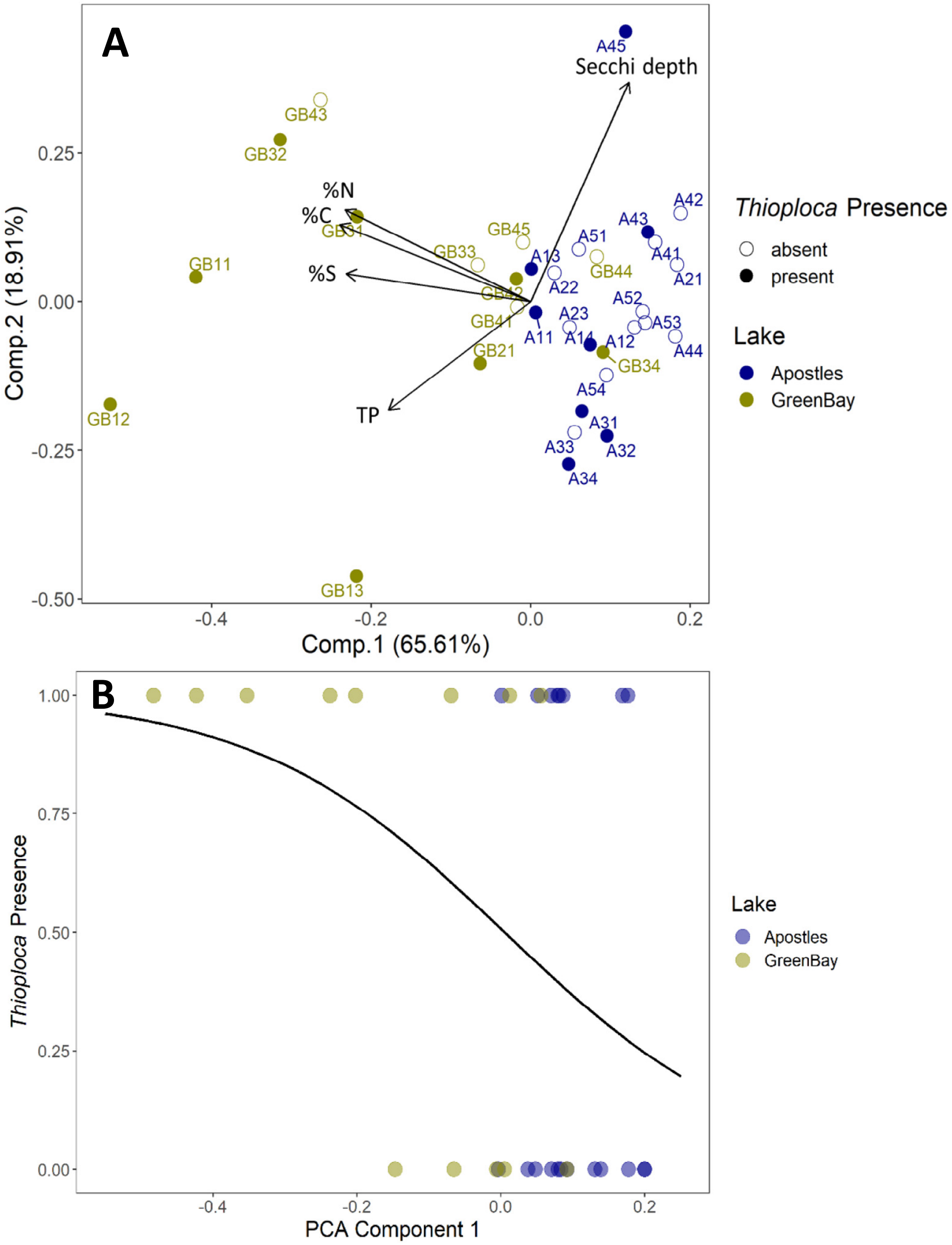

Fig. 3. (A) Principal component analysis plot showing variability in sampling site environmental parameters including Secchi depth, surface water total phosphorus (TP) concentrations, and surface sediment organic carbon, nitrogen, and sulfur (%). Sites with detectable *Thioploca* present are indicated by closed circles, and sites without *Thioploca* are indicated by open circles. (B) Logistic regression of the probability of (detectable) *Thioploca* presence at different values of PCA Component 1.

diffusive oxygen uptake rates (DOU) were relatively similar across the sites (3.82 ± 1.73 mmol m^{-2} day^{-1} (mean ± SD); Table 1). Some of the sediment cores, most notably the GB12 core, showed a distinct absence of porewater ammonia through the depths where *Thioploca* was abundant. The core with the highest *Thioploca* biovolume (A32) also had particularly high porewater ammonia concentrations below the layer of *Thioploca*.

There was no clear correlation between *Thioploca* biovolume and percent organic C, N, or S in the sediment cores (Fig. 6). The two cores with the highest *Thioploca* biovolume spanned the range of some of the highest and lowest values for sediment organic C, N, and S in our samples, with concentrations at site GB12 falling in the top quartile, and concentrations at site A32 falling in the bottom quartile for N and S and the second quartile for C. The GB12 and GB42 cores exhibited an increase in S below the zone occupied by *Thioploca*.

Discussion

Our results document that *Thioploca* was widespread and often surprisingly abundant across the sampled freshwater sediments. The observed range of *Thioploca* biomass in individual ponar samples (0.5–250 g m^{-2} wet weight) is similar to that recorded in other parts of the Great Lakes (Dermott and Legner, 2002; Dermott et al., 2005) and approaches the densities observed in marine systems

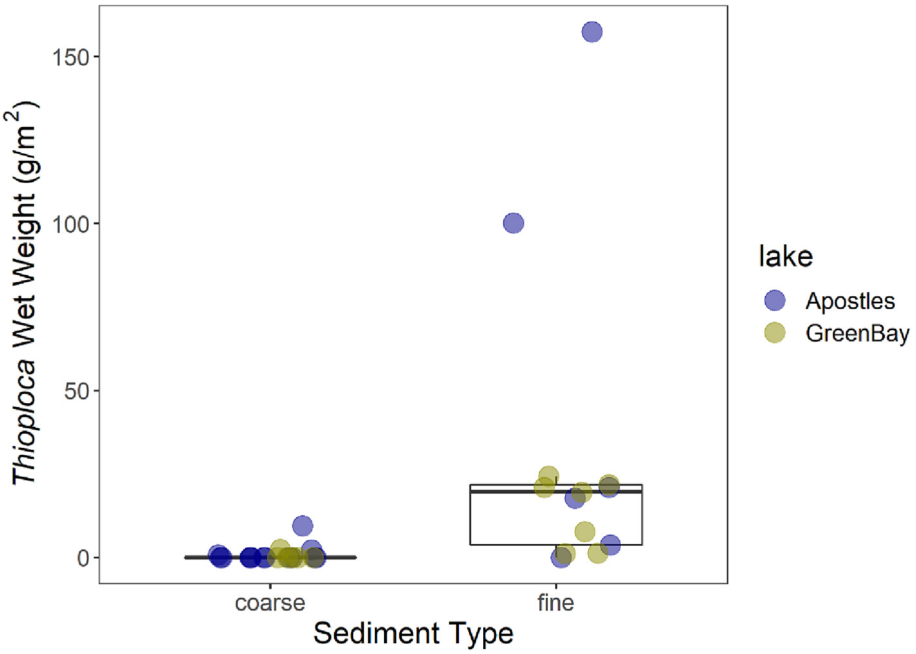

Fig. 4. *Thioploca* wet weight (g/m^{-2}) by sediment texture. Sediment was categorized as fine-grained or coarse-grained based on field observations.

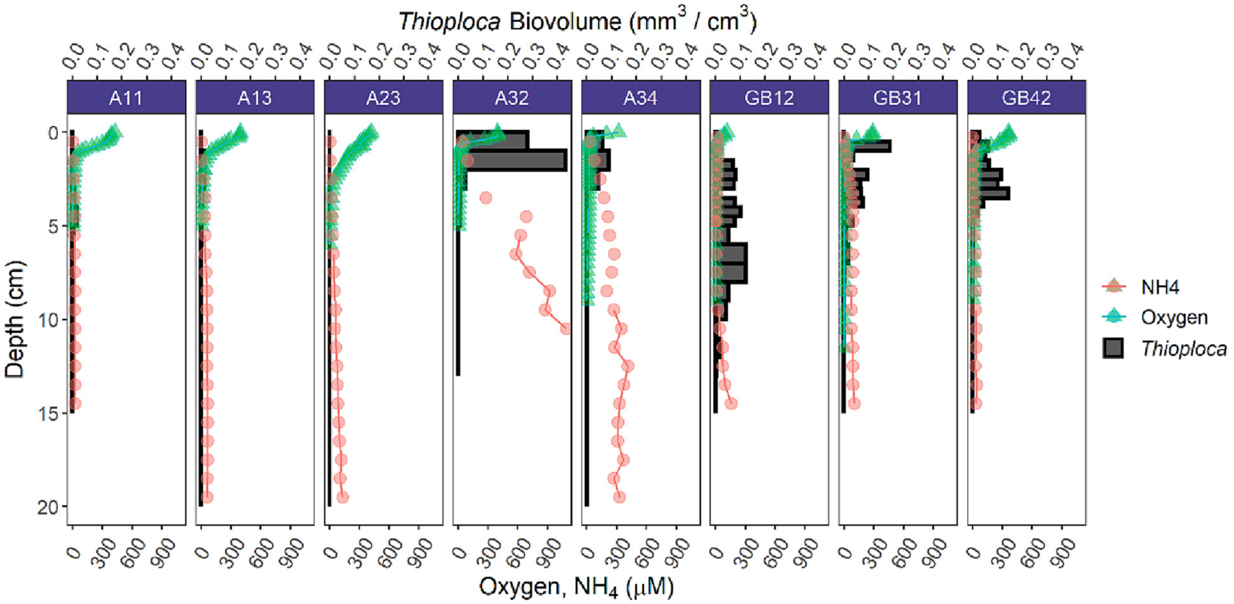

Fig. 5. *Thioploca* biovolume (mm^3 *Thioploca* cm^{-3} sediment as filled, grey bars) and porewater oxygen (triangles) and ammonium (circles) concentrations (μM) in sediment cores collected in June 2019. AI = Apostle Islands; GB = Green Bay. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Total *Thioploca* biovolume and dissolved oxygen uptake rates (DOU) at each site cored.

Site	*Thioploca* Biovolume (mm^3/cm^2)	DOU (mmol/m^2/d)
A11	0.028	4.00
A13	0.032	2.74
A23	0.000	1.08
A32	0.760	4.37
A34	0.197	4.74
GB12	0.664	3.08
GB31	0.323	7.04
GB42	0.320	3.54

which have the highest recorded densities of thioplocas (Table 2). *Thioploca* constituted a significant fraction of the sediment carbon pool. At the site with the most abundant *Thioploca* (A32), C in *Thioploca* (4.4 g C/m^{-2}(–|–)) accounted for approximately 1.5% of the total sediment organic C. (This calculation used the wet-to-dry weight conversion factor in Dermott and Legner (2002), the measured sediment dry weight and organic C content, and approximated the *Thioploca* stoichiometry as CH$_2$O). *Thioploca* abundances at our sites were high compared to the total benthic bacterial biomass observed in other lake sediments. On a carbon basis, the average *Thioploca* biomass was 1.1 g C m-^{2} in the Apostle Islands and 0.34 g C/m^{-2} in Green Bay. For comparison, in several

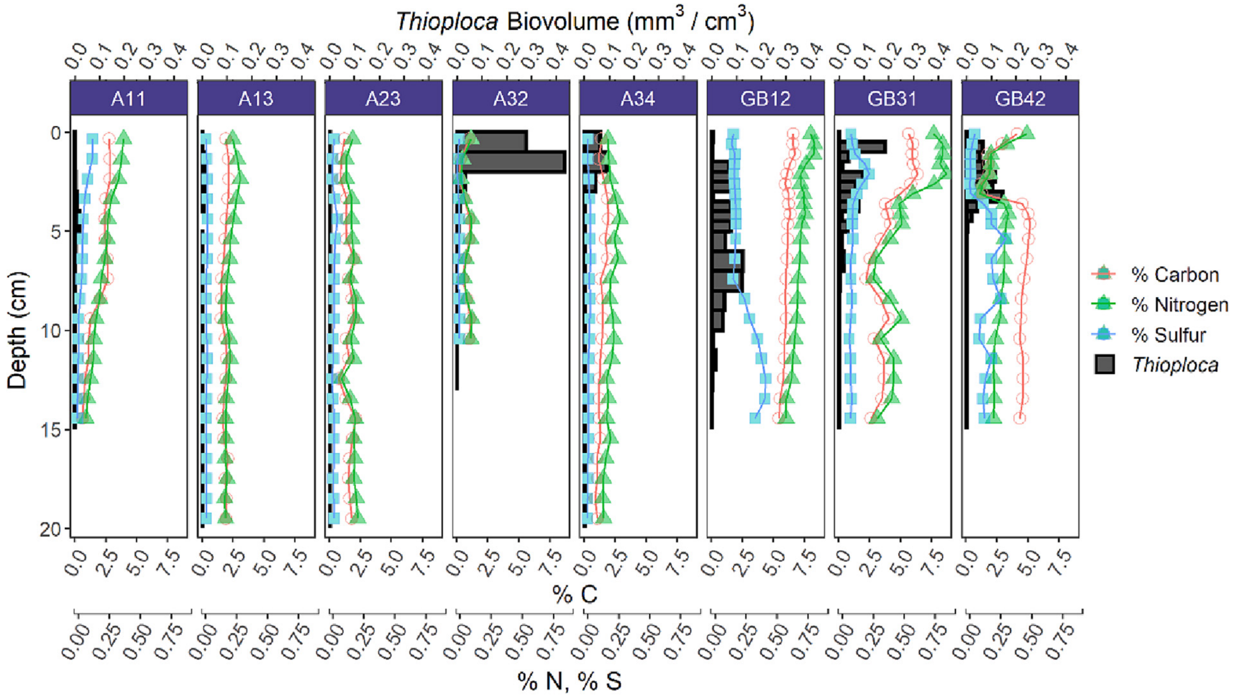

Fig. 6. *Thioploca* biovolume (mm^3 *Thioploca* cm^{-3} sediment as filled, grey bars) and sediment organic carbon (pink circles), nitrogen (green triangles), and sulfur (blue squares) (as % of dry weight) in sediment cores collected in June 2019. AI = Apostle Islands; GB = Green Bay. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Thioploca biomass comparison across freshwater, brackish, and marine ecosystems.

Location	Salinity	Species	Wet weight range (g/m^2)	Source
Lake Superior, Apostle Islands[1]	Freshwater	*Thioploca*	0.5–250 (mean: 39.0)	This study
Lake Ontario, Eastern	Freshwater	*T. ingrica*	0–206	Dermott and Legner 2002
Lake Winnipeg, North Basin	Freshwater	*Thioploca*	198	Dermott et al., 2006
Lake Michigan, Green Bay[2]	Freshwater	*Thioploca*	0.5–93 (mean: 12.4)	This study
Lake Biwa	Freshwater	*Thioploca*	12–50[3]	Kojima et al., 2007
Lake Erie, Eastern	Freshwater	*T. ingrica*	12	Dermott et al., 2005
Lake Superior, Batchawana Bay	Freshwater	*T. ingrica*	10	Dermott et al., 2005
Lake Okotanpe	Freshwater	*Thioploca*	0.003–0.13[4]	Nemoto et al., 2011
Denmark, Hjarbæk Fjord	Brackish	*T. ingrica*	33.8 ± 14.3 (mean ± SD)	Høgslund et al., 2010
Chile	Marine	*Ca.* Marithioploca	≤1000	Gallardo, 1977
Chile, Bay of Concepción	Marine	*Ca.* Marithioploca *araucae* and *chileae*	100–800	Schulz et al., 1996
Chile, Bay of Concepción	Marine	*Ca.* Marithioploca *araucae* and *chileae*	202–774	Fossing et al., 1995
Chile, Bay of Concepción	Marine	*Ca.* Marithioploca	356.4 ± 329.3	Huettel et al., 1996
Central Peruvian Coast	Marine	*Ca.* Marithioploca	≤131.9	Gutiérrez, et al., 2008
Peruvian Coast	Marine	*Ca.* Marithioploca	≤107.6	Rosenberg et al., 1983
Chile, Bay of Concepción	Marine	*Ca.* Marithioploca *araucae* and *chileae*	≤100	Holmkvist et al., 2010
Central Chilean Coast	Marine	*Ca.* Marithioploca	66.0–83.7	Gallardo et al., 2013
Chile, Bay of Concepción	Marine	*Ca.* Marithioploca	≤31.9	Carrasco et al., 1999
Chile, Bay of Concepción	Marine	*Ca.* Marithioploca	≤26[5]	Neira et al., 2001
Central Chilean Coast	Marine	*Ca.* Marithioploca *araucae* and *chileae*	12.66	Gallardo et al., 1995

[1] Values from individual ponar samples, not site averages.
[2] Values from individual ponar samples, not site averages.
[3] Values estimated from Fig. 4 using webplotdigitizer, dry weight converted to wet weight using regression from Dermott & Legner 2002.
[4] Values estimated from Fig. 1 using webplotdigitizer.
[5] Values estimated from Fig. 8 using webplotdigitizer.

lakes in Quebec and Ontario spanning a large trophic gradient, average sediment bacterial biomass C was estimated at 4.96 g C/m^{-2} (range: 0.37–20.33 g C/m^{-2}; Schallenberg and Kalff, 1993), and in an oligotrophic lake in Germany it was 1.5 g C/m^{-2} (Bergtold and Traunspurger, 2005).

At several of our sites, *Thioploca* biomass (wet weight) was considerably higher than the biomass of *Beggiatoa* in marine coastal systems where it was found to contribute significantly to sediment C and sulfur cycling (Mussmann et al., 2003; Jørgensen et al., 2010) (Table 2). The comparison is even stronger if one takes into consideration that, in freshwater *Thioploca,* a greater proportion of the wet weight is active biomass. Marine thioplocas and *Beggiatoa* have large nitrate vacuoles that can constitute 80% of the cell volume, adding to the wet weight but not to active biomass (Fossing et al., 1995). Freshwater *Thioploca*, in contrast, have much smaller or no nitrate vacuoles (Høgslund et al., 2010). *Thioploca* wet weight also includes the sheath material, the proportion of which relative to living biomass can vary (Schulz et al., 1996; Gallardo et al., 2013). Nevertheless, the comparison suggests that, at the observed densities, *Thioploca* is an important element of the sediment microbiota at some Great Lakes locations and may have a strong effect on biogeochemistry in these environments.

The abundance of *Thioploca* at organic-poor sites and the weak correlation with eutrophication indicators (ESM Fig. S1) disagree with the current paradigm derived from marine thioplocas and *Beggiatoa*. Mats of marine thioplocas are typically associated with high sediment organic C content (~5–7 wt%), though they are sometimes found in sediment with as little as ~2–4% organic C (Huettel et al., 1996; Holmkvist et al., 2010). The sediment organic content in the Apostle Islands and Green Bay, as well as other sites with *Thioploca* in the Great Lakes (Dermott et al., 2005), spans a much wider range (~0.1–9% C, ~0.03–0.9% N), and sediment organic C content was not a good predictor of *Thioploca* abundance (Table 2). In fact, the highest *Thioploca* abundances in our study were observed at sites with the lowest sediment organic C values (~1%). There was also no indication of a relationship between *Thioploca* biomass and diffusive oxygen uptake rate (DOU) in the sediment, although DOU was lowest for the A23 site (1.08 mmol m^{-2} day^{-1}), the only cored site that didn't have *Thioploca* (Table 1). Unlike marine thioplocas in the sediments below the Eastern Tropical South Pacific oxygen minimum zone and *Thioploca* in Lake Biwa, which inhabit sediment underlying oxygen-depleted water (Jørgensen and Gallardo, 1999; Nishino et al., 1998), the dissolved oxygen levels in the bottom water at our sites were high (77–100% saturation). Together with the lack of dependence on sulfate reduction rates (see '*Thioploca* biogeochemistry' section below), our observations indicate that drivers of freshwater *Thioploca* distribution are likely different from those of marine thioplocas.

The most consistent pattern observed in our data was that *Thioploca* occurred more frequently and attained higher biomass in fine-grained (muddy) sediment compared to coarse-grained (sandy) sediment. Fine-grained sediment is often associated with depositional areas (Cole and Weigmann, 1983; Evans et al., 1990), whereas coarse-grained sediment indicates erosional areas (Rea et al., 1981). *Thioploca* has been similarly observed in the depositional areas of oligotrophic Lakes Michigan and Huron (unpublished observations, T. Ozersky). Fine-grained sediment had higher organic C content (3.5 ± 2.6 %C (mean ± SD)) than coarse-grained sediment (1.5 ± 1.5 %C) (Welch's *t*-test: $t = -2.55$, $df = 17.42$, $p = 0.020$), even if the *Thioploca* abundance was not directly correlated to the sediment organic matter content (ESM Fig. S1). Other factors may also contribute to higher *Thioploca* biomasses in fine-grained sediment. For example, small marine *Beggiatoa* prefer muddy sediments, as the rigid structure of sandy sediments impedes their movement (Jorgensen, 1977). Similarly, *Thioploca* tended to be absent in sandy sediments in Lake Ontario (Dermott and Legner, 2002). However, in a survey of Danish brackish sediments, *Thioploca ingrica* was confined to river mouth sites of fjords but did not otherwise show a relationship with sediment properties, including texture, and was found in organic-rich sediment of a variety of textures, from fine silt to peat and sand (Høgslund et al., 2010). More research is needed to elucidate the reason for this pattern.

Thioploca distribution was highly variable over small spatial scales. At some of the sites we sampled, biomass varied by an order of magnitude between replicate ponar samples collected within several meters of each other. This is consistent with observations in Lake Baikal, Lake Okotanpe, and Danish brackish water where *Thioploca* distribution is also spatially patchy (Zemskaya et al., 2001; Høgslund et al., 2010; Nemoto et al., 2011). In addition to patchy spatial distribution, *Thioploca ingrica* populations also appear to display high temporal variability in biomass. For example, *Thioploca* was reported in Lake Erie in 1960, but the population had declined to below detection by 1980 (Maier, 1980), and at a site in Lake Ontario, *Thioploca* biomass increased by three orders of magnitude in five years (Dermott and Legner, 2002). The spatial patchiness and temporal variability of *Thioploca ingrica* in lacustrine ecosystems indicate capacity for active population dynamics. As this variability also makes it more difficult to discern *Thioploca* distribution patterns, larger scale studies may be needed to confidently determine the ecological niches and population drivers of freshwater *Thioploca*.

Thioploca span both micro-oxic and anoxic depths within the sediment. In most sediment cores, *Thioploca* filaments were detected continuously from the sediment surface down to a maximum depth ranging between 2 and 10 cm. This distribution is consistent with the canonical motile lifestyle of these bacteria, whereby *Thioploca* trichomes glide within their sheaths, enabling a connection between oxidizing zones and deeper sediments where sulfur may be obtained. *Thioploca ingrica* are additionally capable of anaerobic cell maintenance using internal sulfur globules as electron acceptors (Kojima et al., 2015), enabling their survival for several months in anoxic sediments without access to oxygen or nitrate (Høgslund et al., 2010). This strategy could conceivably underlie the *Thioploca* depth distribution observed at Station GB12, where the *Thioploca* filaments were observed almost entirely in subsurface anoxic sediments, without an obvious connection to the oxygenated sediment surface, or to oxygenated biogenic structures, such as infaunal burrows. It is not yet clear how dynamic the *Thioploca* depth distribution patterns are in these sediments, or whether these distributional patterns have quantitatively important implications for sedimentary biogeochemical cycling.

Role of Thioploca in sediment biogeochemistry

An enigmatic aspect of freshwater *Thioploca* ecology is its energy source. Marine thioplocas inhabit sediments in oxygen minimum zones with high sulfate reduction rates and obtain energy by oxidizing the produced sulfide (Ferdelman et al., 1997). Surface marine sediments, however, have nearly 1000 times more sulfate than Great Lakes sediments (Ferdelman et al., 1997; Fakhraee et al., 2017). Sulfate reduction rates in freshwaters, being limited mainly by availability of reactive organic matter, can sometimes approach those in marine environments (Fakhraee et al., 2017), but sulfide is much less available, as it becomes scavenged by ferrous iron in sediment porewater or reacts with iron oxides to produce elemental sulfur. In fact, sulfate reduction rates do not appear to determine freshwater *Thioploca* abundance. *Thioploca* biomass in the Apostle Islands is higher than in Lake Biwa (Table 2), despite an order of magnitude lower sulfate reduction rates (0.11–1.4 nmol cm^{-3} day^{-1} (Fakhraee et al., 2017) vs. 11–28 nmol cm^{-3} day^{-1} (Kojima et al., 2007)). And while sulfate reduction rates in Lake Superior are much lower than those in *Thioploca*-dominated marine sediments (170–4700 nmol cm^{-3} day^{-1}), the highest *Thioploca* biomasses observed in the Apostle Islands approach the range observed for marine thioplocas (Ferdelman et al., 1997). This discrepancy suggests that freshwater *Thioploca* may be using an alternative energy source.

Such alternatives may include, for example, utilization of the organic S-containing products of organic matter mineralization, or other reactive sulfur intermediates. Remineralization of S compounds serves as a major source of substrates for S cycling in low-sulfate environments. In Lake Superior, it may fully support the in-sediment sulfate reduction, as well as supply a range of more reduced S compounds (Fakhraee et al., 2017). These compounds can then undergo a number of reactive transformations, with thiosulfate ($S_2O_3^{2-}$) and elemental sulfur (S^0) serving as major intermediates (Jørgensen et al., 2019; Findlay and Kamyshny, 2017). The known catabolic ability of freshwater *Thioploca* to use thiosulfate as an electron donor (Kojima et al., 2015) may be enabling it to utilize this energy resource.

Energetics calculations may help constrain the range of possible metabolic pathways. In Lake Superior sediments, sulfate reduction alone produces no more than 0.2 mmol H_2S m^{-2} day^{-1} (Fakhraee et al., 2017), integrated over the entire sediment depth including the aerobic zone. At the in-situ conditions, sulfide oxidation with nitrate as the electron acceptor generates about 740 kJ mol^{-1} H_2S of energy when the product is N_2. If biomass synthesis requires approximately 1000 kJ mol^{-1}C, and at least ~400 kJ mol^{-1}C is dissipated in the process (Heijnen and Van Dijken, 1992), then microbes can synthesize at most 0.53 mol C-biomass per mol H_2S (McCollom and Amend, 2005; Smeaton and Van Cappellen, 2018). Approximating the stoichiometry of *Thioploca* as CH_2O, carbon accounts for roughly one third of the dry weight which is approximately 8.45% of *Thioploca* wet weight (Dermott and Legner, 2002). Thus, *Thioploca* could synthesize approximately 45 mg of wet weight biomass m^{-2} day^{-1} at our Lake Superior sites. We measured an average of approximately 40 g m^{-2} *Thioploca* wet weight in the Apostle Islands. At this estimated rate, it would take about 2.5 years to accumulate the biomass of *Thioploca* we observed. Given that this is a highly conservative estimate that neglects large losses of sulfide to other processes, and that *Thioploca* populations reportedly fluctuate strongly from year to year, this could be an indication that sulfide may not be the only electron source utilized by freshwater *Thioploca*.

Alternatively, sulfate reduction and sulfide oxidation could be happening multiple times within the sediment, similar to the rapid recycling of sulfide that was suggested to occur between marine thioplocas and sulfate-reducing bacteria inhabiting the *Thioploca* sheaths (Jørgensen and Gallardo, 1999). A similar association with sulfate reducers could also be benefitting freshwater *Thioploca*. The sheaths of freshwater *Thioploca* are inhabited by diverse Bacteria, but previous investigations found no associations with any known (canonical) sulfate reducers (Kojima et al., 2006). However, *Thioploca* sheaths from multiple lakes were inhabited by members of the Chloroflexi (Kojima et al., 2006), a diverse and metabolically flexible phylum, with members that have recently been implicated as putatively capable of sulfate and thiosulfate reduction to sulfide (Wasmund et al., 2016; Zheng et al., 2021). The distribution and biogeochemical function of microbes closely associated with freshwater *Thioploca* therefore merits further examination.

In some of our cores (notably GB12 and possibly A32), *Thioploca* biomass appears to be associated with ammonium consumption well below the depths where ammonium can be oxidized by oxygen. The consumption of ammonium is intriguing and substantiates previous observations that the freshwater *Thioploca* do not perform DNRA (Høgslund et al., 2010; Kojima et al., 2015). Marine thioplocas have the genetic capacity to perform DNRA (Høgslund et al., 2009), and so vast regions dominated by mats of these bacteria are sites of net ammonium production (Thamdrup and Canfield, 1996; Otte et al., 1999). Yet, marine thioplocas have also been found in consortia with anammox bacteria, with a net effect of ammonium consumption in these sediments (Prokopenko et al., 2013). Anammox bacteria have also been reported in the sheath community of *Thioploca ingrica* in Lake Baikal around the Posolsk Bank cold methane seep (Chernitsyna et al., 2016). As significant anammox activity (up to 0.040 mmol m^{-2} d^{-1}) has been reported in Lake Superior sediments (Crowe et al., 2017), it is possible that anammox bacteria are responsible for at least some of the ammonium consumption.

Assimilatory consumption of ammonia is unlikely to play a significant role; rather, it is probably being metabolized as an energy source. The upward flux of ammonium from anoxic sediment towards the *Thioploca* layers is estimated at 0.0017–0.028 mmol m^{-2} day^{-1} at our sites. Assuming ammonium production is driven by mineralization of deposited phytoplankton in the sediment at near-Redfield ratios, ammonium production can be estimated as DOU/16 (Table 1). Thus, total estimated ammonium input (upward flux from anoxic sediment plus gross production within the oxic sediment) at our sites ranged 0.2–0.5 mmol m^{-2} day^{-1}. As marine *Thioploca* assimilate up to 2400 nmol C day^{-1} g^{-1} of wet *Thioploca* biomass (Ferdelman et al., 1997; Otte et al., 1999) and are approximately 1.9% N by dry weight after the removal of the excess nitrate stored in the vacuoles (Thamdrup and Canfield, 1996), for ~40 g m^{-2} wet *Thioploca* biomass in the Apostle Islands this would correspond to the N assimilation rate of approximately 0.005 mmol N m^{-2} day^{-1}. This is two orders of magnitude lower than the ammonium fluxes, suggesting a negligible sink by assimilation.

More investigation is needed to improve our understanding of freshwater *Thioploca* N and S dynamics. Measurements of freshwater *Thioploca* growth rates and stoichiometry do not currently exist, which limits bioenergetic understanding of the elemental dynamics of freshwater *Thioploca*. Measurements of nitrate and nitrite profiles in the sediment could also help us better understand these processes. We attempted to measure nitrate and nitrite in the porewater but unfortunately did not get usable results due to analytical error. We documented accumulation of sulfur globules in all examined *Thioploca* samples, suggesting that *Thioploca* are active and important nodes in S cycling in the sediments they inhabit. Understanding of freshwater *Thioploca* metabolism could additionally be improved by measurements of the sulfur content of *Thioploca*, and sulfate, sulfide, and thiosulfate concentrations and turnover rates from sediments inhabited by *Thioploca* in the Great Lakes and other freshwater systems. Such studies could also help understand how *Thioploca* impacts sediment composition (e.g., to what extent are *Thioploca* responsible for some of the variation in N and S content seen in cores GB12 and GB42). Further investigation is also needed to determine whether in low S environments *Thioploca* can use other electron donors, such as acetate (Kojima et al., 2015). Analysis of the microbial communities associated with *Thioploca* is also necessary to identify potential interactions with anammox bacteria and could help reveal whether they form consortia with canonical sulfate reducing bacteria such as *Desulfonema* (Fukui et al., 1999) or other putative sulfur cycling bacteria, e.g., among the Chloroflexi (Wasmund et al., 2016).

Conclusions

The ecology of freshwater *Thioploca* reveals major gaps in our understanding of the microbial metabolisms and the interactions of *Thioploca* with its environment. How does *Thioploca* survive in such low S environments and grow to biomasses comparable with populations of marine thioplocas? What are their microbial associations? And what is the overall impact of these unusual and abundant bacteria on the biogeochemistry of modern freshwater ecosystems? The abundant *Thioploca* we found in the Apostle Islands and Green Bay are among only a few instances of *Thioploca* reports in the Great Lakes (Maier, 1980; Dermott and Legner, 2002; Dermott et al., 2005). However, our results and previous reports suggest that freshwater *Thioploca* is widespread and locally abundant in the Great Lakes and possibly other freshwater systems. *Thioploca* is clearly able to survive and achieve high biomass in the Great Lakes under low nutrient conditions, high water column oxygen concentrations, low sediment organic content, and low sulfate reduction rates. These findings suggest that *Thioploca* could be present and active in a much wider variety of environmental conditions than previously recognized.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary material to this article can be found online at https://doi.org/10.1016/j.jglr.2023.07.003.

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