Biogeochemistry (2016) 131:303–319
DOI 10.1007/s10533-016-0280-y

Ecosystem metabolism and greenhouse gas production in a mesotrophic northern temperate lake experiencing seasonal hypoxia

Kateri R. Salk · Peggy H. Ostrom · Bopaiah A. Biddanda · Anthony D. Weinke · Scott T. Kendall · Nathaniel E. Ostrom

Received: 9 June 2016 / Accepted: 18 November 2016 / Published online: 2 December 2016

Abstract Many lacustrine systems, despite management efforts to control eutrophication, are hypoxic during stratified periods. Hypoxia is a major concern, not only for its impact on aquatic life but also for its potential to stimulate production of the greenhouse gases, methane (CH_4) and nitrous oxide (N_2O). We investigated the drivers of hypoxia in Muskegon Lake, a temperate dimictic freshwater estuary that experiences frequent hypolimnetic mixing due to atmospheric forces, riverine inputs, and intrusion of oxic water from coastal upwelling in Lake Michigan. Primary production and respiration (R) rates obtained from a $\delta^{18}O$ mass balance model were similar to other mesotrophic environments (0.56–26.31 and 0.57–13.15 mmol O_2 m^{-3} day^{-1}, respectively), although high P/R ($\geq$2 in mid-summer) indicated there is sufficient autochthonous production to support hypoxia development and persistence. The isotopic enrichment factor for respiration (ε_{obs}) varied markedly and was least negative in August of both sampling years, consistent with high R rates. Hypoxic conditions were associated with accumulation of N_2O but not CH_4, and emissions of N_2O are among the highest reported from lakes. The average N_2O site preference value of 25.4‰ indicates that the majority of N_2O was produced by nitrification via hydroxylamine oxidation, despite the presence of resilient hypoxia. While it has been hypothesized that denitrification acts as a sink for N_2O in hypoxic lakes, it is clear that Muskegon Lake functions as a strong source of N_2O via nitrification. Further considerations of lakes as global sources of N_2O thus warrant a closer evaluation of nitrification-fueled N_2O production.

Keywords Hypoxia · Nitrous oxide · Site preference · Methane · Nitrification · Ecosystem metabolism

Responsible Editor: Sujay Kaushal.

Electronic supplementary material The online version of this article (doi:10.1007/s10533-016-0280-y) contains supplementary material, which is available to authorized users.

K. R. Salk · P. H. Ostrom · N. E. Ostrom (✉)
Department of Integrative Biology, Michigan State University, East Lansing, MI 48824, USA
e-mail: ostromn@msu.edu

B. A. Biddanda · A. D. Weinke · S. T. Kendall
Annis Water Resources Institute, Grand Valley State University, Muskegon, MI 49441, USA

Introduction

Hypoxia is a major water quality concern for inland waters and is often accompanied by nuisance algal blooms, declines in fisheries, and decreased recreational value (Diaz and Rosenberg 2008). The development of hypoxia is primarily a product of several physical and biological factors in lakes and coastal oceans: (1) a stratified water column, (2) basin shape, and (3) respiration (R) in excess of O_2 supply. Stratification occurs as surface waters become warmer

than bottom waters, and a thermocline isolates the cooler lower water column (hypolimnion) from wind-driven mixing with the atmosphere. When stratification minimizes the influx of O_2 to the hypolimnion, sinking primary production or sediment organic matter drives respiratory O_2 demand. This leads to a depletion of O_2 to hypoxic levels (<63 μM) (CENR 2000). Nutrient-rich agricultural runoff stimulates primary production (GPP) and subsequent R in lakes and coastal environments, which has led to an increase in the extent, intensity, and duration of hypoxia in aquatic systems worldwide (Livingstone and Imboden 1996; Jankowski et al. 2006; Diaz and Rosenberg 2008; Rabalais et al. 2010; Friedrich et al. 2014). Lake Erie and the Gulf of Mexico are examples of two systems in which hypoxia is enhanced when the water column is stratified in the shallow portions of their basins and GPP is high (Rabalais et al. 2010; Scavia et al. 2014). Ecosystem metabolism, often characterized by the ratio of GPP to R (P/R), can thus be an important indicator of the propensity of a system to develop hypoxia (Hanson et al. 2003; Williamson et al. 2008). Systems with high P/R generally display a high degree of eutrophy (del Giorgio and Peters 1994), and given requisite physical conditions, are prone to hypoxia.

Changes in land use and nonpoint source pollution have resulted in increases in the incidence of hypoxia in freshwaters, raising concerns about the production of greenhouse gases, particularly CH_4 and N_2O (Kaushal et al. 2014). Because both CH_4 and N_2O have anaerobic production pathways, there is potential for these gases to accumulate in the hypoxic or anoxic hypolimnia of stratified lakes and be emitted rapidly during storm-driven or seasonal periods of mixing (Michmerhuizen et al. 1996; Riera et al. 1999; Bastviken et al. 2004). CH_4 and N_2O have global warming potentials that are 25 and 298 times higher than CO_2 over a period of 100 years, respectively (IPCC 2007). Thus, small increases in the atmospheric emissions of one or both of these gases could have a dramatic impact on the greenhouse gas budget from lakes. Despite this possibility, CH_4 and N_2O emissions from lakes are markedly understudied relative to CO_2 (Seitzinger et al. 2006; IPCC 2013). Measurements of the rates of atmospheric N_2O emissions from lakes are particularly sparse, with fewer than ten published studies (Lemon and Lemon 1981; Huttunen et al. 2003; Wang et al. 2006; Whitfield et al. 2011; Miettinen et al. 2015; Yang et al. 2015). However, N_2O emissions from lakes have the potential to rival those from reservoirs, rivers, streams, and wetlands (Whitfield et al. 2011; Morse et al. 2012; Audet et al. 2014; Beaulieu et al. 2014), which merits a closer examination of the role of lakes in the global N_2O budget.

Determining the N_2O budget from lakes depends on an understanding of the microbial pathway by which it is produced. N_2O is produced via three microbial processes that occur under a range of redox conditions: denitrification, nitrifier-denitrification, and hydroxylamine oxidation (Wrage et al. 2001). Denitrification is generally restricted to very low O_2 levels, and as a heterotrophic process, requires a carbon supply (Knowles 1982). The microbial denitrification pathway involves a stepwise reduction of nitrate (NO_3^-) to N_2 gas, in which nitrite (NO_2^-), nitric oxide (NO) and N_2O are produced as intermediates. It is estimated that 7–16% of nitrogen applied to the terrestrial biosphere is denitrified in lakes (Seitzinger et al. 2006), of which a small yet variable portion (typically 0.1–6.0%) is not completely reduced to N_2 and released as N_2O (Seitzinger and Kroeze 1998). Nitrifiers reduce NO_2^- by the same pathway as denitrifiers (nitrifier-denitrification), producing N_2O as an intermediate product (Poth and Focht 1985; Wrage et al. 2001). In the third pathway, nitrifiers produce N_2O as a product of the decomposition of hydroxylamine (NH_2OH) (Wrage et al. 2001). This process is autotrophic and, in contrast to denitrification, can occur in oxic environments. In pure cultures of nitrifiers, the production of N_2O is maximized as O_2 declines, although production ceases under anoxic conditions (Goreau et al. 1980; Frame and Casciotti 2010). Therefore, hypoxia provides the ideal conditions for maximal N_2O production, although the precise microbial production mechanism involved is often uncertain.

Stable isotope analyses of N_2O are used to distinguish N_2O production mechanisms (Ostrom and Ostrom 2011). The relative abundance of a stable isotope within a particular material or reservoir is reported in standard delta notation:

$$\delta = \frac{R_{sam} - R_{std}}{R_{std}} \times 1000 \quad (1)$$

where R_{sam} is the isotope ratio of the sample, R_{std} is the isotope ratio of the standard, and δ is reported as per mil (‰). Because N_2O is a linear asymmetric molecule, the isotopic composition of the N atom in the central position (α) and the N atom in the outer position (β) may be distinct. The difference between

$\delta^{15}N^{\alpha}$ and $\delta^{15}N^{\beta}$ in N_2O is referred to as site preference (SP). In contrast to bulk $\delta^{15}N$ and $\delta^{18}O$ values, SP is a conservative tracer of microbial N_2O production mechanisms that is independent of the substrate isotopic composition (Sutka et al. 2003, 2006, 2008; Toyoda et al. 2005). In general, denitrification and nitrifier-denitrification produce N_2O with constant SP values of −10 to 0‰, whereas hydroxylamine oxidation produces N_2O with a SP value of 33–37‰ (Toyoda et al. 2005; Sutka et al. 2006; Frame and Casciotti 2010; Ostrom and Ostrom 2011). N_2O derived from denitrification and nitrifier-denitrification is indistinguishable on the basis of SP, likely because the enzymes catalyzing these processes are identical (Stein 2011). Therefore, production of N_2O from denitrification and nitrifier-denitrification will henceforth be referred to collectively as denitrification. Fractionation in SP has been demonstrated with a purified fungal NO reductase enzyme but not within microbial culture which likely reflects maintenance of NO in cells at low concentration and steady state (Yang et al. 2014). Therefore, SP is expected to hold as a conservative, non-fractionating measure of N_2O production mechanism under in situ environmental conditions. SP analysis has been demonstrated as an effective approach for distinguishing microbial N_2O production from denitrification versus hydroxylamine oxidation in a variety of terrestrial and aquatic environments (e.g., Westley et al. 2006; Yamagishi et al. 2007; Opdyke et al. 2009; Sasaki et al. 2011).

In this study, we examined the relationships among ecosystem metabolism, hypoxia, and greenhouse gas production and atmospheric emissions in Muskegon Lake, MI. The relationship between P/R, the oxygen isotopic enrichment factor for respiration (ε_{obs}), and the development of hypoxia was also determined. In addition, we evaluated the influence of hypoxia and water column mixing on the production and atmospheric fluxes of CH_4 and N_2O. Further, we determined the microbial pathway of N_2O production in Muskegon Lake through SP analysis and related biogeochemical indicators.

Methods

Study site

Muskegon Lake is a 17 km^2 drowned river-mouth lake in western Michigan with a mean depth of 7 m and a maximum depth of 23 m (Freedman et al. 1979; Carter et al. 2006). The lake drains the Muskegon River Watershed, a 7032 km^2 basin comprised mainly of forest and agricultural land (Tang et al. 2005), and discharges directly into Lake Michigan by means of a narrow shipping channel (Fig. 1). The hydraulic residence time of Muskegon Lake is highly dependent on the rate of water inflow from the Muskegon River, ranging from 14 to 70 days with a mean of 23 days (Freedman et al. 1979; Carter et al. 2006). Historical nutrient loading led to eutrophication and water quality degradation of Muskegon Lake, and as a consequence it was designated a Great Lakes Area of Concern in 1985 (Steinman et al. 2008; U.S. EPA 2013). There has been subsequent improvement in water quality, but Muskegon Lake remains characterized by relatively high nutrient concentrations, periodic nuisance algal blooms, and summer hypoxia in the hypolimnion (Steinman et al. 2008; Biddanda 2012).

Buoy observatory measurements

Time series data for water temperature (2, 4, 6, 7, 9, and 11 m), dissolved O_2, pH, chlorophyll *a* fluorescence (2, 5, 8, and 11 m), and wind speed (1 m above water) were obtained for the period of May to November in 2012 and 2013 from the Muskegon Lake Observatory (MLO). The MLO buoy was deployed in the central part of the lake (Fig. 1) and collected water and meteorological data during the ice-free periods. The water depth at the MLO was approximately 12 and 11.25 m in 2012 and 2013, respectively, due to differences in lake water level between the two years. Water sensors at the MLO collected data every 15 to 30 min, and meteorological sensors collected data every 5 min. Additional information on the MLO can be found at www.gvsu.edu/buoy/ and in Biddanda (2012), McNair et al. (2013), and Vail et al. (2015).

Field sampling procedure

Water samples for nutrient, P/R, and greenhouse gas analysis were collected at the MLO (Fig. 1) between 9:00 am and 12:00 noon on an approximately monthly basis from May to September in 2012 and 2013. In addition, intense temporal sampling was conducted

Fig. 1 Muskegon Lake. *Inset* shows the location of Muskegon Lake (*star*) and its watershed (*light gray*) within the state of Michigan, USA. The *white circle* in the middle of the lake represents the location of the Muskegon Lake Observatory Buoy (MLO; www.gvsu.edu/buoy/)

three times over a 4-day period in August 2013. Water samples were taken by Niskin bottle (General Oceanics, Inc., Miami, FL) at 2, 5, 8, and 11 m (2012) or 10.25 m (2013) depth. For nutrient analysis, water was filtered (0.45 μm) and frozen upon return to the lab. Water for determination of $\delta^{18}O$–O_2 and greenhouse gas analyses was transferred into 250 mL glass serum bottles and sealed without headspace with butyl rubber septa. Biological activity was halted by adding 1 mL of saturated $HgCl_2$ solution to each bottle. Water column profiles of temperature, dissolved O_2, pH, and chlorophyll *a* fluorescence were taken using a YSI 6600 sonde (Yellow Springs Instruments, Inc., Yellow Springs, OH).

Ecosystem metabolism measurements

Respiration rates were determined from lake water collected at 2 m using a Niskin bottle. Water from the Niskin bottle was placed into a 20 L carboy and subsequently dispensed into 300 mL acid-washed BOD bottles that were sealed with glass stoppers without headspace. The BOD bottles were incubated in the dark at in situ temperature. Dissolved O_2 concentrations were then measured in triplicate each day for four days from sacrificed bottles. O_2 concentration was measured via Winkler autotitration using a Radiometer Analytical Titralab 650 with platinum combined Ag/AgCl reference electrode (Weinke et al. 2014).

The $\delta^{18}O$ of dissolved O_2 was determined using water from 2, 5, 8, and 11 m into which $HgCl_2$ was added at the time of collection (2012) or from water at 2 m that was preserved with $HgCl_2$ at the same time points as the BOD bottles (2013). Water was transferred from BOD bottles into pre-evacuated 200 mL glass vessels fitted with high vacuum stopcocks according to Emerson et al. (1991) and Roberts et al. (2000). The vessels were stored at room temperature for at least 4 h to allow the water and headspace to come to equilibrium. The headspace was then introduced to an evacuated 3 mL sampling loop and then onto a 5 m packed molecular sieve (5 Å) column (Alltech, Inc., Deerfield, IL) using He carrier gas within a gas chromatograph (HP-5980, Hewlett Packard, Ramsey, MN) interfaced to an Isoprime isotope ratio mass spectrometer (Elementar Americas, Inc., Mount Laurel, NJ) for determination of the $\delta^{18}O$ of O_2. Analytical reproducibility of standards was 0.3‰.

The oxygen isotopic enrichment factor for respiration, ε_{obs}, was determined using a Rayleigh model from two sets of data, (1) in situ observations of $\delta^{18}O$–O_2 by depth in 2012 and (2) four-day bottle incubations from which $\delta^{18}O$–O_2 was measured in 2013:

$$\varepsilon_{obs} = \frac{\delta_{so} - \delta_s}{ln(O_2Sat)} \quad (2)$$

where and δ_s is the in situ $\delta^{18}O$–O_2 value or the $\delta^{18}O$–O_2 value at a given time point in a bottle incubation, δ_{so} is the initial $\delta^{18}O$–O_2 value, and O_2Sat is the fractional saturation of O_2 (Mariotti et al. 1981; Ostrom et al. 2014). When $ln(O_2Sat)$ is regressed against δ_s, the slope is equivalent to ε_{obs}.

Rates of GPP, R, and P/R ratios in the epilimnion were estimated using a steady-state, mass balance model (hereafter the ^{18}O model; Bocaniov et al. 2012):

$$GPP = \left(\frac{F}{Z_m}\right) \times \frac{O_2(\alpha_g \cdot {}^{18:16}O - \alpha_r \cdot {}^{18:16}O) - O_{2s}(\alpha_g \cdot \alpha_s \cdot {}^{18:16}O_a - \alpha_r \cdot {}^{18:16}O)}{\alpha_p \cdot {}^{18:16}O_w - \alpha_r \cdot {}^{18:16}O} \quad (3)$$

$$R = \left(\frac{F}{Z_m}\right) \times \frac{O_2(\alpha_g \cdot {}^{18:16}O - \alpha_p \cdot {}^{18:16}O_w) - O_{2s}(\alpha_g \cdot \alpha_s \cdot {}^{18:16}O_a - \alpha_p \cdot {}^{18:16}O_w)}{\alpha_p \cdot {}^{18:16}O_w - \alpha_r \cdot {}^{18:16}O} \quad (4)$$

where Z_m is the depth of the mixed layer, O_2 is the measured concentration of dissolved O_2, O_{2s} is the concentration of O_{2-} at atmospheric saturation, $^{18:16}O$ is the measured oxygen isotope ratio of dissolved O_2, $^{18:16}O_w$ is the measured oxygen isotope ratio of H_2O, and $^{18:16}O_a$ is the isotopic ratio of atmospheric oxygen (23.5‰). α_g, α_s, α_p, and α_r are the fractionation factors associated with gas transfer (0.9972), gas solubility in water (Benson and Krause 1984), photosynthetic reaction rates of ^{18}O–H_2O to ^{16}O–H_2O (1.000), and respiration ($1 + \varepsilon_{obs}/1000$), respectively. $\delta^{18}O$–H_2O was determined by off-axis integrated cavity output spectroscopy using a Los Gatos Research Liquid Water Isotope Analyzer (Lis et al. 2008). Analytical reproducibility of standards was 0.2‰. When direct measurements of $\delta^{18}O$–H_2O were not available, mean values from the remainder of the sampling period were used.

The oxygen gas transfer rate (F) was calculated according to Wanninkhof (1992):

$$F = k_w(C_w - C_a) \quad (5)$$

where C_w is the dissolved O_2 concentration at the surface (measured at 2 m depth) and C_a is the calculated dissolved O_2 concentration in equilibrium with the atmosphere (Wanninkhof 1992; Walker et al. 2010). The gas transfer coefficient (k_w, in m s^{-1}) is calculated as:

$$k_w = 0.31U_{10}^2\left(\frac{Sc}{600}\right)^{-1/2} \quad (6)$$

where Sc is the Schmidt number for O_2 determined by the kinematic viscosity of freshwater divided by the diffusion coefficient of O_2 (Wanninkhof 1992) and

U_{10} is the wind speed 10 m above the surface determined using the measured wind speed 1 m above the surface and the power law relationship outlined in Walker et al. (2010). Wind speed was measured by the MLO and averaged over the dissolved O_2 residence time in the mixed layer (2–5 days). Because buoy data were not available on 6 May 2013, wind speed data were obtained from instruments at the Muskegon County Airport (8 km from MLO) and adjusted by the roughness lengths of flat land and water (World Meteorological Organization 2008).

Nutrient concentrations and trace gas analysis

Samples collected in 2013 were analyzed for the concentration of nitrate + nitrite (NO_3^-), ammonium (NH_4^+), and soluble reactive phosphorus (SRP). All nutrient analyses were performed according to Standard Methods (APHA 2005). SRP was analyzed spectrophotometrically using the ascorbic acid method, NO_3^- was determined colorimetrically after cadmium reduction, and NH_4^+ was analyzed using the phenate method. NO_3^- and NH_4^+ were analyzed using a wet chemistry continuous flow analyzer (Skalar Analytical B.V., Breda, The Netherlands).

Water samples for CH_4 and N_2O concentration analysis were introduced by syringe injection at atmospheric pressure into glass serum bottles that had been flushed with He prior to sample introduction. The water sample was equilibrated with the remaining headspace overnight by gentle shaking. The headspace was then analyzed by GC-ECD-FID (Shimadzu Greenhouse Gas Analyzer GC-2014, Shimadzu Scientific Instruments, Columbia, MD) for N_2O and CH_4 concentration. The dissolved concentration was calculated based on the headspace equilibrium concentration (Hamilton and Ostrom 2007). Diffusive atmospheric emissions of CH_4 and N_2O (F) were calculated by Eqs. 5 and 6, substituting the measured concentration, saturation concentration, and Schmidt number of CH_4 and N_2O for those of dissolved O_2.

The isotopic composition of N_2O was analyzed upon introduction of sample water into an enclosed 0.75 L glass vessel that was previously purged of atmospheric air using a gentle flow of He. Dissolved gases were subsequently stripped from the water by sparging the sample with He (Sansone et al. 1997), which carried sample gases into a Trace Gas sample introduction system interfaced to an Isoprime isotope ratio mass spectrometer (Elementar Americas, Inc., Mount Laurel, NJ). Analytical reproducibility for replicate samples was 0.5‰ for bulk $\delta^{15}N$ and $\delta^{18}O$, 0.75‰ for $\delta^{15}N_\alpha$ $\delta^{15}N_\beta$, and 1.3‰ for SP.

The microbial origin of N_2O produced in Muskegon Lake was evaluated by several approaches. First, a Keeling plot was employed to calculate the isotopic composition of microbially-produced N_2O (end-member) by regressing the isotopic ratios of N_2O versus the inverse concentration of N_2O in measured samples and the atmospheric end-member (Pataki et al. 2003; Yamagishi et al. 2007). Second, apparent oxygen utilization (AOU; $O_{2saturation} - O_{2measured}$) and ΔN_2O ($N_2O_{measured} - N_2O_{saturation}$) were compared to provide evidence for nitrification-fueled N_2O production (Yoshinari 1976; Nevison et al. 2003; Bange et al. 2010). Third, $\Delta^{18}O$ ($\delta^{18}O–N_2O - \delta^{18}O–O_2$) was calculated to evaluate the relative contributions of hydroxylamine oxidation and denitrification (Ostrom et al. 2000).

Statistical analyses

All statistical analyses were conducted using R statistical software (version 3.0.2). Principal component analysis was performed on 2013 data to analyze correlation among temperature, pH, greenhouse gas concentrations, dissolved O_2, nutrient concentrations, and chlorophyll *a* fluorescence (Online Resource). Prior to principal component analysis, each variable was standardized to equalize variance across variables. Differences in N_2O isotopic composition between the epilimnion and hypolimnion were evaluated by Welch 2-sample *t* tests following tests for distribution normality and equal variance. The relationship between AOU and ΔN_2O was evaluated by linear regression.

Results

Muskegon Lake displays a dimictic stratification pattern, with periods in the spring and fall of homogenous temperature and O_2 concentration throughout the water column (Fig. 2). The summer period is characterized by higher surface water temperatures than in the spring and fall and low concentrations of O_2 in the hypolimnion. When the water column is stratified, the thermocline is located

between 6 and 8 m. A weak to strong thermocline was present on all sampling dates except 6 June 2012, 20 September 2012, and 11 June 2013 (Fig. 3a, e). June sampling dates coincided with wind-driven episodic mixing events, and 20 September 2012 coincided with the fall mixing period. O_2 concentrations in the hypolimnion during stratified periods regularly decreased to hypoxic levels (Figs. 2, 3b, f).

Epilimnetic GPP and R rates obtained by the ^{18}O model ranged from 0.56–26.31 mmol O_2 m^{-3} day^{-1} and 0.57–13.15 mmol O_2 m^{-3} day^{-1}, respectively (Fig. 4). The lowest GPP and R rates occurred during September 2012 and May 2013. The highest GPP rate occurred in August of both years, and the highest R rate occurred in August 2012 and September 2013. P/R ranged from 0.79–2.36, with the highest ratios occurring in August in each year and the lowest ratios occurring during May, June, and September (Fig. 4).

The isotopic enrichment factor for respiration, ε_{obs}, varied by over 10‰ within each sampling season (Table 1). ε_{obs} was most negative in June 2012, May 2013, and September 2013. ε_{obs} was least negative in August of both years. The range in ε_{obs} values was less negative in 2012 when the in situ method was used than in 2013 when the bottle incubation was used.

NH_4^+, NO_3^-, and SRP concentrations ranged from 2.3–44.5, 163.3–373.7, and 0.8–17.5 μg L^{-1}, respectively (Online Resource—Fig. A1). NH_4^+, NO_3^-, and SRP concentrations were negatively correlated with O_2 concentration and chlorophyll *a* fluorescence (Online Resource—Fig. A2), indicating an accumulation of nutrients in the hypolimnion.

CH_4 and N_2O were supersaturated in the water column at all times (1.1–4.8 and 3700–30,000 times atmospheric equilibration concentration, respectively). CH_4 was fairly homogeneous throughout the water column on each sampling date with the exception of a single extremely high concentration at the lowest sampling depth in September 2012 (Fig. 3c). Excluding this point, the highest concentrations of

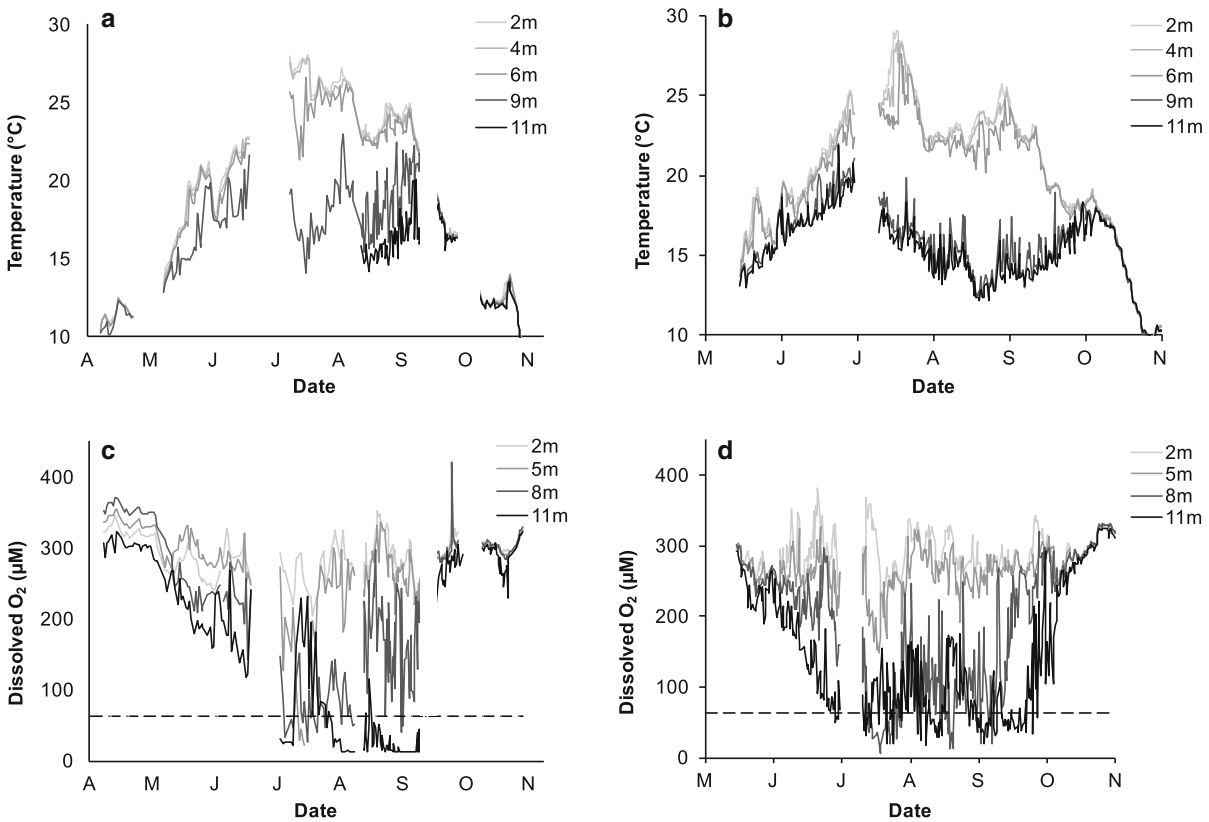

Fig. 2 **a**, **b** Temperature and **c**, **d** dissolved O_2 concentration from April to November in 2012 (*left panel*) and 2013 (*right panel*). Data were collected by the MLO sensors placed at various depths in the water column. Hypoxia (dissolved $O_2 < 63$ μM) is indicated by a *dotted line*

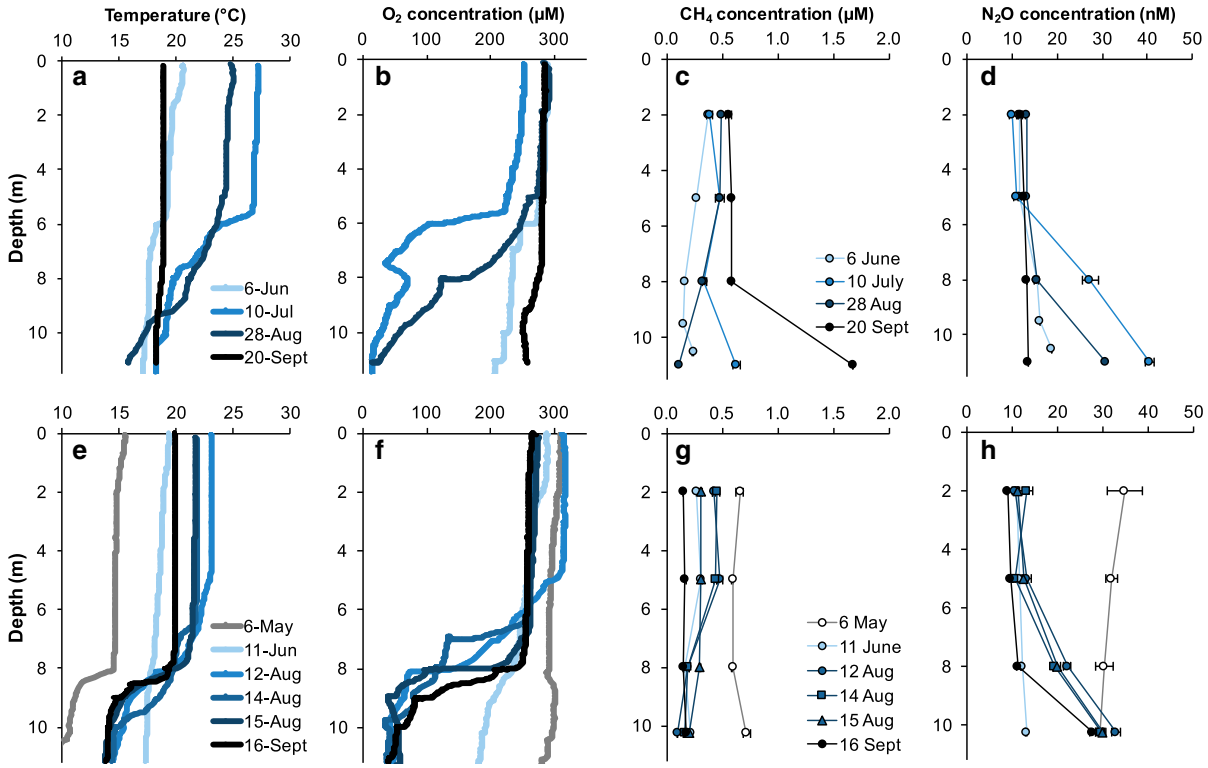

Fig. 3 Water column profiles at the MLO in **a–d** 2012 and **e–h** 2013. Temperature and dissolved O_2 concentration were measured continuously throughout the water column on each sampling date. CH_4 and N_2O concentrations were measured at four depths on each sampling date. *Error bars* represent standard error

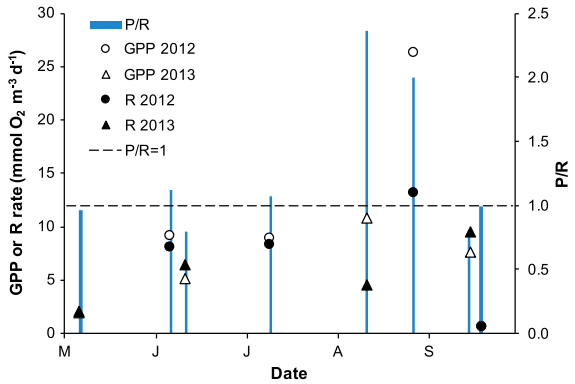

Fig. 4 Primary production (GPP), respiration (R), and P/R in Muskegon Lake in 2012 and 2013. P and R rates (*open* and *closed symbols*, respectively) were calculated according to Bocaniov et al. (2012) on four occasions each year from May through September. P/R ratios (*bars*) were calculated by dividing P by R. The *horizontal line* represents P/R = 1

CH_4 throughout the water column occurred in May 2013 (Fig. 3g). N_2O concentrations were also high throughout the water column in May 2013 (Fig. 3h).

Table 1 ε_{obs} values in Muskegon Lake measured by the in situ (2012) and bottle incubation (2013) methods

Date	ε_{obs} (‰)	Method
6-Jun-12	−16.1	In situ
10-Jul-12	−7.9	In situ
28-Aug-12	−2.3	In situ
20-Sep-12	−4.1	In situ
6-May-13	−21.1	Bottle incubation
12-Aug-13	−11.3	Bottle incubation
16-Sep-13	−23.7	Bottle incubation

Hypolimnetic N_2O concentration were negatively associated with O_2 concentration, displaying accumulation in the hypolimnion during hypoxic periods (Fig. 3).

CH_4 and N_2O atmospheric emissions by diffusive evasion were low yet variable throughout the majority of sampling dates in both years with the exception of two dates during which emissions were 10–100 times

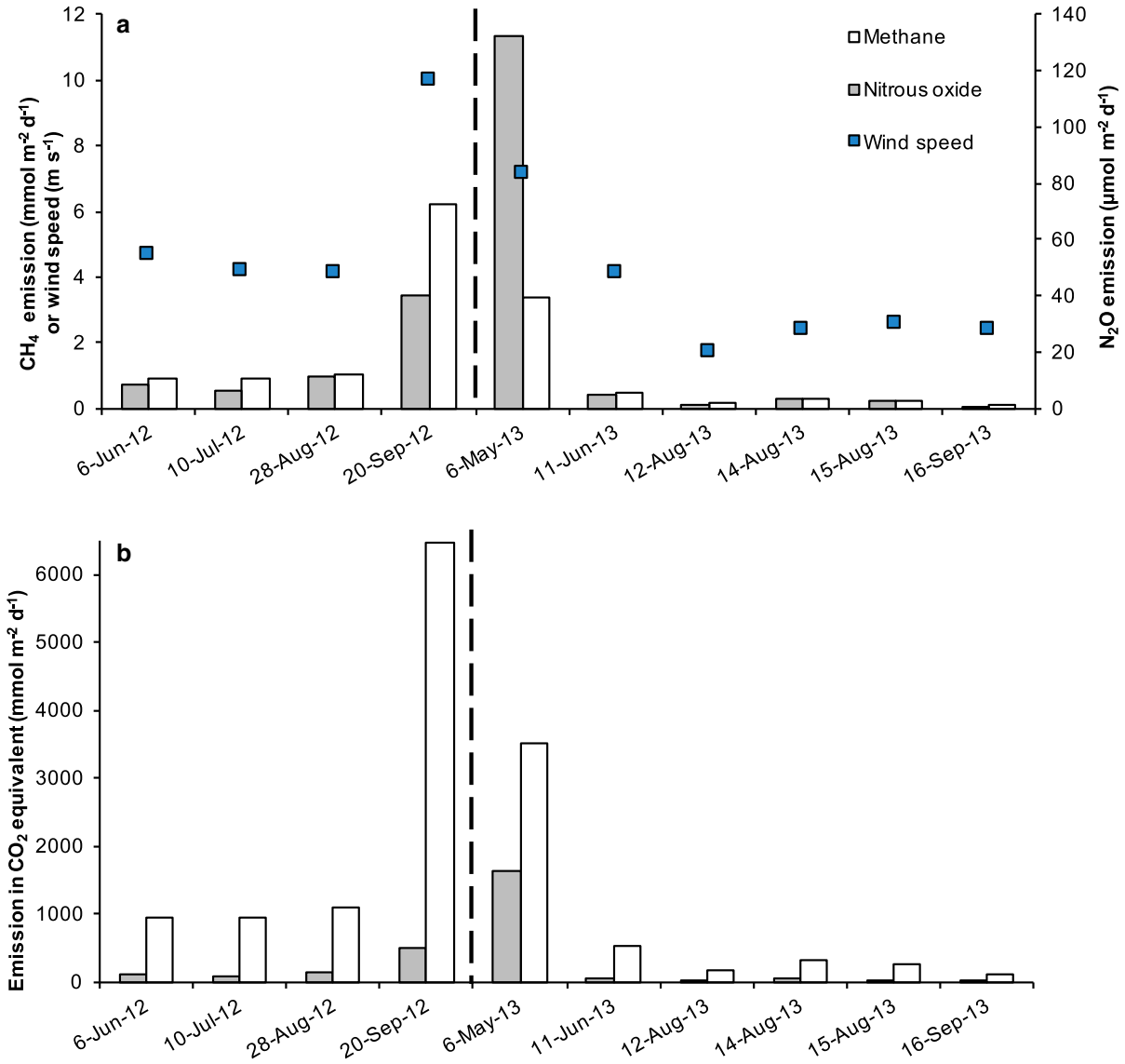

Fig. 5 **a** CH_4 and N_2O emissions and wind speeds for each sampling date in 2012 and 2013 and **b** CH_4 and N_2O emissions converted to CO_2 equivalent for each date. Wind speed was measured by the MLO. CH_4 and N_2O emissions were multiplied by their radiative forcing to obtain CO_2 equivalents (IPCC 2007). The *vertical dashed line* represents the division between the two sampling years

higher than other sampling dates (Fig. 5a). The first period of high emissions occurred in September 2012 during a period of strong wind, and the second occurred in May 2013 when concentrations of both gases were high throughout the water column. When converted to CO_2 equivalents by multiplying emissions by 298 and 25 for N_2O and CH_4, respectively (IPCC 2007), the radiative forcing of CH_4 emission was 2.1–22.9 times that of N_2O (Fig. 5b).

The isotopic composition of N_2O (mean ± SD) in the epilimnion and mixed water column ($\delta^{15}N$: 4.5 ± 0.7‰, $\delta^{18}O$: 50.1 ± 1.9‰, SP: 20.0 ± 2.4‰) and hypolimnion ($\delta^{15}N$: 0.0 ± 0.3‰, $\delta^{18}O$: 54.3 ± 0.9‰, SP: 23.1 ± 1.8‰) were distinct (Table 2). Relative to the epilimnion and mixed water column, Welch 2 sample *t* tests indicated that hypolimnetic $\delta^{15}N$–N_2O values were significantly lower ($t = 15.02$, $df = 9.9$, $p < 0.0001$), $\delta^{18}O$–N_2O

Table 2 Isotopic composition of N_2O in the troposphere (Yoshida and Toyoda 2000), epilimnion/mixed water column in Muskegon Lake, and hypolimnion of Muskegon Lake

Date	Depth (m)	O_2 (μM)	N_2O (nM)	$\delta^{18}O$–N_2O (‰)	$\delta^{15}N$–N_2O (‰)	$\delta^{18}N_\alpha$–N_2O (‰)	$\delta^{18}N_\beta$–N_2O (‰)	N_2O SP (‰)
Tropospheric N_2O				43.7 ± 0.9	7.0 ± 0.6			18.7 ± 2.2
Epilimnion/mixed water column								
11-Jun-13	2	281	10.96	47.9	5.4	14.4	−3.7	18.1
11-Jun-13	5	259	11.47	48.6	4.8	12.7	−3.1	15.8
11-Jun-13	10.25	188	13.05	51.3	3.5	13.8	−6.7	20.6
12-Aug-13	2	317	10.61	48.4	5.1	14.8	−4.6	19.4
12-Aug-13	5	297	13.09	50.3	4.5	16.4	−7.4	23.8
14-Aug-13	2	264	13.11	53.6	5.2	16.2	−5.9	22.0
14-Aug-13	5	262	10.47	49.7	4.1	14.1	−5.9	20.0
16-Sep-13	8	209	11.42	51.0	3.4	13.5	−6.7	20.2
Mean		*260*	*11.77*	*50.1*	*4.5*	*14.5*	*−5.5*	*20.0*
SD		*43*	*1.14*	*1.9*	*0.7*	*1.3*	*1.5*	*2.4*
Hypolimnion								
12-Aug-13	8	146	22.10	54.9	−0.1	11.0	−12.6	25.0
14-Aug-13	10.25	42	29.80	54.7	−0.4	11.2	−11.9	23.1
15-Aug-13	8	99	20.09	55.2	0.3	12.1	−11.5	23.6
15-Aug-13	10.25	57	29.91	53.4	−0.3	11.5	−12.1	23.6
16-Sep-13	10.25	53	27.66	53.4	0.2	10.2	−9.8	20.0
Mean		*79*	*25.91*	*54.3*	*0.0*	*11.2*	*−11.6*	*23.1*
SD		*43*	*4.54*	*0.9*	*0.3*	*0.7*	*1.0*	*1.8*

values were significantly higher ($t = -5.5$, $df = 10.4$, $p < 0.0001$), and SP values were significantly higher ($t = -2.59$, $df = 10.3$, $p < 0.01$). The $\delta^{15}N$, $\delta^{18}O$, and SP of microbially-produced N_2O in Muskegon Lake, determined by the y-intercepts of the Keeling plot, were −3.2, 58.8, and 25.4‰, respectively (Fig. 6). AOU was positively correlated with ΔN_2O (Online Resource—Fig. A3; linear regression, $R^2 = 0.58$, $t = 6.97$, $df = 35$, $p < 0.0001$). The mean $\Delta^{18}O$ value for N_2O was 22.1 ± 6.0‰.

Discussion

Hypoxia development and ecosystem metabolism

Hypoxia is a consequence of extensive nutrient loading, requires the development of stratification, and is predominant in lakes with shallow hypolimnia. In Muskegon Lake, hypoxia was first documented during a period of extensive nutrient loading the late 1970s (Freedman et al. 1979), but hypoxia may be a natural phenomenon in this system. The water column is stratified from June through September or October, but episodic intrusion of oxygenated Lake Michigan water during westerly seiches is demonstrated by increases in hypolimnetic O_2 concentrations by as much as 150 μM on daily to weekly timescales (Fig. 2; B. Biddanda, A. Weinke, S. Kendall, and D. Koopmans, personal communication). The observed decline of hypolimnetic temperature from the onset of stratification to early August, an unusual trend in stratified systems, provides additional evidence of upwelling-induced intrusion of cold Lake Michigan water into Muskegon Lake. Following such hypolimnetic oxygenation events, O_2 concentrations decline rapidly (often within 12 h) to hypoxic conditions (Fig. 2). Thus, hypoxia exhibits resilience in

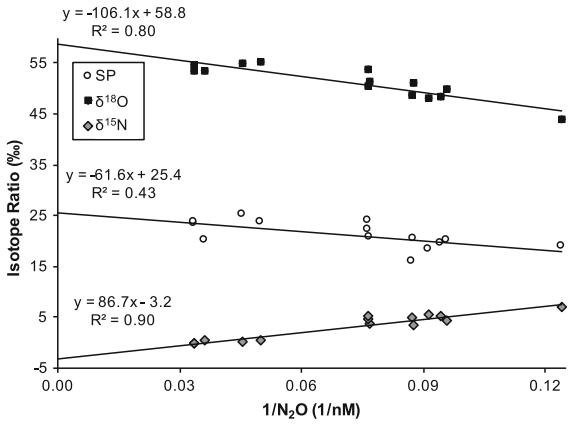

Fig. 6 Linear regression of SP, $\delta^{18}O$, and $\delta^{15}N$ of measured N_2O with the inverse concentration of N_2O at each sampling point. In situ N_2O concentrations and isotopic values are plotted with the atmospheric N_2O concentration and isotopic composition (*rightmost value*; Yoshida and Toyoda 2000). The isotopic composition of microbially produced N_2O is represented as the y-intercept value (Yamagishi et al. 2007)

Muskegon Lake despite a short hydraulic residence time and evidence of the episodic intrusion of cold, oxic water. The persistence of hypoxia in Muskegon Lake points to a strong driving factor for O_2 consumption in the hypolimnion.

The balance of GPP and R and its seasonal variation provides insight into the development of hypoxia. Although rates of GPP and R are lower than many hypoxic systems (Ostrom et al. 2005; Bocaniov et al. 2012), observations of P/R in Muskegon Lake suggest there is sufficient excess primary production to fuel hypolimnetic respiration and hypoxia development. P/R in the epilimnion was ≥ 1 throughout the majority of the sampling period (Fig. 4), demonstrating net autotrophy during the growing season between May and September. Two earlier studies of pelagic metabolism in Muskegon Lake similarly demonstrated a consistent trend of $P/R > 1$ during this time period as well (Weinke et al. 2014; Dila and Biddanda 2015). In particular, GPP was two-fold higher than R in August of both sampling years (2.00 and 2.36 in 2012 and 2013, respectively). A portion of this surplus production from the epilimnion likely sinks into the hypolimnion and fuels water column or sediment respiration in subsequent days to years. Thus, despite evidence of episodic intrusion of oxic water into the hypolimnion, high P/R values indicate a supply of organic matter that reaches the hypolimnion to fuel strong O_2 consumption and persistent hypoxia.

While water column respiration has been demonstrated as the primary driver of hypoxia in systems such as Lake Erie and the Gulf of Mexico (Conroy et al. 2011; McCarthy et al. 2013; Ostrom et al. 2014), respiration in organic matter-rich sediments can enable hypoxia to establish at more moderate nutrient loading than would be expected (Turner et al. 2008). A combination of water column and sediment respiration thus likely fuels hypoxia establishment and resilience in Muskegon Lake. While water quality indicators have neared or exceeded remedial action plan targets for reducing eutrophication (Michigan DEQ 2011), summer hypoxic conditions have persisted and will likely persist for many years. Two potential explanations are that (1) remediation of eutrophication in Muskegon Lake is insufficient to reduce hypoxia or (2) hypoxia is a natural feature of this system.

Seasonal variation in ε_{obs}

The magnitude of isotopic fractionation of O_2 during respiration, ε_{obs}, is generally assumed to be a constant, but substantial variation has been observed in this study and other estuarine and coastal marine ecosystems (Quiñones-Rivera et al. 2007; Lehman et al. 2009; Fry and Boyd 2010; Ostrom et al. 2014). Isotopic fractionation during respiration takes place when O_2 is consumed by oxidative respiratory enzymes such as cytochrome oxidase (Feldman et al. 1959). If diffusion limits the supply of O_2 to the respiratory enzyme, then the expression of isotopic fractionation by the enzyme is reduced (Ostrom et al. 2014). For instance, isotopic discrimination approaching 0‰ has been observed for respiration in sediments where diffusion is expected to be slow, limiting the movement of O_2 into the cell (Brandes and Devol 1997). In contrast, isotopic discrimination in the water column is generally quite large and assumed to be constant (e.g., −21.2‰), because diffusion is not expected to limit the supply of O_2 to the respiratory enzyme (Quiñones-Rivera et al. 2007; Fry and Boyd 2010). In Muskegon Lake, ε_{obs} was evaluated by two approaches: bottle incubation and the in situ method. ε_{obs} values obtained by bottle incubation reflect only water column respiration, whereas ε_{obs} values obtained by the in situ method reflect both water column and sediment respiration. Indeed, the ε_{obs} values obtained from the in situ method were less negative than those obtained from the bottle incubation method (Table 1), likely reflecting a greater influence

of sediment respiration on the ε_{obs} values than in the bottle method. Nonetheless, values for ε_{obs} obtained by the bottle incubation method varied by over 10‰, even in the absence of sediment respiration and were least negative during the summer stratified period in Muskegon Lake. This is in agreement with Ostrom et al. (2014), who proposed that ε_{obs} in the water column might in fact approach 0‰ when R rates are high and that ε_{obs} may correlate with rates of total respiration beneath the thermocline (water column and sediment respiration). Low ε_{obs} values in August are thus indicative of high R, supported by the highest water column R observed at this time in 2012 (Fig. 4).

The observation of marked seasonal variation in ε_{obs} indicates that ε_{obs} in the water column should not be assumed to be constant, as is often the case. For example, if an ε_{obs} value of −21.2‰ had been incorporated into the ^{18}O model rather than the measured value of −11.3‰ in August 2013, the GPP and R rates for Muskegon Lake would have been overestimated by factors of 2.0 and 3.3, respectively. Further, the subsequent calculation of P/R would have yielded a value of 1.41 rather than 2.36. Accurate assessments of ecosystem metabolism by models that utilize ε_{obs} thus require that variation in ε_{obs} be incorporated into P/R models.

Greenhouse gas concentrations and emissions

On a global basis, freshwater ecosystems support a variety of microbial processes that emit CH_4 and N_2O, important greenhouse gases (IPCC 2007). Fluxes of greenhouse gases arising from the lower water column and sediments vary as a function of wind mixing and with the development and deterioration of thermal stratification (e.g., Fallon et al. 1980). In many systems, CH_4 accumulates in anoxic waters beneath the thermocline as a result of anaerobic methanogenesis in both the water column and sediments (Bastviken et al. 2004). This suggests that O_2 and CH_4 should be negatively correlated. Contrary to observations in other temperate dimictic lakes (Fallon et al. 1980; Striegl and Michmerhuizen 1998), profiles of CH_4 in Muskegon Lake revealed no apparent connection between hypoxic conditions and high CH_4 concentrations (Fig. 3). With the exception of one date that occurred during a seasonal mixing event near the sediment–water interface (20 September 2012), accumulation of CH_4 within the hypolimnion was not observed in Muskegon Lake. This lack of hypolimnetic accumulation could relate to low CH_4 production, elevated oxidation of CH_4, or evolution of CH_4 to upper water column and atmosphere (Fallon et al. 1980; Striegl and Michmerhuizen 1998; Huttunen et al. 2006).

Despite the lack of hypolimnetic accumulation, diffusive CH_4 emissions from Muskegon Lake were comparable in magnitude and range to those in other lakes of similar size (Bastviken et al. 2004; Ortiz-Llorente and Alvarez-Cobelas 2012). Owing to strong winds and high epilimnetic concentrations, high emissions of CH_4 were observed in September 2012 and May 2013 (Figs. 3c, g, 5a). While CH_4 emissions were measured at a single location in Muskegon Lake and thus provide limited ability to extrapolate to the lake as a whole, our results indicate that sampling on a seasonal basis is important to capture variability in CH_4 emissions from lakes.

In contrast to CH_4, hypolimnetic production of N_2O was evident by a negative correlation between N_2O and O_2 (Online Resource—Fig. A2), indicating that periods of hypoxia and water column turnover could be strong regulators of N_2O emissions from this system. Indeed, N_2O was markedly supersaturated in the hypoxic hypolimnion, potentially setting the stage for high emissions to the atmosphere during periods of water column turnover. Diffusive emissions of N_2O measured at the MLO were lowest during stratified periods and highest during the development or breakdown of stratification. Periods of exceptionally high emissions, up to two orders of magnitude greater than those during the stratified period, are among the highest reported for natural lakes (Lemon and Lemon 1981; Huttunen et al. 2003; Wang et al. 2006; Whitfield et al. 2011; Miettinen et al. 2015; Yang et al. 2015). In fact, only one study has reported higher atmospheric fluxes of N_2O than those observed in Muskegon Lake (Wang et al. 2006). While limited spatial sampling restricts the ability to evaluate the absolute N_2O emissions from Muskegon Lake, our results illustrate wide variation in N_2O fluxes on seasonal time scales. Total annual fluxes may thus be dominated by a few brief periods of high emissions during mixing events following hypolimnetic accumulation (Fig. 5a), emphasizing the importance of temporal sampling to capture important periods of N_2O emissions from lakes.

When diffusive emissions of CH_4 and N_2O are converted into CO_2 equivalents (IPCC 2007), the radiative forcing of CH_4 always exceeded that of N_2O

in Muskegon Lake (Fig. 5b). However, the radiative forcing of N_2O in this study closely approached that of CH_4 on several occasions, indicating that N_2O is an important contributor to total greenhouse gas emissions from this environment. Nonetheless, the number of publications reporting lacustrine CH_4 emissions is over six-fold greater than those reporting N_2O emissions (Whitfield et al. 2011; Ortiz-Llorente and Alvarez-Cobelas 2012), reflecting a need for greater attention to N_2O in studies that measure greenhouse gas emissions.

Determination of microbial origin of N_2O

The marked differences in the $\delta^{15}N$, $\delta^{18}O$, and SP of N_2O between epilimnetic/mixed water column samples and hypolimnetic samples point to distinct sources, namely atmospheric exchange and microbial N_2O production, respectively (Table 2). It is therefore expected that the isotope composition of N_2O samples collected from Muskegon Lake reflects a mixture between atmospheric and microbially derived N_2O, with microbial production stimulated by hypoxic conditions in the hypolimnion (Goreau et al. 1980; Knowles 1982). Treating the atmospheric isotopic composition (Table 2; Yoshida and Toyoda 2000) and the microbial isotopic N_2O composition (unknown) as isotopic end members in Muskegon Lake, regressing the isotopic composition of measured N_2O by the inverse N_2O concentration yields the isotope composition of microbially-produced N_2O as the y-intercept (Fig. 6; Pataki et al. 2003; Yamagishi et al. 2007). While $\delta^{15}N$–N_2O (−3.2‰) and $\delta^{18}O$–N_2O (58.8‰) values obtained from the mixing model are heavily dependent on the isotopic composition of source material, SP (25.4‰) has been shown to be a conservative tracer, enabling the distinction of production from hydroxylamine oxidation (nitrification) from that by denitrification (Ostrom and Ostrom 2011). SP was therefore used as the primary indicator of the microbial N_2O production mechanism in this study. When SP values of 33 and −10 to 0‰ are considered as end-members for N_2O production via hydroxylamine oxidation and denitrification, respectively (Sutka et al. 2006; Frame and Casciotti 2010), the observed SP of 25.4‰ indicates that 77–82% of in situ production of N_2O is derived from hydroxylamine oxidation.

Because reduction of N_2O by denitrification is likely under low-oxygen conditions and has a marked fractionation effect on its isotopic composition (Popp et al. 2002; Westley et al. 2006; Ostrom et al. 2007; Yamagishi et al. 2007), reduction must be considered as a possible influence on SP in Muskegon Lake that may bias interpretations of microbial sources. If substantial reduction occurs, $\delta^{15}N$ and $\delta^{18}O$ values of N_2O tend to positively correlate because the conversion of N_2O to N_2 preferentially leaves ^{15}N and ^{18}O in the residual N_2O. Similarly, N_2O reduction results in elevated SP values in the residual N_2O pool because the isotopic discrimination is more pronounced in the α than the β position (Westley et al. 2006). However, a negative correlation was found between $\delta^{15}N$–N_2O and $\delta^{18}O$–N_2O in Muskegon Lake (Online Resource—Fig. A4a; linear regression, $R^2 = 0.68$, $t = 4.87$, $df = 11$, $p < 0.001$), which suggests that N_2O reduction is not an important process. Moreover, while the $\delta^{18}O$–N_2O and SP values were positively correlated, the observed slope of 0.78 differed substantially from the expected slope of 0.45 arising from the fractionation of N_2O during reduction (Online Resource—Fig. A4b) (Ostrom et al. 2007; Opdyke et al. 2009). Therefore, we conclude on the basis of stable isotopic indicators that N_2O reduction was not important in removing N_2O from Muskegon Lake, supporting our interpretation of hydroxylamine oxidation as the predominant N_2O production pathway on the basis of SP. Further, while it has been posited that lakes may act as sinks for N_2O under anoxic conditions (Lemon and Lemon 1981; Beaulieu et al. 2014, 2015), N_2O reduction was not demonstrated as an appreciable pathway in Muskegon Lake. A potential explanation for the absence of appreciable N_2O reduction is the evidence of intrusion of oxic water into the hypolimnion; although hypoxia reestablishes quickly in this system, complete anoxia does not develop for long periods, which could inhibit the complete reduction of N_2O by denitrification.

Stoichiometric relationships can also be examined to determine N_2O production pathways. A positive relationship between AOU and ΔN_2O has been attributed to N_2O production by nitrification, as AOU is a tracer of organic matter remineralization that produces the substrates for nitrification (Yoshinari 1976; Nevison et al. 2003; Bange et al. 2010). Indeed, AOU was positively correlated with ΔN_2O, and NO_3^- and NH_4^+ concentrations increased in the hypolimnion throughout the stratified period (Online Resource—Figs. A1, A2), providing a further indication that

nitrification is the primary N_2O production mechanism under hypoxic conditions in Muskegon Lake.

Additional insight into the microbial origin of N_2O can be provided by its $\delta^{18}O$ composition. The oxidation of NH_4^+ to hydroxylamine incorporates an initial oxygen atom from O_2, and further oxidation to NO_3^- derives oxygen from H_2O (Dua et al. 1979; Hollocher et al. 1981; Andersson and Hooper 1983; Kumar et al. 1983). N_2O production by hydroxylamine oxidation therefore reflects the isotopic composition of O_2, whereas denitrification produces N_2O with a $\delta^{18}O$ value influenced both by O_2 and H_2O. Given that O_2 is generally enriched in ^{18}O by at least 23.5‰ relative to H_2O, $\Delta^{18}O$ values are high when hydroxylamine oxidation dominates and are lower when denitrification dominates. For example, Ostrom et al. (2000) observed a shift in $\Delta^{18}O$ values from approximately 23‰ at the surface to approximately 13‰ at 300 m depth in the Pacific Ocean that was interpreted as a transition in N_2O production from hydroxylamine oxidation to denitrification. Similarly, Muskegon Lake $\Delta^{18}O$ values (22.1 ± 6.0‰) are consistent with production via hydroxylamine oxidation.

The microbial origin of N_2O from hydroxylamine oxidation was confirmed by multiple lines of isotopic and stoichiometric evidence, demonstrating that Muskegon Lake is as a nitrification-driven source of N_2O to the atmosphere. Previous studies have shown that denitrification can dominate N_2O production in some eutrophic lakes (Lemon and Lemon 1981; Wang et al. 2006) and that increased availability of organic carbon and nutrients has the capacity to stimulate denitrification (Taylor and Townsend 2010). Further, Beaulieu et al. (2014, 2015) have demonstrated that reservoirs have the capacity to alternate between a source and a sink of N_2O depending on the degree of hypolimnetic anoxia and the timing of water column turnover. Our results conversely indicate the capacity for ample supplies of organic matter and nutrients to fuel N_2O production via denitrification lakes is not ubiquitous. Together, this body of work demonstrates that N_2O dynamics in lakes are more complicated than previously thought, and constraining the role of lakes in the global N_2O budget will entail (1) distinguishing between nitrification and denitrification pathways of N_2O production, (2) evaluating N_2O reduction as a potential sink for N_2O, and (3) investigating water column turnover events as opportunities for brief yet intense periods of N_2O emissions.

Conclusion

Hypoxia persists in Muskegon Lake despite episodic intrusions of oxic water from Lake Michigan into the hypolimnion. We demonstrate net autotrophy in the epilimnion and substantial variation in ε_{obs} in late summer, consistent with delivery of autotrophic material to the hypolimnion followed by high rates of respiration. The presence of hypoxia in Muskegon Lake not only supports N_2O production via nitrification but also results in exceptionally high N_2O emissions to the atmosphere, among the highest reported from lakes, during seasonal water column mixing events. A complete understanding of the impact of hypoxic lakes on the global greenhouse budget will rely on quantification of atmospheric fluxes, particularly during seasonal transitions in stratification. This study adds to the emerging narrative of inland waters as sources of globally significant greenhouse gas emissions to the atmosphere.

Acknowledgements Many thanks to Hasand Gandhi, Leon Gereaux, Adam McMillan, and Alex Dutcher for their assistance in the field and lab. This manuscript has benefitted greatly through feedback from Al Steinman and Steve Hamilton. This material is based upon work supported by the National Science Foundation Graduate Research Fellowship Program under Grant No. DGE1424871 to KRS, the Environmental Protection Agency Great Lakes Restoration Initiative Muskegon Lake Observatory under Grant No. GL00E00460-1 to BAB, and a Michigan State University Water Research Initiation Grant to PHO, NEO, and BAB. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the Environmental Protection Agency or the National Science Foundation.

References

Andersson KK, Hooper AB (1983) O_2 and H_2O are each the source of one O in NO_2^- produced from NH_3 by *Nitrosomonas*: ^{15}NMR evidence. FEBS Lett 164(2):236–240. doi:10.1016/0014-5793(83)80292-0

APHA (2005) Standard methods for the examination of water and wastewater. American Public Health Association, Washington, DC

Audet J, Hoffmann CC, Andersen PM, Baattrup-Pedersen A, Johansen JR, Larsen SE, Kjaergaard Elsgaard L (2014) Nitrous oxide fluxes in undisturbed riparian wetlands located in agricultural catchments: emission, uptake, and controlling factors. Soil Biol Biochem 68:291–299. doi:10.1016/j.soilbio.2013.10.011

Bange H, Freing A, Kock A, Löscher C (2010) Marine pathways to nitrous oxide. In: Smith K (ed) Nitrous oxide and climate change. Earthscan, London, pp 36–62

Bastviken D, Cole J, Pace M, Tranvik L (2004) Methane emissions from lakes: dependence of lake characteristics, two regional assessments, and a global estimate. Glob Biogeochem Cycles 18:GB4009. doi:10.1029/2004GB002238

Beaulieu JJ, Smolenski RL, Nietch CT, Townsend-Small A, Elovitz MS, Schubauer-Berigan JP (2014) Denitrification alternates between a source and sink of nitrous oxide in the hypolimnion of a thermally stratified reservoir. Limnol Oceanogr 59(2):495–506. doi:10.4319/lo.2014.59.2.0495

Beaulieu JJ, Nietch CT, Young JL (2015) Controls of nitrous oxide production and consumption in reservoirs of the Ohio River Basin. J Geophys Res Biogeosci 120:1995–2010. doi:10.1002/2015JG002941

Benson BB, Krause D Jr (1984) The concentration and isotopic fractionation of oxygen dissolved in freshwater and seawater in equilibrium with the atmosphere. Limnol Oceanogr 29(3):620–632

Biddanda BA (2012) Lake sentinel: observatory for ecosystem changes in Muskegon Lake. InterChange, Newsletter of the Regional Math and Science Center, Grand Valley State University. http://www.gvsu.edu/rmsc/interchange/2012-may-connections-awri-651.htm

Bocaniov SA, Schiff SL, Smith REH (2012) Plankton metabolism and physical forcing in a productive embayment of a large oligotrophic lake: insights from stable oxygen isotopes. Freshw Biol 57:481–496. doi:10.1111/j.1365-2427.2011.02715.x

Brandes JA, Devol AH (1997) Isotopic fractionation of oxygen and nitrogen in coastal marine sediments. Geochim Cosmochim Ac 61(9):1793–1801. doi:10.1016/S0016-7037(97)00041-0

Carter GS, Nalepa TF, Rediske RR (2006) Status and trends of benthic populations in a coastal drowned river mouth lake of Lake Michigan. J Great Lakes Res 32:578–595

CENR (2000) Integrated assessment of hypoxia in the Northern Gulf of Mexico. National Science and Technology Council Committee on Environment and Natural Resources, Washington, DC

Conroy JD, Boegman L, Zhang H, Edwards WJ, Culver DA (2011) "Dead Zone" dynamics in Lake Erie: the importance of weather and sampling intensity for calculated hypolimnetic oxygen depleted rates. Aquat Sci 73(2):289–304. doi:10.1007/s00027-010-0176-1

del Giorgio PA, Peters RH (1994) Patterns in planktonic P:R ratios in lakes: influence of lake trophy and dissolved organic carbon. Limnol Oceanogr 39(4):772–787. doi:10.4319/lo.1994.39.4.0772

Diaz RJ, Rosenberg R (2008) Spreading dead zones and consequences for marine ecosystems. Science 321:926–929. doi:10.1126/science.1156401

Dila DK, Biddanda BA (2015) From land to lake: contrasting microbial processes across a Great Lakes gradient of carbon and nutrient inventories. J Great Lakes Res 41:75–85

Dua RD, Bhandari B, Nicholas DJD (1979) Stable isotope studies on the oxidation of ammonia to hydroxylamine by *Nitrosomonas europaea*. FEBS Lett 106(2):401–404. doi:10.1016/0014-5793(79)80541-4

Emerson S, Quay P, Stump C, Wilbur D, Knox M (1991) O_2, Ar, N_2, and ^{222}Rn in surface waters of the subarctic ocean: net biological O_2 production. Glob Biogeochem Cycles 5(1):49–69. doi:10.1029/90GB02656

Fallon RD, Harrits S, Hanson RS, Brock TD (1980) The role of methane in internal carbon cycling in Lake Mendota during summer stratification. Limnol Oceanogr 25(2):357–360. doi:10.4319/lo.1980.25.2.0357

Feldman DE, Yost HT Jr, Benson BB (1959) Oxygen isotope fractionation in reactions catalyzed by enzymes. Science 129:146–147

Frame CH, Casciotti KL (2010) Biogeochemical controls and isotopic signatures of nitrous oxide production by a marine ammonia-oxidizing bacterium. Biogeosciences 7:2695–2709. doi:10.5194/bg-7-2695-2010

Freedman P, Canale R, Auer M (1979) The impact of wastewater diversion spray irrigation on water quality in Muskegon County lakes. Rep. 905/9–79–006-A. U.S. Environmental Protection Agency, Washington, DC

Friedrich J, Janssen F, Aleynik D et al (2014) Investigating hypoxia in aquatic environments: diverse to addressing a complex phenomenon. Biogeosciences 11:1215–1259. doi:10.5194/bg-11-1215-2014

Fry B, Boyd B (2010) Oxygen concentration and isotope studies of productivity and respiration on the Louisiana continental shelf, July 2007. In: Ohkouchi N et al (eds) Earth, life, and isotopes. Kyoto University Press, Kyoto, pp 223–241

Goreau TJ, Kaplan WA, Wofsy SC, McElroy MB, Valois FW, Watson SW (1980) Production of NO_2^- and N_2O by nitrifying bacteria at reduced concentrations of oxygen. Appl Environ Microbiol 40(3):526–532

Hamilton SK, Ostrom NE (2007) Measurements of the stable isotope ratio of dissolved N_2 in ^{15}N tracer experiments. Limnol Oceanogr Methods 5:233–240

Hanson PC, Bade DL, Carpenter SR, Kratz TK (2003) Lake metabolism: relationships with dissolved organic carbon and phosphorus. Limnol Oceanogr 48(3):1112–1119. doi:10.4319/lo.2003.48.3.1112

Hollocher TC, Tate ME, Nicholas DJ (1981) Oxidation of ammonia by *Nitrosomonas europaea*. Definite ^{18}O-tracer evidence that hydroxylamine formation involves a monooxygenase. J Biol Chem 256:10834–10836

Huttunen JT, Juutinen S, Alm J, Larmola T, Hammar T, Silvola J, Martikainen PJ (2003) Nitrous oxide flux to the atmosphere from the littoral zone of a boreal lake. J Geophys Res 108(D14):4421. doi:10.1029/2002JD002989

Huttunen JT, Väisänen TS, Hellsten SK, Martikainen PJ (2006) Methane fluxes at the sediment-water interface in some boreal lakes and reservoirs. Boreal Environ Res 11:27–34

IPCC (2007) Climate Change 2007: the physical science basis. In: Solomon S et al (eds) Contribution of working group I to the fourth assessment report of the Intergovernmental Panel on Climate Change. Cambridge University Press, New York

IPCC (2013) Climate Change 2013: the physical science Basis. In: Stocker TF et al (eds) Contribution of working group I to the fifth assessment report of the Intergovernmental Panel on Climate Change. Cambridge University Press, New York

Jankowski T, Livingstone DM, Bührer H, Forster R, Niederhauser P (2006) Consequences of the 2003 European heat wave for lake temperature profiles, temperature stability, and hypolimnetic oxygen depletion: implications for a warmer world. Limnol Oceanogr 51(2):815–819. doi:10.4319/lo.2006.51.2.0815

Kaushal SS, Mayer PM, Vidon PG, Smith RM, Pennino MJ, Newcomer TA, Duan S, Welty C, Belt KT (2014) Land use and climate variability amplify carbon, nutrient, and contaminant pulses: a review with management implications. U.S. Environmental Protection Agency Paper 234

Knowles R (1982) Denitrification. Microbiol Rev 46(1):43–70

Kumar S, Nicholas DJD, Williams EH (1983) Definitive ^{15}N NMR evidence that water serves as a source of 'O' during nitrite oxidation by *Nitrobacter agilis*. FEBS Lett 152(1):71–74. doi:10.1016/0014-5793(83)80484-0

Lehman MF, Barnett B, Gélinas Y, Gilbert D, Maranger R, Mucci A, Sundby B, Thibodeau B (2009) Aerobic respiration and hypoxia in the Lower St. Lawrence Estuary: stable isotope ratios of dissolved oxygen constrain oxygen sink partitioning. Limnol Oceanogr 54(6):2157–2169

Lemon E, Lemon D (1981) Nitrous oxide in freshwaters of the Great Lakes Basin. Limnol Oceanogr 26(5):867–879. doi:10.4319/lo.1981.26.5.0867

Lis G, Wassenaar LI, Hendry MJ (2008) High-precision laser spectroscopy D/H and $^{18}O/^{16}O$ measurements of microliter natural water samples. Anal Chem 80(1):287–293. doi:10.1021/ac701716q

Livingstone DM, Imboden DM (1996) The prediction of hypolimnetic oxygen profiles: a plea for a deductive approach. Can J Fish Aquat Sci 53:924–932. doi:10.1139/f95-230

Mariotti A, Germon JD, Hubert P, Kaiser P, Letolle R, Tardieux A, Tardieux P (1981) Experimental determination of nitrogen kinetic isotope fractionation: some principles: illustration for the denitrification and nitrification processes. Plant Soil 62(3):413–430. doi:10.1007/BF02374138

McCarthy MJ, Carini SA, Liu Z, Ostrom NE, Gardner WS (2013) Oxygen consumption in the water column and sediments of the Gulf of Mexico hypoxic zone. Estuar Coast Shelf S 123:46–53. doi:10.1016/j.ecss.2013.02.019

McNair JN, Gereaux LC, Weinke AD, Sesselmann MR, Kendall ST, Biddanda BA (2013) New methods for estimating components of lake metabolism based on free-water dissolved-oxygen dynamics. Ecol Model 263:251–263. doi:10.1016/j.ecolmodel.2013.05.010

Michigan DEQ (2011) Stage 2 remedial action plan Muskegon Lake Area of Concern. Michigan Department of Environmental Quality, Lansing

Michmerhuizen CM, Striegl RG, McDonald ME (1996) Potential methane emission from north-temperate lakes following ice melt. Limnol Oceanogr 41(5):985–991. doi:10.4319/lo.1996.41.5.0985

Miettinen H, Pumpanen J, Heiskanen JJ, Aaltonen H, Mammarella I, Ojala A, Levula J, Rantakari M (2015) Towards a more comprehensive understanding of lacustrine greenhouse gas dynamics—two-year measurements of concentrations and fluxes of CO_2, CH_4, and N_2O in a typical boreal lake surrounded by managed forests. Boreal Environ Res 20:75–89

Morse JL, Ardón M, Bernhardt ES (2012) Greenhouse gas fluxes in southeastern U.S. coastal plain wetlands under contrasting land uses. Ecol Appl 22(1):264–280

Nevison C, Butler JH, Elkins JW (2003) Global distribution of N_2O and the ΔN_2O-AOU yield in the subsurface ocean. Glob Biogeochem Cycles 17(4):1119. doi:10.1029/2003GB002068

Opdyke MR, Ostrom NE, Ostrom PH (2009) Evidence for the predominance of denitrification as a source of N_2O in a temperate agricultural soils based on isotopologue measurements. Glob Biogeochem Cycles 23:GB4018. doi:10.1029/2009GB003523, 10.1002/2015GL066235

Ortiz-Llorente MJ, Alvarez-Cobelas M (2012) Comparison of biogenic methane emissions from unmanaged estuaries, lakes, oceans, rivers and wetlands. Atmos Environ 59:328–337. doi:10.1016/j.atmosenv.2012.05.031

Ostrom NE, Ostrom PH (2011) The isotopomers of nitrous oxide: analytical considerations and application to resolution of microbial production pathways. In: Baskaran M (ed) Handbook of environmental isotope geochemistry. Springer, New York, pp 453–476

Ostrom NE, Russ ME, Popp B, Rust TM, Karl DM (2000) Mechanisms of nitrous oxide production in the subtropical North Pacific based on determinations of the isotopic abundances of nitrous oxide and di-oxygen. Chemosphere 2:281–290. doi:10.1016/S1465-9972(00)00031-3

Ostrom NE, Carrick HJ, Twiss MR, Piwinski L (2005) Evaluation of primary production in Lake Erie by multiple proxies. Oecologia 144:115–124. doi:10.1007/s00442-005-0032-5

Ostrom NE, Pitt A, Sutka R, Ostrom PH, Grandy AS, Huizinga KM, Robertson GP (2007) Isotopologue effects during N_2O reduction in soils and in pure cultures of denitrifiers. J Geophys Res 112:G02005. doi:10.1029/2006JG000287

Ostrom NE, Gandhi H, Kamphuis B, DeCamp S, Liu Z, McCarthy MJ, Gardner WS (2014) Oxygen metabolism and water mass mixing in the northern Gulf of Mexico hypoxic zone in 2010. Geochim Cosmochim Acta 140:39–49. doi:10.1016/j.gca.2014.05.020

Pataki DE, Ehleringer JR, Flanagan LB, Yakir D, Bowling DR, Still CJ, Buchmann N, Kaplan JO, Berry JA (2003) The application and interpretation of Keeling plots in terrestrial carbon cycle research. Glob Biogeochem Cycles 17(1):1022. doi:10.1029/2001GB001850

Popp BN, Westley MB, Toyoda S, Miwa T, Dore JE, Yoshida N, Rust TM, Sansone FJ, Russ ME, Ostrom NE, Ostrom PH (2002) Nitrogen and oxygen isotopomeric constraints on the origins and sea-to-air flux of N_2O in the oligotrophic subtropical North Pacific gyre. Glob Biogeochem Cycles 16(4):1064. doi:10.1029/2001GB001806

Poth M, Focht DD (1985) ^{15}N kinetic analysis of N_2O production by *Nitrosomonas europaea*: an examination of nitrifier denitrification. Appl Environ Microbiol 49(5):1134–1141

Quiñones-Rivera ZJ, Wissel B, Justić D, Fry B (2007) Partitioning oxygen sources and sinks in a stratified, eutrophic coastal ecosystem using stable oxygen isotopes. Mar Ecol Prog Ser 342:69–83. doi:10.3354/meps342069

Rabalais NN, Diaz RJ, Levin LA, Turner RE, Gilbert D, Zhang J (2010) Dynamics and distribution of natural and human-caused hypoxia. Biogeosciences 7:585–619. doi:10.5194/bg-7-585-2010

Riera JL, Schindler JE, Kratz TK (1999) Seasonal dynamics of carbon dioxide and methane in two clear-water lakes and two bog lakes in northern Wisconsin, U.S.A. Can J Fish Aquat Sci 56:265–274. doi:10.1139/f98-182

Roberts BJ, Russ ME, Ostrom NE (2000) Rapid and precise determination of the $\delta^{18}O$ of dissolved gaseous dioxygen via gas chromatography-isotope ratio mass spectrometry.

Environ Sci Technol 34(11):2337–2341. doi:10.1021/es991109d

Sansone FJ, Popp BN, Rust TM (1997) Stable carbon isotopic analysis of low-level methane in water and gas. Anal Chem 69(1):40–44. doi:10.1021/ac960241i

Sasaki Y, Koba K, Yamamoto M, Makabe A, Ueno Y, Nakagawa M, Toyoda S, Yoshida N, Yoh M (2011) Biogeochemistry of nitrous oxide in Lake Kizaki, Japan, elucidated by nitrous oxide isotopomers analysis. J Geophys Res 116:G04030. doi:10.1029/2010JG001589

Scavia D, Allan JD, Arend KK et al (2014) Assessing and addressing the re-eutrophication of Lake Erie: central basin hypoxia. J Great Lakes Res 40:226–246. doi:10.1016/j.jglr.2014.02.004

Seitzinger SP, Kroeze C (1998) Global distribution of nitrous oxide production and N inputs in freshwater and coastal marine ecosystems. Glob Biogeochem Cycles 12(1):93–113

Seitzinger S, Harrison JA, Böhlke JK, Bouwman AF, Lowrance R, Peterson B, Tobias C, Van Drecht G (2006) Denitrification across landscapes and waterscapes: a synthesis. Ecol Appl 16(6):2064–2090

Stein LY (2011) Surveying N_2O-producing pathways in bacteria. In: Klotz MG (ed) Methods in Enzymology, vol 486. Elsevier Academic Press, San Diego, pp 131–152

Steinman AD, Ogdahl M, Rediske R, Ruetz CR III, Biddanda BA, Nemeth L (2008) Current status and trends in Muskegon Lake, Michigan. J Great Lakes Res 34(1):169–188. doi:10.3394/0380-1330(2008)34[169:CSATIM]2.0.CO;2

Striegl RG, Michmerhuizen CM (1998) Hydrologic influence on methane and carbon dioxide dynamics at two north-central Minnesota lakes. Limnol Oceanogr 43(7):1519–1529

Sutka RL, Ostrom NE, Ostrom PH, Gandhi H, Breznak JA (2003) Nitrogen isotopomer site preference of N_2O produced by *Nitrosomonas europaea* and *Methylococcus capsulatus* Bath. Rapid Commun Mass Spectrom 17(7):738–745. doi:10.1002/rcm.968

Sutka RL, Ostrom NE, Ostrom PH, Breznak JA, Gandhi H, Pitt AJ, Li F (2006) Distinguishing nitrous oxide production from nitrification and denitrification on the basis of isotopomer abundances. Appl Environ Microbiol 72(1):638–644. doi:10.1128/AEM.72.1.638-644.2006

Sutka RL, Adams GC, Ostrom NE, Ostrom PH (2008) Isotopologue fractionation during N_2O production by fungal denitrification. Rapid Commun Mass Spectrom 22(24):3989–3996. doi:10.1002/rcm.3820

Tang Z, Engel BA, Pijanowski BC, Lim KJ (2005) Forecasting land use change and its environmental impact at a watershed scale. J Environ Manag 76:35–45. doi:10.1016/j.jenvman.2005.01.006

Taylor PG, Townsend AR (2010) Stoichiometric control of organic carbon-nitrate relationships from the soil to the sea. Nature 464:1178–1181. doi:10.1038/nature08985

Toyoda S, Mutobe H, Yamagishi H, Yoshida N, Tanji Y (2005) Fractionation of N_2O isotopomers during production by denitrifier. Soil Biol Biochem 37(8):1535–1545. doi:10.1016/j.soilbio.2005.01.009

Turner RE, Rabalais NN, Justic D (2008) Gulf of Mexico hypoxia: alternate states and a legacy. Environ Sci Technol 42:2323–2327. doi:10.1021/es071617k

U.S. EPA (2013) Great lakes areas of concern: Muskegon Lake. U.S. Environmental Protection Agency, Washington, DC

Vail J, Meyer A, Weinke A, Biddanda BA (2015) Water quality monitoring: lesson plan for exploring time-series data. J Mich Sci Teach Assoc 60(1):37–48

Walker JT, Stow CA, Geron C (2010) Nitrous oxide emissions from the Gulf of Mexico hypoxic zone. Environ Sci Technol 44(5):1617–1623. doi:10.1021/es902058t

Wang H, Wang W, Yin C, Wang Y, Lu J (2006) Littoral zones as the "hotspots" of nitrous oxide (N_2O) emission in a hyper-eutrophic lake in China. Atmos Environ 40:5522–5527. doi:10.1016/j.atmosenv.2006.05.032

Wanninkhof R (1992) Relationship between wind speed and gas exchange over the ocean. J Geophys Res 97(C5):7373–7382. doi:10.1029/92JC00188

Weinke AD, Kendall ST, Kroll DJ, Strickler EA, Weinert ME, Holcomb TM, Defore AA, Dila DK, Snider MJ, Gereaux LC, Biddanda BA (2014) Systematically variable planktonic carbon metabolism along a land-to-lake gradient in a Great Lakes coastal zone. J Plankton Res 36:1528–1542. doi:10.1093/plankt/fbu066

Westley MB, Yamagishi H, Popp BN, Yoshida N (2006) Nitrous oxide cycling in the Black Sea inferred from stable isotope and isotopomers distributions. Deep Sea Res Pt II 53:1802–1816. doi:10.1016/j.dsr2.2006.03.012

Whitfield CJ, Aherne J, Baulch HM (2011) Controls on greenhouse gas concentrations in polymictic headwater lakes in Ireland. Sci Total Environ 410–411:217–225. doi:10.1016/j.scitotenv.2011.09.045

Williamson CE, Dodds W, Kratz TK, Palmer MA (2008) Lakes and streams as sentinels of environmental change in terrestrial and atmospheric processes. Front Ecol Environ 6(5):247–254. doi:10.1890/070140

World Meteorological Organization (2008) Guide to meteorological instruments and methods of observation. WMO-No. 8. World Meteorological Organization, Geneva

Wrage N, Velthof GL, van Beusichem ML, Oenema O (2001) Role of nitrifier denitrification in the production of nitrous oxide. Soil Biol Biochem 33(12–13):1723–1732. doi:10.1016/S0038-0717(01)00096-7

Yamagishi H, Westley MB, Popp BN, Toyoda S, Yoshida N, Watanabe S, Koba K, Yamanaka Y (2007) Role of nitrification and denitrification on the nitrous oxide cycle in the eastern tropical North Pacific and Gulf of California. J Geophys Res 112:G02015. doi:10.1029/2006JG000227

Yang H, Gandhi H, Ostrom NE, Hegg EL (2014) Isotopic fractionation by a fungal P450 nitric oxide reductase during the production of N_2O. Environ Sci Technol 48:10707–10715. doi:10.1021/es501912d

Yang H, Andersen T, Dörsch P, Tominaga K, Thrane J-E, Hessen DO (2015) Greenhouse gas metabolism in Nordic boreal lakes. Biogeochemistry 126:211–225. doi:10.1007/s10533-015-0154-8

Yoshida N, Toyoda S (2000) Constraining the atmospheric N_2O budget from intramolecular site preference in N_2O isotopomers. Nature 405:330–334. doi:10.1038/35012558

Yoshinari T (1976) Nitrous oxide in the sea. Mar Chem 4:189–202. doi:10.1016/0304-4203(76)90007-4