RESEARCH ARTICLE

Key Points:
- Epilimnion N_2O in reservoirs is positively related to nitrite and nitrate
- Nitrate predicts whether hypoxic hypolimnia are a net N_2O source or sink
- Mixing events can lead to pulses of N_2O production in reservoirs

Controls on nitrous oxide production and consumption in reservoirs of the Ohio River Basin

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Abstract Aquatic ecosystems are a globally significant source of nitrous oxide (N_2O), a potent greenhouse gas, but estimates are largely based on studies conducted in streams and rivers with relatively less known about N_2O dynamics in reservoirs. Due to long water residence times and high nitrogen (N) loading rates, reservoirs support substantial N processing and therefore may be particularly important sites of N_2O production. Predicting N_2O emissions from reservoirs is difficult due to complex interactions between microbial N processing in the oxygen-poor hypolimnion and oxygen-rich epilimnion. Here we present the results of a survey of N_2O depth profiles in 20 reservoirs draining a broad range of land use conditions in four states in the U.S. Nitrous oxide was supersaturated in the epilimnion of 80% of the reservoirs and was undersaturated in only one, indicating that reservoirs in this region are generally a source of N_2O to the atmosphere. Nitrous oxide was undersaturated in the hypolimnion of 10 reservoirs, supersaturated in 9, and transitioned from supersaturation to undersaturation in 1 reservoir that was monitored periodically from midsummer to fall. All reservoirs with a mean hypolimnion nitrate concentration less than $50\,\mu g\,N\,L^{-1}$ showed evidence of net N_2O consumption in the hypolimnion. All reservoirs sampled during lake turnover supported N_2O production throughout the water column. These results indicate that N_2O dynamics in reservoirs differ widely both among systems and through time but can be predicted based on N and oxygen availability and degree of thermal stratification.

1. Introduction

Nitrous oxide (N_2O) is a potent greenhouse gas [*Myhre et al.*, 2013] that also contributes to the depletion of ozone in the upper stratosphere [*Ravishankara et al.*, 2009]. In 2011, atmospheric N_2O was 19% above its 1750 level, and anthropogenic emissions constitute 35% to 45% of the global total [*Myhre et al.*, 2013], primarily from the conversion of agricultural nitrogen (N) to N_2O in soils and waters via microbially mediated N transformations.

Denitrification and nitrification have been identified as the major N_2O-producing pathways in soils and waters [*Firestone and Davidson*, 1989; *Thuss et al.*, 2014]. Denitrification is a form of anaerobic respiration in which nitrate (NO_3^-) is sequentially reduced to nitrite (NO_2^-), nitric oxide (NO), nitrous oxide (N_2O), and dinitrogen (N_2). Nitrous oxide can be released to the environment when the rate of N_2O formation exceeds that of N_2O reduction to N_2. Conversely, denitrification can be a net N_2O sink when the rate of N_2O reduction exceeds that of N_2O production. Controls on denitrification rates include oxygen levels, N and carbon (C) availability, temperature, and pH [*Knowles*, 1982]. Nitrification is an aerobic, chemolithoautotrophic process in which ammonium (NH_4^+) is sequentially oxidized to NO_2^- and NO_3^-. Hydroxylamine, which is produced as an intermediate product during nitrification, can decompose to form N_2O which can be released to the environment. Controls on nitrification rates include NH_4^+, organic C, pH, and temperature [*Bianchi et al.*, 1999; *Strauss et al.*, 2004].

Nitrous oxide emissions from soils have been intensively studied for decades [*Stehfest and Bouwman*, 2006], but emissions from aquatic ecosystems have received much less attention. Recent estimates indicate that river networks may be the source of ~10% of global anthropogenic N_2O emissions [*Beaulieu et al.*, 2011], but most investigations of aquatic N_2O emissions have focused on streams [*Baulch et al.*, 2011; *Beaulieu et al.*, 2009; *Beaulieu et al.*, 2008] and rivers [*Beaulieu et al.*, 2010; *Rosamond et al.*, 2011; *Venkiteswaran et al.*, 2014] with relatively less known about N_2O emissions from lentic ecosystems (lakes and reservoirs) [*Guérin et al.*, 2008; *Hendzel et al.*, 2005; *Huttunen et al.*, 2002]. Due to their long water residence time, however, lentic

ecosystems have been long recognized as systems where extensive N processing can occur [*Wetzel*, 2001], suggesting they may be important N_2O sources. Among lentic ecosystems, reservoirs may support particularly high N_2O emissions due to higher N loading and processing rates than lakes [*Harrison et al.*, 2009].

Studies from lotic ecosystems indicate that N_2O emissions tend to increase with N loading, but it is unclear whether this pattern holds for lentic systems. Unlike lotic ecosystems, which tend to be oxygenated and well mixed, reservoirs can become thermally stratified leading to the development of well-oxygenated shallow waters (epilimnion) and hypoxic deep waters (hypolimnion) which support different types of microbial N transformations. Nitrous oxide biogeochemistry in oxygen-deprived hypolimnetic waters is primarily controlled by denitrification. The factors that determine whether denitrification is a net source or sink for N_2O in the hypolimnion are unclear, though nitrate availability appears to be important [*Beaulieu et al.*, 2014b]. Nitrification is the major N_2O-producing pathway in oxic epilimnion, but NH_4^+ availability (the nitrification substrate) can be very low, even in systems with high N loading, due to NH_4^+ assimilation by phytoplankton [*Smith et al.*, 2014; *Tõnno et al.*, 2005]. The area of rapidly decreasing dissolved oxygen concentration (i.e., oxycline) located at the interface between the hypoxic hypolimnion and oxic epilimnion is yet another distinct biogeochemical zone where the products of aerobic and anaerobic metabolic processes mix, allowing for high rates of N_2O production. For example, ammonium (NH_4^+) produced from organic matter decomposition in the hypolimnion can be rapidly nitrified at the hypoxic-oxic interface. Similarly, NO_3^- produced via nitrification in the epilimnion can be subsequently denitrified at the hypoxic-oxic interface through coupled nitrification-denitrification. Within the transitional oxycline, nitrification and denitrification are occurring at the limits of their oxygen tolerance which can lead to a large fraction of denitrified/nitrified N being converted to N_2O [*Firestone et al.*, 1980; *Goreau et al.*, 1980], further enhancing N_2O production rates. Several studies have reported middepth N_2O maxima near the oxycline in stratified freshwaters [*Deemer et al.*, 2011; *Knowles et al.*, 1981; *Mengis et al.*, 1997; *Yoh et al.*, 1988], but these middepth N_2O maxima are often transient [*Beaulieu et al.*, 2014b] or fail to develop altogether [*Yoh et al.*, 1988].

While previous research has provided considerable insight into N_2O biogeochemistry, a broad synthesis of the factors controlling N_2O distributions is lacking, in part because of the lack of comparable data collected across systems that represent a range of morphologies and land use conditions. Here we present the results of a cross-site survey of N_2O depth profiles in midlatitude/low-elevation reservoirs draining land from four states in the U.S. within the Ohio River Basin. Sixteen of the reservoirs were sampled once during the period of thermal stratification, and three were sampled while the epilimnion and hypolimnion were mixing. One reservoir was sampled several times during the period of thermal stratification and once while the epilimnion and hypolimnion were mixing.

The study objective was to use the range of physiochemical characteristics among the different reservoirs to identify key factors controlling the distribution of N_2O in reservoir waters. We hypothesized that in the epilimnion, N_2O would be supersaturated due to persistent production via nitrification and negligible consumption via denitrification. The degree of N_2O saturation would be determined by the balance between N_2O production and evasion to the atmosphere. Below the thermocline in hypoxic hypolimnetic waters, we hypothesized that denitrification would be a net N_2O source when NO_3^- concentrations are high, leading to N_2O supersaturation, and a net N_2O sink when NO_3^- concentrations are low, leading to N_2O undersaturation. We hypothesized that middepth N_2O maxima would be associated with the oxycline and would be more likely to occur when thermal stratification was relatively weak, allowing for greater exchange of solutes across the hypoxic-oxic boundary. During the annual erosion of stratification that occurs in the fall, we hypothesized that the mixing of NH_4^+-rich hypolimnetic waters with oxygenated surface waters would result in a pulse of nitrification and elevated dissolved N_2O concentrations.

2. Methods

2.1. Study Sites

We sampled 20 reservoirs in the Ohio River Basin draining portions of Indiana, Ohio, Kentucky, and Tennessee (Figure 1). The reservoirs are operated by the United States Army Corps of Engineers (USACE) and are used for numerous purposes including drinking water supply, flood control, water quality, and recreation. The surface areas and storage volumes of the reservoirs ranged from 1–43 km^2 and 0.4–42.6 $\times 10^6 \, m^3$, respectively
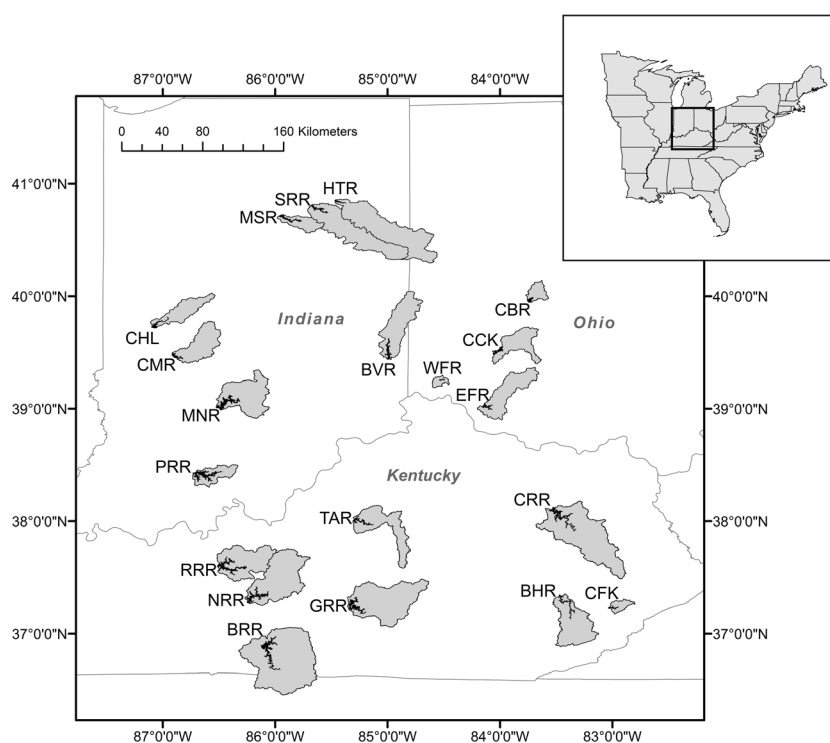
Figure 1. Location of the reservoirs sampled in this study. Reservoirs and watersheds are represented with black and grey fills, respectively.

(Table 1). The study sites include reservoirs that drain pasture lands (e.g., SRR, 80% pasture), croplands (e.g., 80% cropland in HTR), and forested land (e.g., 84% forest in BHR) and one reservoir drains a developed watershed (i.e., WFR, 74% urban). More information about the reservoirs, including operation plans and historic water levels can be found at the USACE website (http://www.lrl.usace.army.mil/Missions/CivilWorks/WaterInformation.aspx).

2.2. Field Sampling

We sampled 19 reservoirs once during the summer and early fall of 2013 (see Table 1 for sample dates). One reservoir, East Fork Lake (EFR; also known as William H. Harsha Lake), was sampled on four dates between 24 July 2013 and 29 October 2013. All lakes were sampled at their deepest point. Water temperature and dissolved oxygen (DO) were measured at 0.3 m intervals near the thermocline, defined as the plane of maximum rate of temperature decrease [*Wetzel*, 2001], and at 1.5 m intervals throughout the rest of the water column using a data sonde with optical dissolved oxygen sensor (YSI 6920 V2, Yellow Springs, OH, USA). Water samples were collected from 20 depths ranging from 0.2 m above the lakebed to 0.1 m below the water surface using a horizontal Van Dorn sampler (Wildlife Supply Company, Yulee, FL, USA). Atmospheric pressure was measured using a barometer (YSI MDS 650, Yellow Springs, OH, USA).

A water sample for dissolved gas analysis was collected from each sampling depth by transferring water from the Van Dorn sampler into 140 mL glass serum vials using a length of tubing. The serum bottles were filled from the bottom, allowed to overflow by 3 times their volume, preserved with 100 μL of a saturated mercury chloride solution, and sealed with a grey butyl septa. Samples were stored at 5°C until analysis.

A water sample for nutrient analysis was collected from each depth by withdrawing 30 mL of water from the Van Dorn sampler using a 60 mL syringe. The water was field filtered (0.45 μm pore size) into an acid washed 30 mL high-density polyethylene bottle, stored on ice and analyzed within 24 h, or frozen and analyzed within 7 days.

2.3. Sample Processing and Analysis

Nitrous oxide was extracted from the water samples using headspace equilibration [*Ioffe and Vitenberg*, 1984]. Forty milliliters of ultrahigh purity helium was transferred into the serum bottle, while an equivalent

Table 1. Reservoir and Watershed Characteristics for the 20 Systems Included in This Study

Reservoir	Latitude	Longitude	Date	Reservoir Surface Area (km²)	Storage Volume (10⁶ m³)	Max Depth (m)	Watershed Area (km²)	Watershed Land Use (%)			
								Crops	Pasture	Urban	Forest
Buckhorn Lake (BHR)	37°20'19.11"N	83°28'14.58"W	2013-08-12	5	1.6	16.7	1072	0.1	0.2	5.3	84.3
Barren River Lake (BRR)	36°53'31.26"N	86°7'21.14"W	2013-08-06	39	15.4	16.7	1500	5.6	43.9	6.8	38.5
Brookville Lake (BVR)	39°26'34.22"N	85°0'0.49"W	2013-08-18	21	33.8	31.9	1031	52.9	9.0	12.1	22.5
C.J. Brown (CBR)	39°56'54.74"N	83°44'45.18"W	2013-08-28	8	7.2	10.3	225	62.8	16.0	6.6	8.7
Caesar Creek (CCK)	39°29'8.77"N	84°3'36.79"W	2013-09-25	11	21.9	31.0	643	71.3	6.6	6.4	13.0
Carr Creek Lake (CFK)	37°13'44.00"N	83°1'57.00"W	2013-08-14	2	3.8	20.0	153	0.0	0.0	6.7	76.3
C.M. Harden (CHL)	39°43'10.04"N	87°4'17.79"W	2013-09-26	8	3.8	16.4	588	72.9	5.5	5.8	12.9
Cagles Mill (CMR)	39°29'13.96"N	86°54'53.58"W	2013-10-08	6	6.3	10.9	796	61.5	7.8	5.5	22.5
Cave Run Lake (CRR)	38°7'5.96"N	83°31'49.56"W	2013-09-05	31	41.0	21.8	2186	0.6	8.5	4.1	78.3
East Fork Lake (EFR)	39°1'17.40"N	84°9'6.89"W	2013-07-24, 2013-08-27, 2013-09-24, and 2013-10-29	8	19.2	32.8	882	53.6	9.2	7.0	27.8
Green River Lake (GRR)	37°15'3.00"N	85°20'17.00"W	2013-08-05	33	38.0	23.4	1797	4.7	24.6	3.7	60.8
J.E. Roush (HTR)	40°50'47.53"N	85°28'2.89"W	2013-09-09	3	1.0	6.6	1778	80.4	3.6	7.3	6.6
Monroe Lake (MNR)	39°0'28.81"N	86°30'55.11"W	2013-08-20	43	42.6	15.4	1166	3.5	4.2	2.1	81.9
Mississinewa Lake (MSR)	40°42'51.42"N	85°57'21.83"W	2013-09-10	13	5.4	18.5	347	65.8	1.8	9.6	14.0
Nolin Lake (NRR)	37°16'45.00"N	86°14'49.00"W	2013-08-08	23	14.9	24.0	1582	13.8	29.0	6.5	45.6
Patoka Lake (PRR)	38°26'2.39"N	86°42'16.62"W	2013-08-22	35	35.8	12.7	448	3.7	11.5	4.0	67.0
Rough River Lake (RRR)	37°37'6.00"N	86°29'59.00"W	2013-09-17	20	7.0	18.5	1127	8.1	31.6	4.1	50.5
Salamonie (SRR)	40°48'25.00"N	85°40'38.00"W	2013-10-02	11	2.6	17.6	1473	0.0	80.0	6.9	9.2
Taylorsville Lake (TAR)	38°0'10.20"N	85°18'20.40"W	2013-09-16	12	18.8	21.5	939	3.9	39.7	6.1	44.9
West Fork (WFR)	39°15'38.38"N	84°29'50.83"W	2013-08-29	1	0.4	3.6	98	0.1	1.0	74.3	0.7

volume of water was allowed to escape the bottle through a vent needle. The bottle was shaken for ≥ 1 h, and 25 mL of headspace gas, at 1 atm, was withdrawn from the serum bottle and transferred to a pre-evacuated 12 mL glass vial (Exetainer, LabCo, UK).

Nitrous oxide was measured using a gas chromatograph (Bruker 450, Billerica, MA, USA) equipped with an electron capture detector and autosampler (CTC Analytics, PAL-xt, Zwingen, Switzerland). A 90:10 mixture of argon and methane was used as the carrier gas. The original dissolved N_2O concentration was calculated from the measured headspace concentration, the temperature specific Bunsen coefficient, and a mass balance for the headspace equilibration system as described in *Beaulieu et al.* [2012].

The equilibrium dissolved gas concentration at the time of sampling was calculated from the global average atmospheric N_2O concentration (327 ppb) [*Tans and Keeling*, 2013], the measured barometric pressure, and the water temperature. Dissolved N_2O concentration is presented as a saturation ratio, defined as the ratio of measured to equilibrium concentration. Equilibrium N_2O concentration ranged from 193–363 μg N_2O-N L^{-1}, depending on water temperature and atmospheric pressure.

Soluble reactive phosphorus (SRP), nitrite (NO_2^-), nitrate (NO_3^-) + NO_2^-, and ammonium (NH_4^+) were measured with automated colorimetry (Lachat Instruments QuickChem 8000 Flow Injection Autoanalyzer, Loveland, CO, USA) [*Sardina*, 2000; *Smith*, 2001; *Wendt*, 1995].

2.4. Statistics and Data Analysis

We calculated the thermocline strength index (TSI) as an indicator of thermal resistance to mixing of hypolimnetic and epilimnetic waters near the thermocline [*Horne and Goldman*, 1994].

$$TSI = \Delta T / \Delta h \tag{1}$$

where ΔT and Δh are the differences in water temperature (°C) and water depth (m), respectively, between the top and bottom of the metalimnion. We used logistic regression to determine if the probability of the occurrence of a middepth N_2O maximum was related to TSI.

We categorized the epilimnion and hypolimnion of each lake as a site of N_2O production, N_2O consumption, or negligible N_2O processing based on the mean dissolved N_2O saturation ratio in each layer. We considered mean saturation ratios greater than 1.2 and less than 0.8 to be indicative of production and consumption, respectively. We assumed that saturation ratios between these values were indicative of negligible N_2O processing. In three instances the mean N_2O saturation ratio in the hypolimnion exceeded 0.8 due to a few depths with high dissolved N_2O concentrations, despite clear evidence of N_2O consumption throughout much of the water layer, and these reservoirs were classified as site of hypolimnion N_2O consumption. Lakes where the maximum N_2O saturation occurred at middepths were also identified (in the sense of *Priscu et al.* [2008]).

Our analysis assumes that N_2O saturation ratios > 1.2 or < 0.8 are indicative of biological production or consumption; however, abiotic factors can also cause the saturation ratio to deviate from 1.0, such as the seasonal warming of waters [*Venkiteswaran et al.*, 2014]. For example, water that equilibrated with the atmosphere at 5°C then warmed to 30°C would have a saturation ratio of 2.3 if no air-water gas exchange occurred during the process. Gas exchange in the well-mixed epilimnion is relatively rapid, however. Assuming a conservative gas exchange rate of 2 cm h^{-1} [*Cole and Caraco*, 1998], the dissolved gas content of a 3 m thick epilimnion would equilibrate with the atmosphere in ~6 days, which is sufficiently rapid to allow excess gas formed by rising water temperatures to off-gas to the atmosphere. While no gas exchange occurs between the hypolimnion and atmosphere, water temperatures in the hypolimnion are relatively stable, often increasing by no more than 3°C during the summer [*Beaulieu et al.*, 2014b], which would increase the saturation ratio by ~0.1.

We calculated the difference in mean nutrient concentrations between the epilimnion and hypolimnion of all stratified lakes and used a paired t test to determine if the differences were statistically significant. We assessed relationships between dissolved N_2O and potential controlling variables using simple linear regression unless the relationship was clearly nonlinear, in which case we modeled the relationship using a two-parameter exponential model:

$$y = a(1 - e^{-cx}) \tag{2}$$

where y and x are the dependent and independent variables, respectively, and a and c are constants. This model was chosen because it can fit a wide range of asymptotic nonlinear relationships and is not

Table 2. Thermocline Stratification Index (TSI), Water Temperature, Dissolved Oxygen (DO), Nitrite + Nitrate ($NO_{2.3}$), Nitrite (NO_2^-), Soluble Reactive Phosphorus (SRP), and Ammonium (NH_4^+) at the Study Sites[a]

Reservoir	Date	TSI (°C m^{-1})	Strata	Water Temperature (°C)	DO (mg L^{-1})	$NO_{2.3}$ (µg N L^{-1})	NO_2^- (µg N L^{-1})	SRP (µg P L^{-1})	NH_4^+ (µg N L^{-1})
Buckhorn Lake (BHR)	8/12/2013	0.9	epilimnion	28.0	8.1	17	2	23	8
			hypolimnion	22.4	0.4	19	5	29	247
Barren River Lake (BRR)	8/6/2013	0.5	epilimnion	27.5	9.7	24	11	12	15
			hypolimnion	22.5	0.3	387	55	27	142
Brookville Lake (BVR)	8/18/2013	4.1	epilimnion	22.9	3.8	1075	75	11	95
			hypolimnion	11.1	0.4	1676	61	30	239
C.J. Brown (CBR)	8/28/2013	2.1	epilimnion	23.2	1.0	6	5	16	258
			hypolimnion	21.1	0.2	5	5	118	3458
Caesar Creek (CCK)	9/25/2013	1.9	epilimnion	21.0	4.2	1202	25	9	165
			hypolimnion	12.5	0.3	947	6	29	1118
Carr Creek Lake (CFK)	8/14/2013	1.9	epilimnion	24.8	3.4	13	2	23	3
			hypolimnion	14.2	0.2	102	15	31	64
C.M. Harden (CHL)	9/26/2013	2.4	epilimnion	22.2	6.2	308	50	9	274
			hypolimnion	16.3	0.2	20	5	46	3718
Cagles Mill (CMR)	10/8/2013		mixed	21.2	2.4	147	3	11	320
Cave Run Lake (CRR)	9/5/2013	1.8	epilimnion	26.8	8.6	12	6	18	6
			hypolimnion	20.0	0.3	11	7	23	224
East Fork Lake (EFR)	7/24/2013	3.1	epilimnion	27.6	5.5	23	8	19	15
			hypolimnion	17.2	0.2	385	69	94	99
	8/27/2013	1.4	epilimnion	25.6	3.9	5	5	17	16
			hypolimnion	16.5	0.2	38	6	232	673
	9/24/2013	0.7	epilimnion	22.8	5.7	83	33	30	173
			hypolimnion	13.5	0.2	32	4	194	1256
	10/29/2013	NA[b]	mixed	16.1	0.6	269	32	122	288
Green River Lake (GRR)	8/5/2013	2.7	epilimnion	26.3	9.0	24	10	13	28
			hypolimnion	15.1	0.1	263	27	26	256
J.E. Roush (HTR)	9/9/2013	NA[b]	mixed	24.3	6.9	531	81	13	36
Monroe (MNR)	8/20/2013	2.5	epilimnion	25.5	6.3	8	2	15	16
			hypolimnion	17.8	0.2	7	5	42	892
Mississinewa (MSR)	9/10/2013	1.4	epilimnion	25.1	8.8	1784	99	12	27
			hypolimnion	22.5	0.5	996	23	44	908
Nolin Lake (NRR)	8/8/2013	2.0	epilimnion	26.8	7.7	16	8	7	11
			hypolimnion	20.4	0.2	526	82	28	313
Patoka Lake (PRR)	8/22/2013	4.9	epilimnion	25.4	5.4	10	3	8	8
			hypolimnion	15.5	0.2	9	4	19	372
Rough River Lake (RRR)	9/17/2013	0.8	epilimnion	25.2	5.8	13	1	10	18
			hypolimnion	20.5	0.3	4	1	28	765
Salamonie (SRR)	10/2/2013	NA[b]	mixed	20.3	2.7	1782	3	11	15
Taylorsville Lake (TAR)	9/16/2013	0.8	epilimnion	25.0	4.6	16	1	13	13
			hypolimnion	18.6	0.3	13	2	274	1728
West Fork (WFR)	8/29/2013	2.2	epilimnion	28.0	7.6	9	8	6	8
			hypolimnion	25.7	0.8	9	4	43	622

[a]Mean values are presented for the epilimnion and hypolimnion if the reservoir was thermally stratified during sampling; otherwise, the values represent means for the mixed water column.
[b]NA: TSI not calculated for mixed systems.

assumed to represent the underlying mechanisms (supporting information Text S1 and Data Set S1). All data analyses and nonlinear model fitting were conducted in R [*R Development Core Team*, 2015].

3. Results

3.1. Stratification and Temperature

All reservoirs sampled in July, August, and September were thermally stratified, with the exception of HTR (Table 2 and Figure S15a). HTR is among the smallest reservoirs included in the study (Table 1), and 127 temperature profiles collected from the reservoir between May and September of 2000–2010 (J. L. Young, unpublished data, 2013, available from USACE) indicate that the reservoir rarely stratifies, possibly
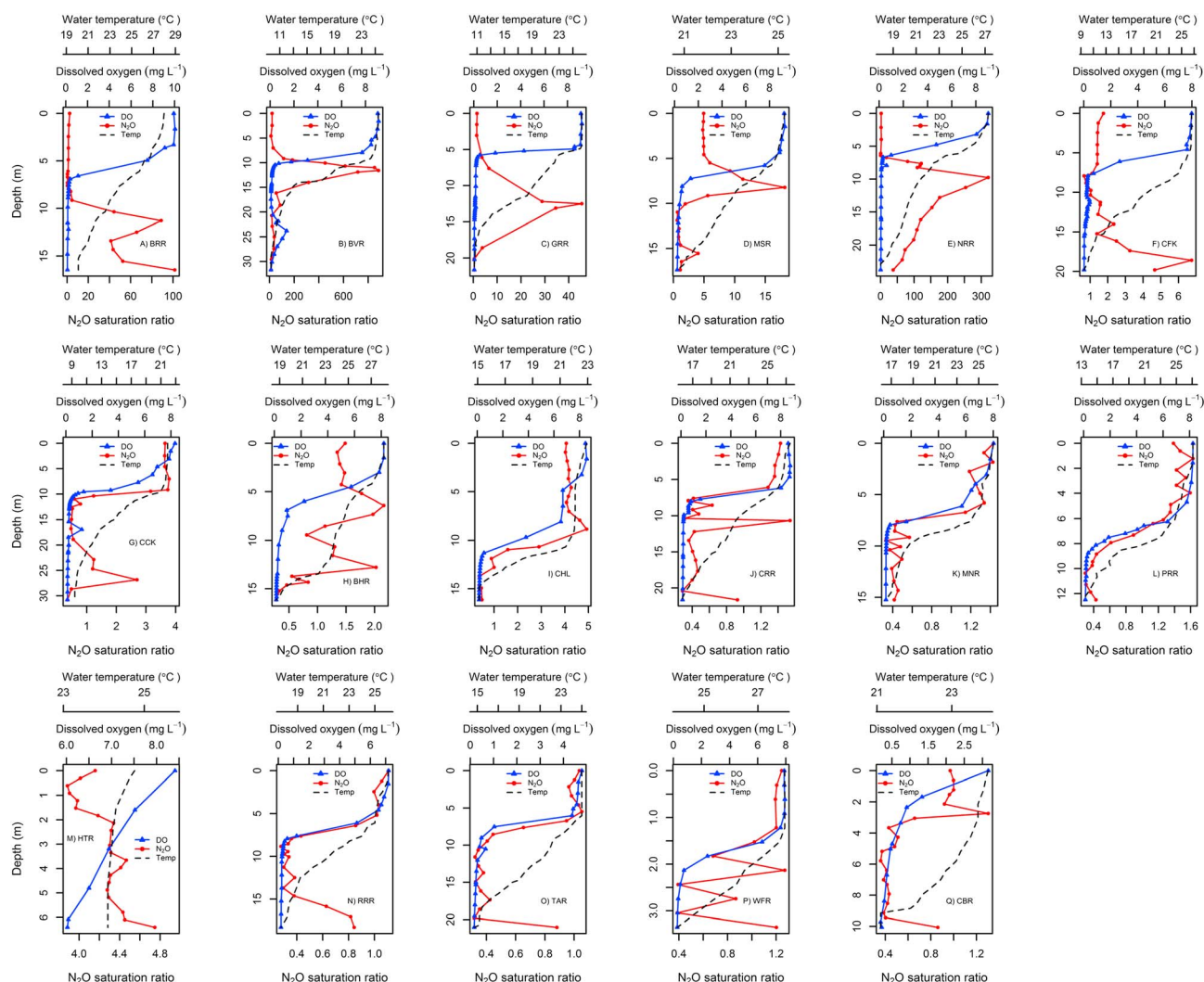
Figure 2. (a–q) Depth profiles of water temperature, dissolved oxygen, and nitrous oxide (N$_2$O) saturation ratio in the deepest location in each of the reservoirs that were sampled during the onetime sampling campaign between July and August of 2013. See Table 1 for a definition of the lake name acronyms listed in each panel.

due to a short water residence time. Overall, the mean epilimnion temperature of the reservoirs sampled between July and September was 25.2°C (range: 21.0–28.°C). The mean hypolimnion temperature was 18.3°C and decreased with increasing reservoir depth (Table 2). The mean TSI value was 1.9°C m^{-1} (range: 0.4–4.9°C m^{-1}) for reservoirs sampled during the period of thermal stratification (Table 2). Thermal stratification was completely absent, or the thermocline had sank close to the sediment-water interface, in the three reservoirs sampled in October (CMR, EFR, and SRR), likely because cooler temperatures caused lake waters to circulate after a prolonged period of thermal stratification, a process typically referred to as lake turnover.

Depth profiles of nutrient concentrations and water temperature for each lake are presented in the supporting information.

3.2. Dissolved Oxygen

Dissolved oxygen was nearly depleted from the hypolimnion of all thermally stratified reservoirs (mean hypolimnion DO = 0.28 mg L^{-1}, Table 2). The mean DO saturation in the epilimnion of the stratified reservoirs was 74.6% (Table 2), though DO saturation exceeded 100% at one or more depths in the epilimnion of all but three stratified reservoirs (Figures 2, 4, and S1–23).

Table 3. Mixing Status, Presence or Absence of a Middepth N_2O Maximum, and Net Effect of Biological Processing on Dissolved N_2O Concentration in the Epilimnion and Hypolimnion of Each Reservoir[a]

Reservoir	Sample Date	Mixing Status	N_2O Classification[a]		Middepth N_2O Maximum Present
			Hypolimnion	Epilimnion	
Reservoirs Sampled Once					
Buckhorn Lake (BHR)	8/12/2013	stratified	consumption	production	
Barren River Lake (BRR)	8/6/2013	stratified	production	production	x
Brookville Lake (BVR)	8/18/2013	stratified	production	production	x
C.J. Brown (CBR)	8/28/2013	stratified	consumption	consumption	
Caesar Creek (CCK)	9/25/2013	stratified	consumption	production	x
Carr Creek Lake (CFK)	8/14/2013	stratified	production	production	x
C.M. Harden (CHL)	9/26/2013	stratified	consumption	production	
Cagles Mill (CMR)	10/8/2013	fall turnover	production throughout water column		
Cave Run Lake (CRR)	9/5/2013	stratified	consumption	production	
Green River Lake (GRR)	8/5/2013	stratified	production	production	x
J.E. Roush (HTR)	9/9/2013	mixed[b]	production throughout water column		
Monroe (MNR)	8/20/2013	stratified	consumption	production	
Mississinewa (MSR)	9/10/2013	stratified	production	production	x
Nolin Lake (NRR)	8/8/2013	stratified	production	production	x
Patoka Lake (PRR)	8/22/2013	stratified	consumption	production	
Rough River Lake (RRR)	9/17/2013	stratified	consumption	neutral	
Salamonie (SRR)	10/2/2013	fall turnover	production throughout water column		
Taylorsville Lake (TAR)	9/16/2013	stratified	consumption	neutral	
West Fork (WFR)	8/29/2013	stratified	consumption	neutral	
Reservoir Sampled Repeatedly					
East Fork Lake (EFR)	7/24/2013	stratified	production	production	x
	8/27/2013	stratified	consumption	production	
	9/24/2013	stratified	consumption	production	
	10/29/2013	fall turnover	production throughout water column		

[a]N_2O classification: Classification is based on deviation of dissolved N_2O concentration from equilibrium values. Water layers with a mean saturation ratio < 0.80 or > 1.2 were defined as supporting N_2O consumption and production, respectively. Intermediate values indicated that biogeochemical processing had a negligible effect on dissolved N_2O concentration, and these water layers were classified as neutral. Lakes sampled during fall turnover were not stratified and did not have epilimnion and hypolimnion. These lakes all showed evidence of N_2O saturation throughout the water column.
[b]HTR rarely stratifies and was well mixed when sampled.

Dissolved oxygen was undersaturated throughout the water column in the three reservoirs sampled during fall turnover (dissolved oxygen <5.1, <4.7, and $< 2.0 \, mg \, L^{-1}$ in SRR, CMR, and EFR, respectively).

3.3. Nutrients

In thermally stratified reservoirs, the mean $NO_2^- + NO_3^-$ (referred to as $NO_{2.3}$ hereafter) concentration ranged from 5.8 to 1351 $\mu g \, N \, L^{-1}$ (mean = 294 $\mu g \, N \, L^{-1}$, Table 2) across the reservoirs and did not differ consistently between the epilimnion and hypolimnion ($p = 0.55$).

Nitrite was below detection (1.8 $\mu g \, N \, L^{-1}$) in 22% of the samples and constituted an average of 37% of the $NO_{2.3}$. Elevated NO_2^- was observed near the oxycline in several lakes, however, with a maximum value of 200 $\mu g \, N \, L^{-1}$ observed in NRR (Figure S18c).

Soluble reactive phosphorus and NH_4^+ concentrations were consistently greater in the hypolimnion than in the epilimnion of stratified lakes ($p < 0.003$) with maximum values reaching 490 $\mu g \, SRP \, L^{-1}$ and 5720 $\mu g \, N \, L^{-1}$ (Figures S11f, 4e).

3.4. Nitrous Oxide

Of the 17 reservoirs that were sampled once between June and September, 13 showed evidence of N_2O production in the epilimnion (mean N_2O saturation = 29.5 and median = 1.6; Table 3 and Figures 2a–2m), 3 were at equilibrium (mean N_2O saturation = 1.04; Figures 2n–2p), and 1 showed evidence of net N_2O consumption (mean N_2O saturation = 0.65; Figure 2q). Six of the reservoirs showed evidence of N_2O production in the hypolimnion (mean saturation ratio = 35.5, median = 23.6; Figures 2a–2f), and 10 showed evidence of net N_2O consumption (mean saturation = 0.60; Figures 2g–2l and 2n–2q).
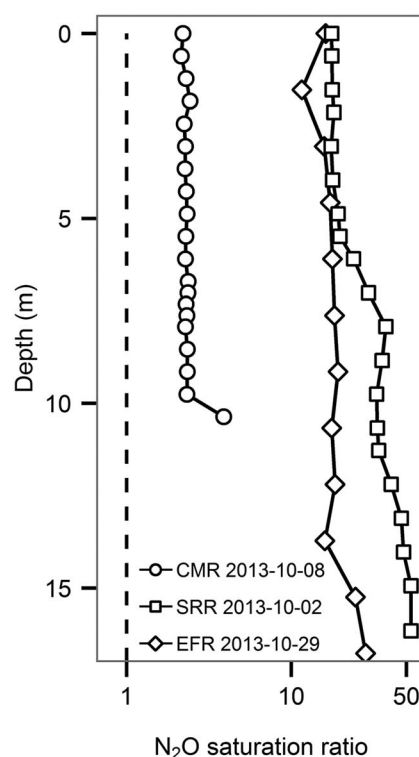
Figure 3. Depth profiles of the nitrous oxide (N_2O) saturation ratio in the deepest location of each of the three reservoirs sampled during lake turnover in the fall. The dashed vertical line is drawn at an N_2O saturation ratio of 1, indicating dissolved N_2O is in equilibrium with the atmosphere.

3.4.1. Middepth Dynamics

Seven reservoirs (BRR, CCK, MSR, BVR, NRR, CFK, and GRR) that were sampled once between June and September exhibited middepth N_2O maxima that occurred between the oxycline and the sediment-water interface (Figures 2a–2g and Table 3). The maximum N_2O saturation ratio in the depth profiles at each lake ranged from 3.8 to 884 and included the highest values yet reported for a freshwater lake. In MSR and BVR the N_2O maximum occurred within a few feet of the oxycline, but in the other four lakes the N_2O maximum was well below the oxycline.

3.4.2. Reservoirs Sampled During Turnover

Three reservoirs were sampled in October during turnover (CMR, SRR, and EFR). Nitrous oxide was supersaturated throughout the water column in all three lakes with mean saturation ratios of 2.4, 30.7, and 14.0 at CMR, SRR, and EFR, respectively (Figure 3).

3.4.3. EFR Sampled Through Time

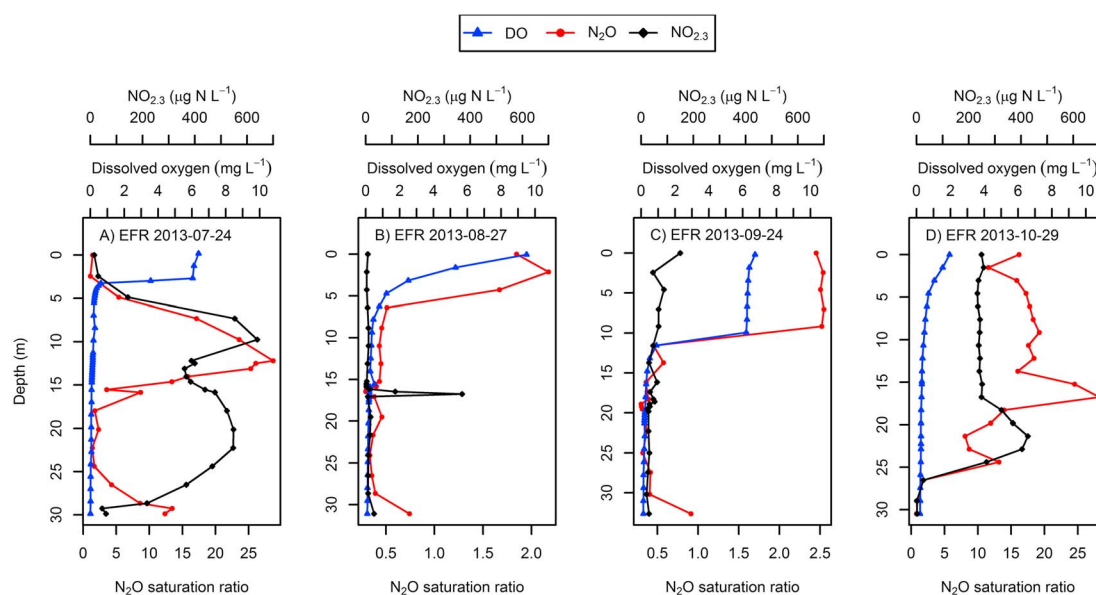During the first sampling date at EFR (24 July 2013) the N_2O profile was characterized by a middepth N_2O maximum located 4.8 m below the oxycline with a maximum saturation ratio of 28.2 (Figure 4a). Nitrous oxide saturation declined with increasing depth to 1.4 at 22 m, at which point N_2O saturation increased to a maximum of 13.5 near the sediment-water interface.

By late August (27 August 2013) the entire hypolimnion was undersaturated with N_2O (mean = 0.4) while the epilimnion remained supersaturated (mean = 1.9, Figure 4b). The decrease in hypolimnion N_2O was accompanied by a substantial reduction in $NO_{2.3}$ to a mean of 38 $\mu g \, N \, L^{-1}$. By late September (24 September 2013) the thermocline had begun to drop (Figure S12a), though the epilimnion remained supersaturated with N_2O (mean = 2.5) and the hypolimnion remained undersaturated (mean = 0.4) (Figure 4c).

The 29 October 2013 sampling at EFR occurred during lake turnover and the water column was nearly isothermal to a depth of 23 m where a weak thermocline persisted (Figure S13a). The mean N_2O saturation ratio above and below the thermocline was 16.3 and 0.6, respectively. Similarly, $NO_{2.3}$ remained low below the thermocline (mean = 26 $\mu g \, N \, L^{-1}$) but increased to a mean of 277 $\mu g \, N \, L^{-1}$ above the thermocline. Dissolved oxygen was low throughout the water column, with a maximum value of only 2.0 $mg \, L^{-1}$, which occurred at the air-water interface.

3.5. Relationship Between N_2O and Controlling Factors

We found no relationships between N_2O saturation and NH_4^+, but mean epilimnion N_2O saturation exhibited an asymptotic relationship with mean epilimnion NO_3^- ($p < 0.001$, $r^2 = 0.76$) and NO_2^- ($p < 0.001$, $r^2 = 0.83$) (Figures 5a and 5b). Hypolimnion N_2O production and consumption was predicted by mean hypolimnion $NO_{2.3}$ concentration. With only one exception (CCK), all hypolimnion with a mean $NO_{2.3}$ concentration less than 50 $\mu g \, N \, L^{-1}$ were a net N_2O sink and those with mean $NO_{2.3}$ greater than 50 $\mu g \, N \, L^{-1}$ were a net N_2O source (Figure 6). The threshold is insensitive to whether NO_3^- or $NO_{2.3}$ is used as the predictor variable because NO_2^- composed a small proportion of $NO_{2.3}$ in the hypolimnion. The relationship with NO_2^- is as

Figure 4. Depth profiles of the nitrous oxide (N_2O) saturation ratio, dissolved oxygen, and nitrate + nitrite ($NO_{2.3}$) at the deepest location in EFR on (a) 24 July 2013, (b) 27 August 2013, (c) 24 September 2013, and (d) 29 October 2013.

described for $NO_{2.3}$, except that the threshold occurred at 10 µg N L^{-1}. Finally, the probability of observing a middepth N_2O maximum was not related to the thermocline stability index ($p = 0.50$).

4. Discussion

The objective of our study was to determine the factors controlling N_2O distributions in reservoir waters. We hypothesized that (1) nitrous oxide would be supersaturated in reservoir epilimnion and that the degree of N_2O saturation would be related to rates of nitrification and gas exchange, (2) denitrification in hypoxic hypolimnion would be a net N_2O source when NO_3^- concentrations were high, leading to N_2O supersaturation, and a net N_2O sink when NO_3^- concentrations were low, leading to N_2O undersaturation, (3) middepth N_2O maxima would be associated with the oxycline and be more frequently observed in weakly stratified reservoirs, and (4) erosion of thermal stratification during the fall would result in elevated N_2O concentrations. In support of our first hypothesis, N_2O was supersaturated or in equilibrium with the atmosphere in
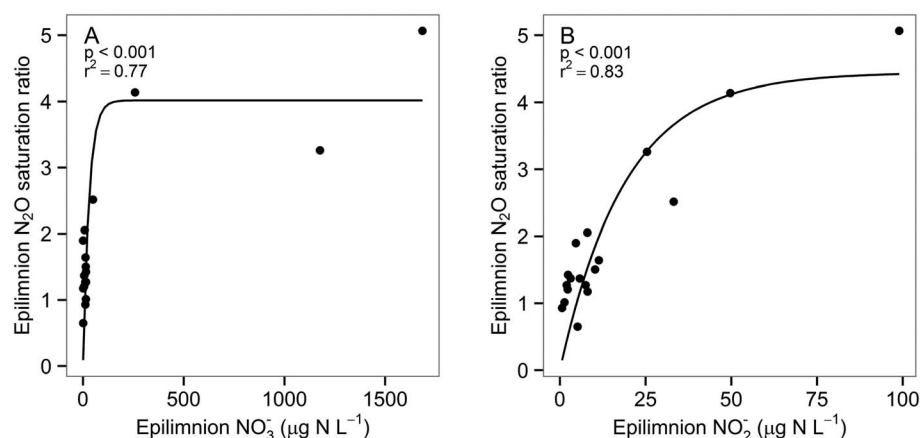

Figure 5. Relationship between the mean nitrous oxide (N_2O) saturation ratio and (a) mean nitrate (NO_3^-) and (b) mean nitrite (NO_2^-) in the epilimnion of the stratified reservoirs during the onetime sampling campaign between July and September of 2013.
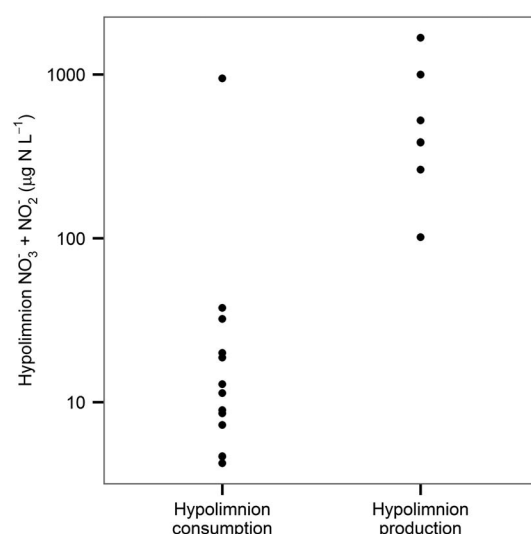
Figure 6. Mean hypolimnion nitrate (NO_3^-) plus nitrite (NO_2^-) by hypolimnion nitrous oxide (N_2O) production status category (i.e., N_2O production or consumption). All hypolimnion with evidence of net N_2O production had a mean $NO_{2.3}$ value greater than $50\,\mu g\,N\,L^{-1}$.

the epilimnion of all but one reservoir and the degree of saturation correlated with NO_2^- and NO_3^- concentrations, suggesting that nitrification was the dominant production pathway. With only one exception (CCK), all hypolimnion with a mean $NO_{2.3}$ concentration less than $50\,\mu g\,N\,L^{-1}$ were a net N_2O sink and those with mean $NO_{2.3}$ greater than $50\,\mu g\,N\,L^{-1}$ were a net N_2O source (Figure 6), providing support for our second hypothesis. Middepth N_2O maxima were observed near the oxycline in two lakes and well below the oxycline in five lakes, providing mixed support for our third hypothesis. Finally, differences in N_2O concentrations between shallow and deep waters disappeared when hypolimnetic and epilimnetic waters mixed during the

fall season and N_2O became supersaturated throughout the water column, lending support to our fourth hypothesis. Collectively, these results indicate that the wide range of N_2O distribution patterns observed in reservoir waters sampled can be predicted by the presence or absence of thermal stratification and the degree of inorganic N availability.

4.1. Epilimnion N$_2$O Dynamics

Nitrous oxide emissions from lakes and reservoirs are partially regulated by the N_2O concentration gradient between water surface and the atmosphere. Therefore, patterns in N_2O emissions will be linked to the degree of N_2O supersaturation in the epilimnion. Nitrous oxide was supersaturated in the epilimnion of 80% of the reservoirs sampled during the summer and undersaturated in only one, indicating that most of the reservoirs were a source of N_2O to the atmosphere. These results are consistent with reports from a wide variety of lakes and reservoirs [*Deemer et al.*, 2011; *Guérin et al.*, 2008; *Liu et al.*, 2011; *Mengis et al.*, 1997; *Yoh et al.*, 1983], suggesting that most lentic systems are a source of N_2O to the atmosphere.

We observed a positive relationship between mean epilimnion N_2O concentration and both NO_2^- and NO_3^- concentrations. Positive correlations between N_2O and $NO_{2.3}$ have been reported for a variety of freshwater ecosystems including small streams [*Baulch et al.*, 2011; *Beaulieu et al.*, 2008], small rivers [*Stow et al.*, 2005], lakes [*Hashimoto et al.*, 1993], and reservoirs [*Liu et al.*, 2011]. In streams and rivers this correlation is generally attributed to higher rates of sediment denitrification at higher $NO_{2.3}$ concentrations leading to elevated rates of N_2O production [*Baulch et al.*, 2011; *Beaulieu et al.*, 2008; *Stow et al.*, 2005]. Denitrification is unlikely to be an important source of N_2O in the well-oxygenated epilimnion of reservoirs, however, and a more likely explanation for the correlation between these solutes is that they are being produced simultaneously by nitrification. While nitrification can produce all three solutes, N_2O saturation correlated more strongly with NO_2^- than NO_3^- in this study (Figures 5a and 5b), possibly because other sources of NO_3^-, such as river inputs of watershed-derived NO_3^-, obscured the relationship between NO_3^- and N_2O production. Nitrite concentrations in river waters are exceedingly low, and in situ nitrification is the most important source of NO_2^- in the epilimnion of lakes and reservoirs. The correlation between N_2O and NO_2^- is also consistent with the physiology of nitrification. The first step of the nitrification pathway is the oxidation of NH_4^+ to hydroxylamine (NH_2OH), from which both NO_2^- and N_2O are formed. If the NO_2^- is not immediately oxidized to NO_3^-, reduced to N_2O or N_2 (nitrifier-denitrification), or assimilated into biomass, NH_4^+ oxidation should result in the simultaneous accumulation of N_2O and NO_2^-. The correlation between N_2O and NO_2^- in oxic waters is therefore partially regulated by the rate of NO_2^- consumption following NH_4^+ oxidation. *Sasaki et al.* [2011] used N_2O istopomers to demonstrate that nitrification was the dominant source of N_2O in the

oxic waters of Lake Kizaki, Japan, but found no relationship between N_2O and NO_2^-; rather, N_2O was strongly correlated with NO_3^-. The likely explanation for this pattern is that NO_2^- was rapidly oxidized to NO_3^- following NH_4^+ oxidation. Therefore, the correlation between N_2O and NO_2^- in oxic waters may differ among lakes and reservoirs based on the rates of NO_2^- and N_2O transport and transformations. Nevertheless, the N_2O-NO_2^- correlation reported here is consistent with the growing body of evidence indicating that nitrification is the dominant N_2O production mechanism in oxic lake and reservoir waters.

The relationship between N_2O saturation and NO_2^- and NO_3^- in the epilimnion was asymptotic, which may reflect differences in the water residence time of these nitrification products. The primary factor determining the water residence time of NO_3^- and NO_2^- is the rate of biological uptake (i.e., algal assimilation and denitrification), while the residence time of N_2O will be primarily determined by evasion to the atmosphere. When rates of N_2O evasion exceed those of NO_3^- and NO_2^- uptake, dissolved N_2O concentrations will become depleted relative to that of NO_3^- and NO_2^-. This imbalance is likely to be most severe at high dissolved N_2O concentrations, when the rate of N_2O evasion is greatest, and these differences in water residence time may be the cause of the asymptotic relationship observed between these nitrification products (Figure 5).

4.2. Hypolimnion N_2O Dynamics

Dissolved N_2O was either under or supersaturated in the hypolimnion of all stratified reservoirs (Table 3), reflecting the ubiquitous influence of biological processes on N_2O distributions in lentic ecosystems. The major biological processes affecting N_2O in freshwaters are denitrification and nitrification [*Firestone and Davidson*, 1989]. The low dissolved oxygen content of the hypolimnion waters (mean $= 0.28\,mg\,L^{-1}$ for the hypolimnion of all stratified reservoirs; mean DO at the sediment-water interface of all stratified reservoirs $= 0.13\,mg\,L^{-1}$) largely precludes the possibility that ammonium oxidation was a large source of N_2O in the hypolimnion. Although pure cultures of nitrifiers have been shown to oxidize NH_4^+ at dissolved oxygen levels as low as $0.18\,mg\,L^{-1}$ in the laboratory [*Goreau et al.*, 1980], field investigations indicate that nitrification ceases at $\sim 1.0\,mg\,L^{-1}$ in the hypolimnion of stratified lakes [*Sasaki et al.*, 2011]. Therefore, the biological process most likely to consume or produce N_2O in hypoxic hypolimnion waters is denitrification, which has been shown to occur at and below DO levels of $\sim 0.2\,mg\,L^{-1}$ in reservoirs [*Beaulieu et al.*, 2014b; *Deemer et al.*, 2011; *Grantz et al.*, 2012].

Whether denitrification is a net source or sink of N_2O depends on the relative activity rates of the denitrification enzymes that lead to the production (nitric oxide reductase) and consumption (nitrous oxide reductase) of N_2O. Although each step in the sequential reduction of NO_3^- to N_2 yields energy, the final reduction of N_2O to N_2 is the least energetic reaction, yielding less than 20% of the total energy yield of NO_3^- reduction to N_2 [*Koike and Hattori*, 1975; *Richardson et al.*, 2009]; there is therefore little disadvantage for the cell in not reducing N_2O to N_2, particularly when NO_3^- availability is not limiting. Under conditions of high NO_3^- availability, the enzyme production rates may be greater for the high-energy yielding reduction of NO_3^- to N_2O than for the low-energy yielding reduction of N_2O to N_2, resulting in elevated N_2O production. Under low $NO_{2.3}$ concentrations, however, the opposite may be true, and rates of N_2O reduction may exceed that of N_2O production. Under these conditions, denitrification may convert extracellular N_2O to N_2, thereby reducing N_2O concentrations in the water column. These energetics may explain why denitrification in the hypolimnion was always a net N_2O sink when $NO_{2.3}$ was less than $50\,\mu g\,N\,L^{-1}$ but was typically a net source when $NO_{2.3}$ exceeded this threshold (Figure 6). This pattern is consistent with reports that low-nutrient ($NO_{2.3} < 2\,\mu g\,N\,L^{-1}$) boreal reservoirs are persistent N_2O sinks [*Hendzel et al.*, 2005].

While the energetics of denitrification offers a plausible explanation as to why denitrification may switch from an N_2O source to a sink under conditions of low $NO_{2.3}$ availability, it is unclear why this switch occurs near a $NO_{2.3}$ threshold of $50\,\mu g\,N\,L^{-1}$. One possibility is that below $50\,\mu g\,N\,L^{-1}$ competition for nitrate with organisms that dissimilate nitrate to ammonium (DNRA) becomes sufficiently severe that denitrifiers become nitrate limited. This has been shown in several tropical estuaries where denitrification rates were 0 or extremely low and DNRA was the dominant nitrate reduction pathway when $NO_{2.3} < 100\,\mu g\,N\,L^{-1}$ [*Dong et al.*, 2011; *Dunn et al.*, 2013], possibly because DNRA generates more energy per mol of nitrate metabolized than denitrification under nitrate limiting conditions [*Dong et al.*, 2011]. DNRA has received

less attention in freshwater lentic ecosystems than in estuaries or coastal waters, but DNRA has been shown to compose up to 34% of total dissimilatory nitrate removal in an oligotrophic mountain lake [*Washbourne et al.*, 2011], suggesting that DNRA can be a quantitatively important process in lakes and reservoirs.

The relationship between $NO_{2.3}$ availability and dissolved N_2O in the hypolimnion observed in the cross-site survey was also apparent in the four depth profiles collected from EFR between July and October (Figure 4). The hypolimnion at EFR functioned as a net N_2O source in July, when the mean hypolimnion $NO_{2.3}$ was 385 $\mu g\,N\,L^{-1}$ (Table 2), but switched to a net N_2O sink by August when the mean hypolimnion $NO_{2.3}$ fell to 38 $\mu g\,N\,L^{-1}$. This pattern persisted until lake turnover in the fall. A similar pattern was also observed in EFR during the summers of 2012 [*Beaulieu et al.*, 2014b] and 2014 (J. J. Beaulieu, unpublished data, 2013), indicating that denitrification in the hypolimnion of this agricultural reservoir consistently transitions from a net N_2O source to a sink in response to falling $NO_{2.3}$ availability during the period of thermal stratification.

While other factors such as carbon availability [*Weier et al.*, 1993], trace metals [*Twining et al.*, 2007], and sulfide [*Sorensen et al.*, 1980] have been shown to influence the production of N_2O via denitrification, the clear threshold between $NO_{2.3}$ and N_2O production/consumption found in this work may serve as a basis for predicting N_2O dynamics in reservoirs. There are exceptions to this pattern, however. For example, CCK exhibited net N_2O consumption through most of the hypolimnion, despite having $NO_{2.3}$ concentrations in excess of 500 $\mu g\,N\,L^{-1}$. Similarly, *Deemer et al.* [2011] reported sustained N_2O supersaturation in the hypolimnion of a stratified reservoir with a $NO_{2.3}$ concentration of 50 $\mu g\,N\,L^{-1}$, which is the approximate threshold where our data suggest that denitrification begins to consume N_2O. Together, these data suggest that $NO_{2.3}$ availability is the primary determinant of the net effect of denitrification on dissolved N_2O concentrations in hypoxic hypolimnion but that other factors can modify the general relationship reported here.

4.3. Middepth N_2O Maximum

Eight reservoirs exhibited a middepth N_2O maximum during at least one site visit. In BVR and MSR the middepth maximum co-occurred with a decrease in DO along the oxycline (Figures 2b and 2d). The maximum saturation ratio observed in BVR (884) is, to the best of our knowledge, the highest yet reported for a natural freshwater system (see *Knowles et al.* [1981] and *Yoh et al.* [1988] for reported saturation ratios of 626 and 132, respectively). The oxycline is a zone of strong biogeochemical gradients where nitrification and denitrification may occur at the extreme limits of their oxygen tolerance, which can lead to high yields of N_2O from both processes [*Firestone et al.*, 1980; *Goreau et al.*, 1980]. The oxycline is also a highly dynamic zone, where the location of the aerobic and anaerobic interface can shift over hourly to daily time scales in response to diurnal temperature cycles, wind induced mixing [*MacIntyre et al.*, 2001], or internal seiches [*Mortimer*, 1953] which can lead to short-term increases in N_2O production. For example, the injection of oxygenated waters into previously hypoxic areas may result in an enhanced denitrification N_2O yield because the N_2O-reducing enzyme is more sensitive to oxygen than the N_2O producing enzyme [*Codispoti*, 2010; *Firestone and Tiedje*, 1979; *Otte et al.*, 1996]. Similarly, the mixing of oxic and hypoxic waters can promote the coupling of aerobic and anaerobic metabolic processes, leading to enhanced N_2O production rates. The presence of a middepth N_2O maxima near the oxycline was not related to the thermocline stability index (TSI), however, suggesting that the extent of mixing at the hypoxic-oxic interface at the time of sampling did not determine the presence or absence of a middepth N_2O maximum. A possible explanation for this finding is that the observed middepth N_2O maxima may be a residual signal of enhanced N_2O production that occurred during a past mixing event.

While the presence of a N_2O maxima in close proximity to an oxycline has been reported for a wide range of lakes [*Knowles et al.*, 1981; *Mengis et al.*, 1997; *Yoh et al.*, 1988], the pattern is often transient and was only observed twice in this study. Nevertheless, the highest levels of dissolved N_2O observed in freshwater lakes have been in association with the oxycline, and future research could better resolve the underlying biology using a combination of isotopic and molecular approaches [*Priscu et al.*, 2008].

Six of the reservoirs exhibited a middepth N_2O maximum located well below the thermocline in the hypoxic hypolimnion during at least one site visit (Figures 2a, 2c, 2e–2g, and 4a). These localized areas of elevated N_2O
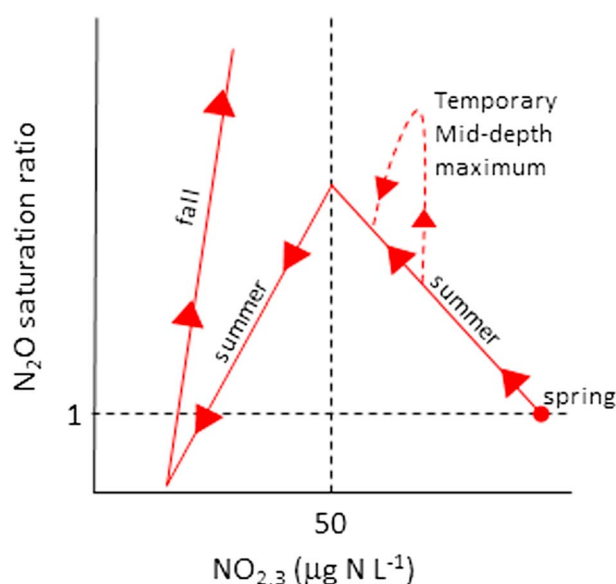
Figure 7. Schematic representation of temporal trends in nitrous oxide (N_2O) and nitrate plus nitrite ($NO_{2.3}$) concentrations in the hypolimnion of reservoirs from the onset of thermal stratification in the spring to turnover in the fall. The vertical dashed line represents a $NO_{2.3}$ concentration of approximately $50\,\mu g\,N\,L^{-1}$ where the hypolimnion switches from a net N_2O source to a N_2O sink. The horizontal dashed line represents a dissolved N_2O concentration that is in equilibrium with the atmosphere (i.e., N_2O saturation ratio = 1).

production are sufficiently distant from the thermocline that they are unlikely to be the result of variable oxygen availability, as may occur closer to the oxycline. Rather, they must result from another peculiarity in the chemical environment. One feature these reservoirs share is relatively high $NO_{2.3}$ concentrations in the hypolimnion (max $NO_{2.3}$ = 999, 1705, 672, 784, 162, and 639 in BRR, CCK, GRR, NRR, CFK, and EFR, respectively). It therefore appears that the coincidence of low DO and high $NO_{2.3}$ are required to generate a middepth N_2O maximum in the hypolimnion. Other factors which may further promote the development of a middepth N_2O maximum, but were not measured in this study, include low inorganic copper availability which is needed for the synthesis of N_2O reductase [*Twining et al.*, 2007] and high sulfide which can inhibit N_2O reductase activity [*Knowles*, 1982].

4.4. Turnover

Three lakes (CMR, SRR, and EFR) were sampled during turnover, the seasonal mixing of waters that occurs following the loss of thermal stratification in the fall. All three reservoirs contained high levels of dissolved N_2O throughout the mixed water column (Figure 3), with saturation ratios as high as 53 in SRR. The elevated levels of N_2O observed during lake turnover could reflect the release of N_2O that accumulated in the hypolimnion during stratification, as is commonly observed with methane [*Beaulieu et al.*, 2014a; *Schubert et al.*, 2012], or could be derived from new N_2O production resulting from the nitrification of NH_4^+ released from the hypolimnion. Since CMR and SRR were only sampled once, we cannot determine whether high levels of N_2O were present in the reservoir prior to turnover. EFR was sampled several times, however, and the hypolimnion contained little N_2O immediately prior to lake turnover (24 September 2013, Figure 4c); therefore the high levels of N_2O observed during turnover likely represent new N_2O production resulting from the nitrification of hypolimnetic NH_4^+ which had accumulated to nearly $1\,mg\,N\,L^{-1}$ at a depth of 23 m (i.e., the depth of the mixed water column on 29 October 2013; Table 2 and Figure S12e) by late September. The occurrence of nitrification is also consistent with the reappearance of $NO_{2.3}$ in the mixed water column and low levels of dissolved oxygen, which may have been reduced by the oxygen demand of nitrifiers.

The dissolved N_2O saturation levels observed in EFR during turnover exceeded those observed during any previous sampling trip, particularly in the epilimnion where the saturation ratio at the water surface was 16. This represents a strong concentration gradient between the atmosphere and water surface which likely resulted in the highest level of N_2O emissions to the atmosphere during the study, suggesting that lake turnover may be an important period of N_2O emission in lentic ecosystems. *Liu et al.* [2011] also reported elevated N_2O emissions associated with nitrification during lake turnover in a large reservoir in China, suggesting the pattern may be widespread.

5. Conclusions

We propose a conceptual model for describing N_2O dynamics in reservoir hypolimnia based on the results of the cross-site survey (Figure 7). In the early stages of thermal stratification (i.e., spring), $NO_{2.3}$ is in relative abundance and dissolved N_2O is near atmospheric equilibrium (Figure 7). As the season progresses, N_2O

accumulates in the hypoxic hypolimnion while $NO_{2.3}$ concentrations decrease, presumably due to incomplete denitrification. This phase may be accompanied by the development of a temporary middepth N_2O maximum, particularly in high $NO_{2.3}$ systems. Nitrous oxide continues to accumulate in the hypolimnion until $NO_{2.3}$ falls below a threshold of approximately 50 μg N L^{-1}, at which point the hypolimnion becomes a net N_2O sink, likely due to N_2O reduction to N_2 via denitrification. If the N_2O reduction phase persists long enough, the hypolimnion can become undersaturated with N_2O. Late in the season, thermal stratification begins to erode and oxygenated surface water mixes into the hypoxic hypolimnion resulting in a large increase in N_2O concentrations throughout the water column, likely due to nitrification of NH_4^+ that had accumulated in the hypolimnion.

While these observations provide a basis for predicting N_2O dynamics across reservoirs, the data must be interpreted within the context of the sample population. The reservoirs included in this study spanned a broad range of morphometric and watershed conditions, but were all located in the Midwestern U.S. and had hypoxic hypolimnion. Since oxygen is an important driver of N cycling processes, the generalizations reported here may not apply to less productive systems with oxygenated hypolimnion. To better understand the role of reservoirs as a N_2O source, this work should be expanded to include reservoirs across a broader range of trophic states and should include sampling during periods of lake turnover.

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