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The relative importance of anammox and denitrification to total N_2 production in Lake Erie

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ABSTRACT

Production of dinitrogen gas via microbially mediated anaerobic ammonium oxidation (anammox) and denitrification plays an important role in removal of fixed N from aquatic ecosystems. Here, we investigated anammox and denitrification potentials via the ^{15}N isotope pairing technique in the helium flushed bottom water (~0.2 m above the sediment) of Sandusky Bay, Sandusky Subbasin, and Central Basin in Lake Erie in three consecutive summers (2010—2012). Potential rates of anammox (0–922 nM/day) and denitrification (1 to 355 nM/day) varied greatly among sampling sites during the 3 years we studied. The relative importance of anammox to total N_2 production potentially ranged from 0 to 100% and varied temporally and spatially. Our study represents one of the first efforts to measure potential activities of both anammox and denitrification in the water column of Lake Erie and our results indicate the Central Basin of Lake Erie is a hot spot for N removal through anammox and denitrification activities. Further, our data indicate that the water column, specifically hypolimnion, and the surface sediment of the Lake Erie Central Basin are comparatively important for microbially mediated N removal.

Introduction

Anaerobic ammonium oxidation (anammox) is a microbially mediated process that regenerates dinitrogen (N_2) from fixed nitrogen (N) in natural environments; this function has previously been thought to be attributable only to denitrification. Since its first discovery in bioreactors of wastewater treatment systems in the 1990s (Mulder et al., 1995; Van de Graaf et al., 1995), anammox has been identified in a variety of environments, including marine, terrestrial, and freshwater ecosystems (e.g. Thamdrup and Dalsgaard, 2002; Rysgaard et al., 2004; Schubert et al., 2006; Crowe et al., 2017).

The number of freshwater studies on the relative contribution of anammox in N removal is consistently increasing (Yoshinaga et al., 2011; Wenk et al., 2013; Zhu et al., 2015). These studies suggest that similar to marine environments (Dalsgaard et al., 2005), anammox is ubiquitous to freshwater systems and its importance to N_2 production may vary spatially and temporally. For example, in a tropical lake (Lake Tanganyika), up to 13% of N_2 production was attributable to anammox (Schubert et al., 2006); whereas this value reached 30% in an alpine lake (Lake Lugano) (Wenk et al., 2013). Temporal variations of anammox activities were identified in a coastal upwelling zone off central Chile, where anammox and denitrification contributed almost equally to N_2 production in spring, but in fall only denitrification was observed (Galán et al., 2014).

In spite of the potential importance of anammox in freshwater systems, our understanding of this process in the Laurentian Great Lakes is very limited (Crowe et al., 2017). Yet, the Great Lakes are the largest freshwater lake system on Earth, accounting for ~17% of the surface freshwater in the world (Reynolds, 1996). Lake Erie, the smallest and shallowest of the Laurentian Great Lakes, has been experiencing cyanobacterial harmful algal blooms (CyanoHABs) with increased frequency, intensity, and spatial extent (Brittain et al., 2000; Ouellette et al., 2006). One consequence of CyanoHABs is formation of transient hypoxic microzones in the usually oxygenated western basin of Lake Erie (Millie et al., 2009). CyanoHABs are also present in the Sandusky Subbasin and Central Basin of Lake Erie (Ouellette et al., 2006), but oxygen limitation there in the hypolimnion is mainly induced by seasonal stratification of the water column (Bouffard et al., 2013). These low oxygen zones and microzones in Lake Erie may serve as suitable habitats for anammox bacteria and denitrifiers that will impact N availability.

N availability is suggested as a co-limiting factor, along with phosphorus availability, for primary production in Lake Erie (North et al., 2007; Chaffin et al., 2013; Steffen et al., 2014). However, unlike the repeatedly identified linkage between phosphorus loading and cyanobacterial growth (Michalak et al., 2013), evidence of N limitation on cyanobacterial biomass is not readily apparent (Kane et al., 2014). N loading in Lake Erie is high and has increased in recent years (Chaffin et al., 2014; Ohio EPA Lake Erie Monitoring Program, http://

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www.epa.ohio.gov/dsw/lakeerie/index.aspx), most likely due to the extensive use of N-rich fertilizers in the Lake Erie catchment (Richards and Baker, 1993; Kumar et al., 2007). Nitrate/nitrite concentration in Lake Erie, however, remained relatively constant (Ohio EPA Lake Erie Monitoring Program). This indicates an increasing N removal from Lake Erie by assimilatory (uptake by phytoplankton and other organisms) and/or dissimilatory (N removal through production of N_2O and N_2) processes. Given the observation of expanding oxygen limiting zones and microzones in Lake Erie, we hypothesized that increased removal of N from Lake Erie is contributed by enhanced reduction of fixed N to N_2 by anammox and/or denitrification. To test this hypothesis, we employed the ^{15}N isotope pairing technique to examine the anammox and denitrification potentials and their potential regulating environmental factors in Lake Erie.

Methods

Sample collection

Water samples were collected once per year from the bottom (~0.2 m above the sediment) of Lake Erie using a peristaltic pump in the Sandusky Bay (SB), Sandusky Subbasin (SS), and Central Basin (CB) in August of three consecutive years, 2010, 2011, and 2012 (Fig. 1). Environmental variables including temperature (T) and dissolved oxygen saturation (DO) were determined in situ with a Hydrolab H_2O multidata Sonde (Hydrolab Corp., Austin, TX, USA).

For the ^{15}N isotope pairing technique, water was immediately transferred to three 250 mL acid washed BOD glass bottles via Tygon tubing (Cole Parmer, Vernon Hills, IL, USA) by placing the tubing at the bottom of the BOD bottles. After the water overflowed ~750 mL, BOD bottles were capped and stored on ice before returning to the lab within 3 h after sample collection. In addition, another 1 L of whole water was filtered through 3 μm and 0.2 μm pore-size membrane filters (Millipore Filter Corp., Bedford. MA, USA). Cells collected on the 0.2 μm filters were frozen at −80 °C before DNA extraction. Filtrates were collected and stored at −20 °C for analysis of dissolved organic carbon (DOC), dissolved nitrogen (DN), nitrate/nitrite (NO_x^-), and ammonium (NH_4^+) concentrations.

^{15}N isotope pairing analysis

Isotope pairing analysis was performed as described previously with minor modifications (Dalsgaard et al., 2003). Briefly, whole water samples (250 mL each) individually received three different sets of ^{15}N compounds, including: 1) 5 μmol of $Na^{15}NO_3$, 2) 2.5 μmol of $^{15}NH_4Cl$; 3) 5 μmol of $Na^{15}NO_3$ + 2.5 μmol of $^{14}NH_4Cl$ (Sigma-Aldrich, Saint Louis, MO, USA; final concentrations were 20, 10 and 20 + 10 μM, respectively). After flushing with helium for 20 min, the amended water filled up a set of exetainer tubes (Labco, High Wycombe, Buckinghamshire, UK). Tubes were incubated in the dark at room temperature (25 °C) for 7 days for water samples from 2010. N_2 production was completed in 2 days for 2010 samples, thus incubation was kept to 2 days for 2011 and 2012 samples. At the beginning and end of the incubation, 9 exetainer tubes (3 each from the three amendments) were sacrificed by replacing 5 mL of water with 5 mL of helium gas followed by $ZnCl_2$ treatment to stop biological activity. Sacrificed tubes were stored at 4 °C until headspace gas analysis. Production of $^{15}N^{14}N$ and $^{15}N^{15}N$ in the headspace of exetainer tubes was measured at the Stable Isotope Facility at the University of California, Davis, USA. Anammox and denitrification N_2 production rate was calculated as described by Thamdrup and Dalsgaard (2002). Due to the possibility that the anammox in the $^{15}NH_4^+$ experiments might have been stimulated by the added ammonium, the concentration of N_2 produced by anammox and denitrification was determined based on the $^{15}NO_3^-$ incubation (Dalsgaard et al., 2003). Rates of anammox and denitrification were calculated from the linear regression of isotopic N_2 concentrations as a function of time. Equations used were:

$$N_2\text{-denitrification} = P_{30} \times {F_N}^{-2},$$

$$N_2\text{-anammox} = {F_N}^{-1}\left[P_{29} + 2 \times \left(1 - {F_N}^{-1}\right) \times P_{30}\right],$$

where N_2-denitrification represents the N_2 production by denitrification, N_2-anammox represents the N_2 production by anammox, F_N represents the ^{15}N fraction in NO_3^-, it is calculated for each sample based on measured nitrate concentration: $F_N = [^{15}N] / ([^{15}N] + [\text{nitrate}])$, and P_{29} and P_{30} represent the determined total mass of $^{29}N_2$ and $^{30}N_2$ production, respectively (Thamdrup and Dalsgaard, 2002).

Environmental variable analysis

DOC and DN were determined with a Shimadzu TOC/TN analyzer (TOC-VCPN; Shimadzu Corp., Tokyo, Japan) based on combustion oxidation/infrared detection and combustion chemiluminescence detection methods, respectively (Clesceri et al., 1999). Concentrations of NO_x (NO_3- + NO_2^-) were measured using a cadmium reduction method with a Lachat (QuikChem FIA+ 8000Series, Loveland, CO, USA) (Clesceri et al., 1999). Concentrations of NH_4^+ were determined with a spectrometer based on color reactions (Strickland and Parsons, 1968).

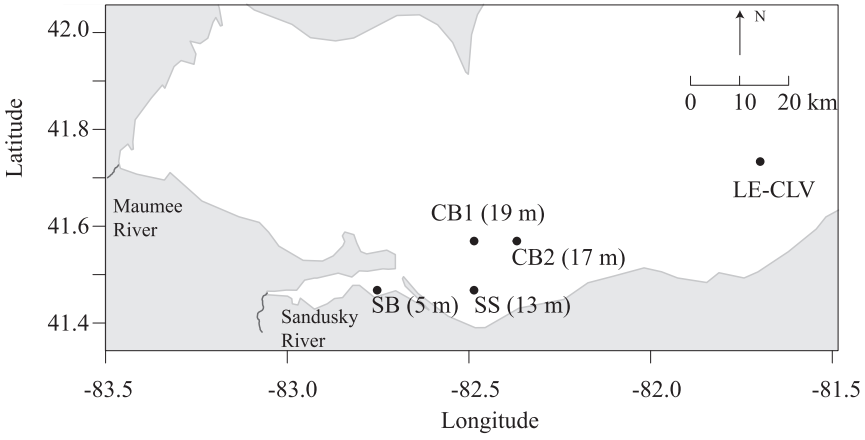

Fig. 1. The sampling sites in SB, SS, CB1, and CB2 of Lake Erie in August of 2010, 2011, and 2012. The depth of water column at each site is listed in the parentheses. The location of NOAA-GLERL LE-CLV buoy (45164) is also marked in the map.

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