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Chlorophyll nitrogen isotope values track shifts between cyanobacteria and eukaryotic algae in a natural phytoplankton community in Lake Erie

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ABSTRACT

Chlorophylls are produced by all photosynthetic organisms and are ideal targets for compound-specific isotopic studies of phytoplankton. In laboratory cultures, the difference between the nitrogen (N) isotope ratio ($\delta^{15}N$ value) of chlorophyll and the $\delta^{15}N$ value of biomass, known as ε_{por}, varies taxonomically, yielding potential applications for studying productivity in modern and ancient environments. Here we take advantage of the annual cyanobacterial bloom in Lake Erie, USA, to demonstrate ε_{por} patterns in a natural community. The resulting time series shows that environmental observations are similar to laboratory cultures: predicted ε_{por} endmember values range from 4.6‰ to 7.4‰ for eukaryotic algae, and −18‰ to −21‰ for cyanobacteria. Because the range and sensitivity of ε_{por} is similar between laboratory and natural settings, the data support the use of ε_{por} as a reliable tracer of the relative contributions of cyanobacteria and eukaryotic algae to nutrient utilization and primary production in lacustrine environments.

1. Introduction

Along with phosphorus (P), nitrogen (N) is a major limiting nutrient for primary production in aquatic ecosystems, where N cycling is largely driven by microbial transformations that convert N between its oxidized and reduced forms. Each of these transformations, including N-fixation, nitrification and denitrification, has an associated kinetic isotope effect that describes the ^{15}N content of the product relative to the substrate in the reaction (summarized in Sigman et al., 2009). N isotope ratios ($\delta^{15}N$ values) of phytoplankton biomass in surface waters therefore record an integrated signal of the sources and forms of N used for algal growth. $\delta^{15}N$ values of bulk organic matter can be preserved long-term in the geological record and serve as a tracer for variations in redox state (Jenkyns et al., 2001; Godfrey and Falkowski, 2009), major modes of primary production (Calvert et al., 1992; Struck et al., 2000), and nutrient source availability in the euphotic zone (Hodell and Schelske, 1998; Teranes and Bernasconi, 2000). However, diagenesis both before and after burial can alter the bulk N pool that is ultimately preserved, complicating interpretations of sedimentary isotope data (Sigman et al., 1999; Robinson et al., 2004, 2012; Tesdal et al., 2013).

To complement bulk analyses, an additional approach is to use compound-specific $\delta^{15}N$ analysis of organic molecules that are not affected by diagenesis (Chicarelli et al., 1993; Sachs et al., 1999; Chikaraishi et al., 2008; Higgins et al., 2009; Ren et al., 2009). Chlorophylls are frequent biomarker targets because they are an essential part of the photosynthetic apparatus and are therefore specific for surface water processes. Importantly, the N bonds in chlorophylls are not altered by diagenesis (Louda and Baker, 1986), and therefore the diagenetic products of chlorophylls retain the original isotopic signature (Tyler et al., 2010). Even accounting for the minor isotope effect likely associated with the formation of metalloporphyrins in sediments (Junium et al., 2015), compound-specific $\delta^{15}N$ measurement of chlorophylls and their degradation products are a more direct tracer of N utilization by phytoplankton than $\delta^{15}N$ values of bulk organic matter alone.

For chlorophyll $\delta^{15}N$ values to be useful, however, the isotopic relationship between chlorophyll and total biomass must be well understood. Several previous studies have examined patterns of biosynthetic N isotope fractionation in cultured organisms (Sachs et al., 1999; Beaumont et al., 2000; Higgins et al., 2011; Tsao

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et al., 2012). This parameter, defined as ε_{por}, represents the isotopic offset between cellular biomass and chloropigment: $\varepsilon_{por} \approx \delta^{15}N_{biomass} - \delta^{15}N_{chloropigment}$. This definition follows the convention that positive values of ε correspond to depletion of the heavy isotope relative to the starting material (Hayes, 2001).

ε_{por} appears to be taxonomically diagnostic for three main algal groups (Fig. 1). The eukaryotic algae have a median ε_{por} value of 5.5 ± 1.6‰, indicating that they biosynthesize chlorophyll that is ~5.5‰ depleted in ^{15}N relative to cellular biomass. Marine cyanobacteria have a median ε_{por} value of 0.2 ± 2.5‰, showing on average no fractionation between biomass and chlorophyll. Finally, the freshwater cyanobacteria have a median ε_{por} value of −10.3 ± 2.6‰, meaning the chlorophyll is enriched, or isotopically "heavy", relative to biomass. The ε_{por} signature of freshwater cyanobacteria is especially striking, given that biosynthetic reactions usually yield products that are isotopically depleted relative to reactants (Hayes, 2001).

These patterns in ε_{por} hold true across a wide range of culture experiments, regardless of the N substrate and its isotopic composition (NO_3^-, NH_4^+, or N_2-fixing; Higgins et al., 2011) or whether grown in batch or chemostat culture (Tsao et al., 2012). In other words, while N-assimilating enzymes do fractionate each N substrate differently and therefore affect the whole cell $\delta^{15}N$ value, the isotopic offset between chlorophylls and cellular biomass remains the same within each algal group. This independence from the type(s) of N substrate, combined with the significant differences between the eukaryotic and cyanobacterial endmembers for ε_{por}, makes ε_{por} a useful tool for reconstructing phytoplankton export production in modern and ancient marine environments (Higgins et al., 2012; Shen et al., 2018). However, to date the use of ε_{por} has been limited, because the physiological and/or biochemical basis for the observed taxonomic differences remains unknown. In addition, the patterns in ε_{por} are primarily a conclusion of laboratory culture studies.

Environmental evidence for these patterns remains limited. An early study reported two measurements of ε_{por} from a cyanobacteria-dominated freshwater lake in Japan (Katase and Wada, 1990), where the ε_{por} values of approximately −13‰ and −16‰ can now be interpreted as consistent with ε_{por} values for freshwater cyanobacterial cultures. However, recent studies have documented a marine intertidal cyanobacterial (*Lyngbya* sp.) mat with an ε_{por} value of −10.1‰ (Fulton et al., 2012, close to the freshwater cyanobacterial endmember), and a freshwater meromictic lake dominated by *Synechococcus* sp. with an ε_{por} value of −0.4‰ (close to the marine cyanobacterial endmember; Fulton et al., 2018). These results suggest that patterns of ε_{por} may be more variable in natural systems than in culture, and that exploration of additional environmental settings is needed.

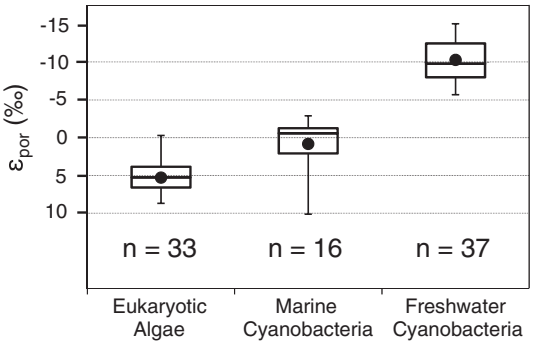

Fig. 1. Summary of ε_{por} values ($\varepsilon_{por} = \delta^{15}N_{biomass} - \delta^{15}N_{chloropigment}$) obtained from previous studies of pure cultures (Sachs et al., 1999; Beaumont et al., 2000; Higgins et al., 2011; Tsao et al., 2012), where n = number of experiments. In each box, the black circle indicates the average, the central line indicates the median, and the bottom and top edges of the box indicate the 25th and 75th percentiles, respectively. The whiskers extend to the most extreme data points.

To further establish the environmental expression of ε_{por}, we sought a system with clearly distinguishable phytoplankton community endmembers. Lake Erie is an ideal location, because it experiences an annual shift in the phytoplankton community from eukaryotic phytoplankton in the early summer to cyanobacteria in the late summer/early fall (Bridgeman et al., 2012). Lake Erie is the shallowest and most productive of the Laurentian Great Lakes and has experienced significant eutrophication over the last half-century. Despite initial improvements achieved by the reduction of total P inputs to the lake via the Great Lakes Water Quality Agreement, the lake has become eutrophic since the mid-1990s, as evidenced by increased water column hypoxia (Zhou et al., 2013; Del Giudice et al., 2018) and by large annual cyanobacterial harmful algal blooms (CHABs) dominated by toxin-forming strains of the non-diazotrophic cyanobacteria *Microcystis* sp. (Michalak et al., 2013; Berry et al., 2017). The intensity of these blooms has increased in the last decade (Stumpf et al., 2012), resulting in several blooms that impacted the drinking water supply for the city of Toledo (Michalak et al., 2013; Carmichael and Boyer, 2016).

In this study, we took advantage of this annual CHAB event in Lake Erie to investigate whether ε_{por} values track the transition in the phytoplankton community over the course of the bloom. We hypothesized that ε_{por} should shift from positive values (^{15}N-depleted chlorophyll) during pre-bloom, eukaryote-dominated conditions in the early summer to more negative values (^{15}N-enriched chlorophyll) during the height of the CHAB in late summer and into fall.

2. Materials and methods

2.1. Sample collection

Weekly samples were collected from the water column at station WE2 (41°46′ N, 83°0′ W) in the western basin of Lake Erie, USA (Fig. 2), from June through October 2017, for a total of 18 time points. Twenty liters of depth-integrated water was collected with a peristaltic pump by slowly moving a weighted Tygon tube up and down from the surface of the water column to one meter from lake bottom (0–5 m depth on average). The water was filtered through 0.7 μm GF/F filters (142 mm diameter, Whatman), which were frozen and stored at −80 °C. Approximately 10% of each filter was

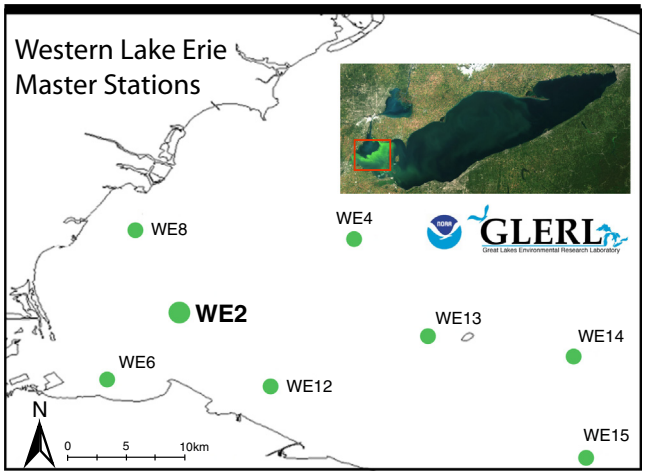

Fig. 2. Map of Great Lakes Environmental Research Laboratory (GLERL) master stations in western Lake Erie, highlighting the location of station WE2. Inset is a satellite image of Lake Erie in late July 2017 showing the location of the CHAB in the western basin (photo credit: NOAA GLERL).

reserved for bulk $\delta^{15}N$ analysis, while the remainder was extracted for chlorophyll $\delta^{15}N$ analysis. If the sample needed to be filtered onto multiple GF/F filters, a fraction for bulk analysis was reserved from each filter to ensure representative sampling. For chlorophyll analysis, these multiple filters were combined for extraction, except in a few cases where they were analyzed separately to verify that variability between filters for a single time point was low. Detailed sample information is available in Supplementary Table S1.

2.2. Phytoplankton community composition

Phytoplankton community composition was determined using a submersible FluoroProbe (bbe Moldaenke GmbH, Germany), which monitors in situ chlorophyll fluorescence. The FluoroProbe quantifies four broad 'spectral groups' of chlorophyll *a*-containing phytoplankton (expressed in μg Chl*a* L^{-1}), based on differences in their accessory light harvesting pigments that result in characteristic fluorescence fingerprints (Beutler et al., 2002; Johnsen and Sakshaug, 2007; Escoffier et al., 2015). Phytoplankton groups that can be distinguished are: (i) green algae (ii) cyanobacteria, (iii) diatoms/dinoflagellates/chrysophytes and (iv) cryptophytes. In Lake Erie, group iii is dominated by diatoms, and hereafter will be referred to as the diatom group. Fluorescence profiles of pigment concentration for each group were depth-integrated for the top 5 m and normalized to the total chlorophyll concentration to give the fraction of chlorophyll contributed by each group.

2.3. Chlorophyll extraction and $\delta^{15}N$ analysis

Chlorophyll was extracted from GF/Fs in a 2:1 (v/v) mixture of dichloromethane/methanol (DCM/MeOH) with vortexing (1 min) followed by sonication in an immersion bath (10 min) and incubation in the dark at 4 °C for at least 2 h. Filter pieces were removed using centrifugation and filtration, and the extract concentrated under N_2 gas using a Turbovap II (Zymark). Silica gel columns were prepared by adding glass wool, dry Na_2SO_4, and silica gel to 5″ glass pipets, and combusted before use. The concentrated chlorophyll extracts were added to the silica columns and eluted using DCM/MeOH (2:1, v/v).

The extracts were further purified for chlorophyll analyses using an HPLC (Agilent 1200 series) equipped with multi-wavelength UV/Vis detector. Using a method modified from Higgins et al. (2009), samples were injected onto two ZORBAX SIL columns (4.6 × 250 mm, 5 μm) connected in series and eluted at 1 mL min^{-1} using the gradient described in Supplementary Table S2. Chlorophylls were identified using absorbance spectra and comparison to an authentic chlorophyll *a* standard (Sigma-Aldrich), and collected using time-based fraction collection from 13 to 17 min. Representative HPLC chromatograms are shown in Supplementary Fig. S1. Purified chlorophyll fractions were dried under N_2 gas and reconstituted in DCM.

Chlorophyll $\delta^{15}N$ values were analyzed according to the methods in Higgins et al. (2009). Briefly, chlorophyll was oxidized in DCM in quartz tubes under UV light in a biosafety cabinet for 6 h, dried, and then oxidized chemically using re-crystallized 0.05 M $K_2S_2O_8$ dissolved in fresh 0.15 M NaOH. Purity of isolated chlorins was assessed by comparing measured oxidized NO_3^- yield and predicted NO_3^- yield. Predicted NO_3^- was calculated by conversion from HPLC peak area using chlorophyll standards (as in Higgins et al., 2009). All samples that were collected and processed as single filters gave linear correspondence (slope 1.0, $R^2 = 0.84$) between measured oxidized NO_3^- yield and predicted NO_3^- yield (Supplementary Fig. S2). Three samples that represent combined GF/F filter extracts fell below the 1:1 line (i.e., low NO_3^- yield), likely due to artifacts from extra sample handling. No samples fell significantly above the line, indicating there was no N contributed by non-UV sensitive sources such as amines.

Oxidized NO_3^- concentration was measured using a chemiluminescent NO_x analyzer (Teledyne NO/NOx Analyzer 200E), and $\delta^{15}N$ values were measured using the denitrifier method (Sigman et al., 2001), on a Delta V Advantage isotope ratio mass spectrometer with a custom built purge and trap system. Isotopic measurements were standardized to the N_2 reference scale using standard reference materials IAEA N3 and USGS 34. $\delta^{15}N$ values were corrected for a N blank originating from the HPLC solvent and from the oxidizing reagent, according to Higgins et al. (2009).

2.4. Bulk $\delta^{15}N$ analysis

Subsamples of GF/F filters for bulk isotopic analysis were placed into tin capsules (Costech) and dried at 50 °C overnight. Dry capsules were folded and crushed, and analyzed on a Thermo Scientific Flash IRMS Elemental Analyzer with EA Isolink, coupled to a Delta V Advantage IRMS through a Conflo IV universal interface. Sample $\delta^{15}N$ values were calculated using in-house laboratory standards as well as standard reference materials USGS40 and USGS41a.

3. Results

The composition of the phytoplankton community at station WE2 is shown in Fig. 3A. Uncertainty in the classification of each phytoplankton group by Fluoroprobe is estimated as ±5% based on previous literature (Escoffier et al., 2015). In early July the community is mostly eukaryotic, with cyanobacteria contributing <20% of the total chloropigments. Over the summer, the relative fraction of cyanobacteria increases, with the exception of a brief period in early September, when the cyanobacteria temporarily decrease to 30% before returning to high abundance. By October, cyanobacteria account for >70% of the chloropigment fluorescence.

Bulk and chlorophyll $\delta^{15}N$ values for each sampling date are shown in Fig. 3B (Supplementary Table S3 contains the numerical values and calculated uncertainties). Throughout the summer, the bulk biomass $\delta^{15}N$ values spanned from 4.4‰ to 9.3‰ (average 7.1‰ ± 1.6‰), indicating that the surface water nitrogen sources and total nitrogen budget were relatively isotopically stable. In contrast, chlorophyll $\delta^{15}N$ values spanned a time-evolving range from −4‰ to 23‰, i.e., a seasonal shift of >25‰. The corresponding values of ε_{por} track the change from a eukaryote-dominated to cyanobacteria-dominated community, transitioning from mostly positive values in July to negative values in September and October (Fig. 3C). Specifically, ε_{por} is strongly correlated with the percentage of cyanobacteria in the community (Fig. 4), becoming more negative as the contribution from cyanobacteria increases. We used both an ordinary least-squares and an orthogonal least-squares regression to predict the range for eukaryotic and cyanobacterial endmembers for ε_{por}. The ordinary regression (using only the uncertainty in $\delta^{15}N$) predicts that ε_{por} equals 4.6‰ when cyanobacteria are absent and −18.4‰ when cyanobacteria make up 100% of the community (Fig. 4), while the orthogonal regression incorporates the average uncertainties in both the Fluoroprobe and ε_{por} measurements (5% and 0.8‰, respectively) and predicts respective endmembers of 7.4‰ and −21.6‰. Although sediment resuspension did occur at our sampling site, a comparison of turbidity measurements with both chlorophyll $\delta^{15}N$ and ε_{por} values showed no correlation with either, indicating that sediment resuspension of chlorophyll degradation products had no effect on the observed ε_{por} relationship (Supplementary Fig. S3).

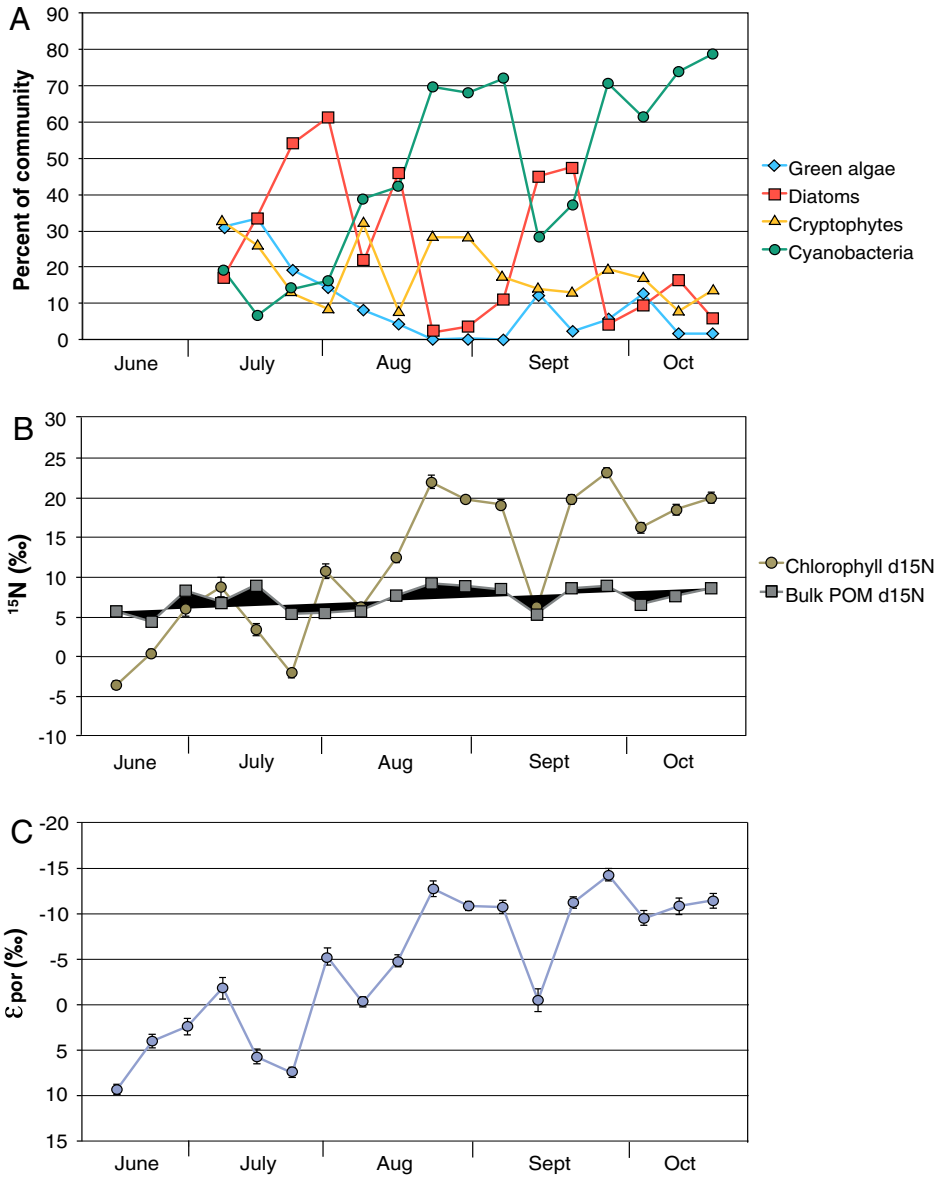

Fig. 3. Community composition as measured by Fluoroprobe (A), $\delta^{15}N$ values of chlorophyll and bulk particulate organic matter (B), and calculated ε_{por} values (C) over the summer. For ease of viewing error bars are not shown in (A), but uncertainty in Fluoroprobe measurements is estimated at ±5%. In (B) and (C), if not visible, error bars are smaller than the size of the symbols.

4. Discussion

The correlation between ε_{por} and community composition in Lake Erie supports the hypothesis that ε_{por} is robustly linked to higher-level taxonomy. Both the chlorophyll $\delta^{15}N$ (ε_{por}) and FluoroProbe data target the pigment pool specifically, rather than algal biomass and/or biovolume. This common focus on the same analyte likely explains why the two types of data exhibit such a strong correlation, both qualitatively (e.g., the simultaneous change in both % cyanobacteria and ε_{por} indicated by the brief community excursion in September, Fig. 3), as well as quantitatively (Fig. 4). Good correspondence between ε_{por} and the proportion of pigments originating from cyanobacteria vs eukaryotic organisms is to be expected if ε_{por} is indeed tied to biosynthetic differences in chlorophyll N isotope fractionation that vary among major algal groups.

Importantly, the ε_{por} endmember values predicted by the Lake Erie data are consistent with the values from laboratory cultures, both in range and magnitude (e.g., Sachs et al. 1999; Higgins et al., 2011). They also agree broadly with the one prior field observation (Katase and Wada, 1990). The eukaryotic endmember is defined as the scenario where cyanobacteria are completely absent from the phytoplankton community (y-intercept, Fig. 4). Although not realized in this natural community, as the cyanobacterial population never reaches 0%, the predicted range for the extrapolated eukaryotic ε_{por} endmember (4.6–7.4‰) overlaps with the median ε_{por} value obtained from eukaryotic cultures grown in the laboratory (5.5 ± 1.6‰, Fig. 1). The predicted range for the extrapolated cyanobacterial ε_{por} endmember (−18.4‰ to −21.6‰) also is in the same direction as – although significantly more fractionated than – the literature values for cultured cyanobacteria and the data of Katase and Wada (1990). It is possible that cyanobacteria growing naturally in freshwater systems may exhibit even greater ^{15}N-enrichment of chlorophyll relative to biomass than when grown in lab cultures, although the reasons for this are currently unknown.

The general agreement between culture studies and the environment is consistent with the idea that there is a fundamental biosynthetic or physiological explanation for why chlorophyll *N*

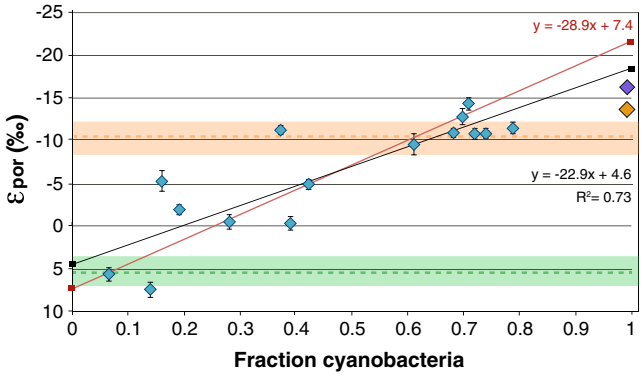

Fig. 4. The correlation between Lake Erie ε_{por} values (blue diamonds) and the fraction of the community consisting of cyanobacteria. Data are fit with an ordinary linear least-squares regression (black line) and an orthogonal (Deming) least-squares regression (red line). The green shaded region indicates the median and standard deviation of ε_{por} from eukaryotic lab cultures (5.5 ± 1.6‰), while the orange shaded region indicates the same from cyanobacterial lab cultures (−10.3 ± 1.8‰). The orange diamond indicates the most extreme ε_{por} value observed to date in lab cultures of cyanobacteria (−14.1‰), while the purple diamond indicates the ε_{por} value of −16‰ observed in a freshwater lake dominated by cyanobacteria (Katase and Wada, 1990). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

isotopes are fractionated differently among major algal groups, independent of culture conditions or the original N source (NO_3^-, NH_4^+, or N_2; Higgins et al., 2011). This property suggests ε_{por} should be a reliable tracer that can be used across nearly all biogeochemical conditions or nutrient regimes, because shifts in ε_{por} appear to be influenced primarily by the phytoplankton group and not by other site-specific environmental factors such as redox state, temperature, or nutrient supply (e.g., N sources, see Supplementary Fig. S4). Exceptions may be unusual systems with large contributions from anoxygenic phototrophs (e.g., Fayetteville Green Lake, Fulton et al., 2018). Although the reason for the difference in characteristic ε_{por} values remains unknown, the biosynthetic pathway for chlorophylls is understood to be the same in both cyanobacteria and eukaryotic phytoplankton (Beale, 1999; Sachs et al., 1999). This implies that the expression of different ε_{por} values requires either a different kinetic isotope effect for a critical enzyme or a fundamental shift in the balance of branch points around a biosynthetic intermediate (Hayes, 2001). In either case, tetrapyrroles are synthesized from the amino acid glutamate; thus, a better understanding of ε_{por} values will require further investigation of the specific cellular fates of this amino acid.

Importantly, the dominant cyanobacterial species in Lake Erie, *Microcystis,* do not fix N_2. In a system where N_2-fixing cyanobacteria are important, the bulk cellular $\delta^{15}N$ value would shift to reflect the minimal fractionation that occurs with N_2 fixation. The ε_{por} values, however, would shift in parallel, as the isotopic offset between chlorophyll and cellular biomass is constant within these major algal groups. This would also be true for other N cycling processes (e.g., denitrification) that result in N isotope fractionation in the water column. Therefore ε_{por} measurements provide integrated information on both the N sources used by phytoplankton (as reflected by individual bulk and chlorin isotope values) and the dominant phototroph type (as indicated by the ε_{por} value), independent from other approaches that sample different timescales such as DNA sequencing. Additionally, results from contemporary studies of ε_{por} values in the water column can be linked directly to interpretations of the sedimentary record, providing continuity between these types of data.

Because the Lake Erie ε_{por} data directly reflect the relative ratio of cyanobacteria to eukaryotic algae in the total community, the results support the application of ε_{por} as a proxy for the contribution of these groups to primary production and N cycling in aquatic environments. While we chose Lake Erie as a study site specifically because it had a well-defined phytoplankton community, there are many systems where the community is more complex or difficult to define in terms of major contributors to primary production and nitrogen cycling, where ε_{por} could also be a useful tracer. For example, benthic algae in riverbeds are important primary producers but their contribution to riverine food webs is still poorly quantified. Recent work examining the isotopic composition of periphyton in riverbeds noted apparently anomalous enriched chlorophyll $\delta^{15}N$ values relative to bulk $\delta^{15}N$ values (Ishikawa et al., 2015), a signature that we can now verify likely indicates the presence of cyanobacteria. ε_{por} could also be applied in coastal wetlands and estuaries, which are among the most productive ecosystems on Earth; they contain a variety of primary producers that are important in supporting secondary production, including benthic microalgae, as well as both marine and freshwater phytoplankton species. Coastal ecosystems are also vulnerable to anthropogenic changes in nutrient concentration and stoichiometry, with many experiencing community and food web shifts over the past several decades in which the role of N is likely important, but not well quantified (e.g., the San Francisco Bay Estuary; Glibert et al., 2011). Alongside existing stable isotope techniques, ε_{por} could be used to elucidate the relationship between N dynamics and the evolution of phytoplankton communities in these complex ecosystems.

This proxy also has applications in paleoenvironmental studies, and indeed has already been applied to the study of ancient marine sediments as a (paleo)-population tracer (Higgins et al., 2012; Gueneli et al., 2018; Shen et al., 2018). Using ε_{por} for population reconstruction requires accounting for some uncertainty in the obtained values for the cyanobacterial contribution. However, even a relatively rough estimate of community composition can allow the use of isotope mixing models to quantitatively reconstruct N cycling (see Higgins et al., 2012 for an example of this in a marine environment). In addition, the larger isotopic separation between the eukaryotic and the freshwater cyanobacterial endmembers could facilitate a more sensitive utilization of ε_{por} in freshwater sedimentary records, perhaps yielding more accurate population reconstructions than in marine environments. Importantly, the large range of ε_{por} values observed in Lake Erie and the linear relationship between ε_{por} values and the fraction of cyanobacteria in the community implies that the cyanobacterial contribution should still be detectable even in lakes where cyanobacteria do not currently form blooms (e.g., Lake Superior).

A specific application is the use of ε_{por} values from Holocene sediment cores to study the history of cyanobacterial expansion in response to eutrophication and anthropogenic alteration of the N cycle, which is a concern in numerous freshwater lakes around the world. Understanding the relationship between nutrient supply and phytoplankton communities in freshwater environments is critical to understanding the factors that drive phytoplankton community shifts and for water quality management efforts, especially for environments heavily impacted by agriculture and/or the effects of impending climate change. For example, a previous study used bulk ^{13}C and ^{15}N isotope measurements of organic matter in sediment cores to reconstruct historic productivity changes in Lake Ontario due to anthropogenic activities (Hodell and Schelske, 1998). However, the authors were unable to explain whether the observed trends in bulk organic matter $\delta^{15}N$ values after 1970 were due to anthropogenic changes in N sources, phytoplankton community shifts, or both. Use of ε_{por} measurements in this setting could potentially resolve this question.

ε_{por} could also be used to look further back into the pre-anthropogenic past, where the influences of climate and precipitation patterns on the phytoplankton community in lakes are still not

well understood. For example, in Lake Erie, recent work showed the intensity of cyanobacterial blooms to be strongly influenced by spring precipitation events that increase river discharge and therefore nutrient inputs to the lake, a synergistic interaction between agriculture practices and climate change (Michalak et al., 2013). Whether similar relationships existed in the pre-anthropogenic past remains to be explored. The evolution of the phytoplankton community over the last 100 to 150 years in response to eutrophication is mostly inferred through studies utilizing N isotopes of bulk organic matter and the sedimentary record of diatoms (Allinger and Reavie, 2013; Hobbs et al., 2016).

Other paleolimnological proxies such as pigment distributions have also been used to successfully document changes in cyanobacterial abundance in other lakes (Taranu et al., 2015). Because of selective preservation of certain pigment types, however, fossil pigment concentrations in sediments alone may be an unreliable measure of the relative abundance of phytoplankton groups (Leavitt, 1993; Leavitt and Findlay, 1994). In contrast, ε_{por} values provide a more reliable, quantitative proxy for the relative importance of cyanobacteria to primary production, organic matter export, and N cycling, which is preserved in the sediment record.

Although there are clearly environmental exceptions to the patterns defined in cultures that have yet to be explained, it is possible that an intertidal mat (Fulton et al., 2012) and the chemocline of a meromictic lake (Fulton et al., 2018) represent unique biogeochemical habitats for cyanobacteria, such that biosynthesis of chlorophyll is different from the planktonic communities in well-mixed lacustrine environments like the Great Lakes. Future work should aim to sample a diverse range of environments to explore this possibility. The data from Lake Erie suggest, however, that changes in ε_{por} throughout the sediment record in the Great Lakes can be used to indicate how (or if) the relative contribution of cyanobacteria to primary production shifted under pre-anthropogenic conditions in response to climate patterns and nutrient supply and assist in differentiating between natural and anthropogenic influences on phytoplankton community dynamics.

5. Conclusions

This study demonstrates that the chlorophyll ^{15}N fractionation patterns observed in laboratory cultures are also observed in a natural phytoplankton community. ε_{por} values can therefore be used to quantify the relative importance of cyanobacteria and eukaryotic algae in past and present lacustrine environments. Future work should aim to elucidate the biosynthetic or physiological mechanism that underlies these characteristic N isotope patterns among major algal groups, as understanding how phytoplankton differ in their acquisition and intracellular partitioning of N may have both evolutionary and biogeochemical implications.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.orggeochem.2018.12.006.

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