Whole-ecosystem oxygenation experiments reveal substantially greater hypolimnetic methane concentrations in reservoirs during anoxia

Alexandria G. Hounshell, Ryan P. McClure, Mary E. Lofton, Cayelan C. Carey
Department of Biological Sciences, Virginia Tech, Blacksburg, Virginia

Scientific Significance Statement
Oxygen concentrations in the bottom waters (hypolimnia) of lakes and reservoirs are an important regulator of the production and accumulation of dissolved greenhouse gases, such as carbon dioxide and methane. As the prevalence of hypolimnetic anoxia increases globally due to land use and climate change, it is expected that hypolimnetic methane concentrations will increase. We manipulated hypolimnetic oxygen at the whole-ecosystem scale in a eutrophic reservoir over three summers and found higher methane concentrations under anoxic vs. oxic conditions for both the experimental reservoir and an upstream reference reservoir, but similar carbon dioxide concentrations. Our whole-ecosystem experiments indicate that greater prevalence of hypolimnetic anoxia will likely increase hypolimnetic methane concentrations and increase the hypolimnetic global warming potential of lake and reservoir ecosystems in the future.

Abstract
Lakes and reservoirs globally produce large quantities of methane and carbon dioxide in their sediments, which accumulate in the hypolimnia (bottom waters) during thermally stratified conditions. A key parameter controlling hypolimnetic greenhouse gas concentrations is dissolved oxygen. Land use and climate change have increased hypolimnetic anoxia worldwide in lakes and reservoirs, which is expected to affect their methane and carbon dioxide concentrations. We conducted whole-ecosystem oxygenation experiments to assess the effects of oxygen concentrations on dissolved hypolimnetic greenhouse gas concentrations in comparison to a reference reservoir and calculated the maximum hypolimnetic global warming potential in both reservoirs over three summers. We observed significantly greater hypolimnetic methane under anoxic conditions but similar...
Lakes and reservoirs play a substantial role in the global carbon (C) cycle and serve as important sites of C transformation, burial, and export to downstream ecosystems (Cole et al. 2007; Tranvik et al. 2018). In particular, lakes and reservoirs contribute a significant proportion of global C-based greenhouse gases (GHGs: carbon dioxide, CO$_2$; methane, CH$_4$), despite their relatively small surface area (Downing et al. 2006; Bastviken et al. 2011; Deemer et al. 2016). Despite their importance for the global C cycle, it remains unclear how lake and reservoir CO$_2$ and CH$_4$ dynamics will respond to anthropogenic activities, including land use and climate change, which are altering the major drivers of their production (e.g., dissolved oxygen, temperature, organic C quality, primary production; Wik et al. 2016; Bartosiewicz et al. 2019; Beaulieu et al. 2019).

Important determinants of the magnitude of lake and reservoir CO$_2$ and CH$_4$ diffusive emissions are the production and subsequent concentrations of dissolved CO$_2$ and CH$_4$ in the water column (Cole et al. 2010), which may be changing in response to land use and climate change. Specifically, eutrophication, warmer temperatures, and enhanced thermal stratification have increased the prevalence of hypolimnetic anoxia (dissolved oxygen [DO] < 1 mg L$^{-1}$) worldwide (Butcher et al. 2015; Jenny et al. 2016). Greater organic matter deposition at the sediments due to increased nutrient concentrations, coupled with a longer thermally stratified period and decreased mixing with the epilimnion, promote hypolimnetic anoxia (Jenny et al. 2016). Hypolimnetic anoxia has substantial implications for hypolimnetic GHG production and concentrations, as most CO$_2$ and CH$_4$ in lakes and reservoirs with anoxic hypolimnia are produced near the sediment–water interface or within the top few millimeters of sediment (Bartosiewicz et al. 2019; Vachon et al. 2019).

It is predicted that hypolimnetic CH$_4$ concentrations will increase with greater hypolimnetic anoxia (Encinas Fernández et al. 2014; Vachon et al. 2019). Diffusive CH$_4$ fluxes measured during fall turnover support this prediction. Large fluxes of CH$_4$ are observed from ecosystems with anoxic hypolimnia (Encinas Fernández et al. 2014), with smaller CH$_4$ fluxes from oxic hypolimnia (López Bellido et al. 2009), suggesting greater accumulation of hypolimnetic CH$_4$ under anoxic vs. oxic conditions over a summer stratified-period. Similarly, modeling studies have shown higher CH$_4$ in the hypolimnia of lakes in response to anoxia (Bartosiewicz et al. 2019). Given that CH$_4$ is a 34x more potent greenhouse gas than CO$_2$ over a 100-yr horizon (Myhre et al. 2013), greater prevalence of hypolimnetic anoxia may thus increase the global warming potential (GWP) of lakes and reservoirs.

Changes in hypolimnetic CO$_2$ concentrations as a result of increasing hypolimnetic anoxia are less certain. It is predicted that hypolimnetic CO$_2$ concentrations would be lower in anoxic vs. oxic conditions due to less energetically favorable redox pathways (Stumm and Morgan, 1996; Hulthe et al., 1998) and lower organic matter mineralization rates (Bastviken et al. 2004). However, a whole-ecosystem, under-ice oxygenation experiment observed comparable CO$_2$ concentrations for both experimentally oxygenated and anoxic hypolimnia (Huttunen et al., 2001), suggesting that CO$_2$ may still accumulate in hypolimnia during anoxic conditions.

For waterbodies with anoxic hypolimnia, there is significant variability in the fraction of hypolimnetic GHGs that are directly emitted to the atmosphere vs. oxidized in the water column (CH$_4$: Bastviken et al. 2008; Vachon et al. 2019; Saarela et al. 2020) or taken up by phytoplankton (CO$_2$: Balmer and Downing, 2011; Engel et al. 2019). Encinas Fernández et al. (2014) estimated that up to ~50% of the total hypolimnetic CH$_4$ stored in anoxic hypolimnia is directly released to the atmosphere during turnover. Other studies have estimated the release of hypolimnetic CH$_4$ to the atmosphere as much lower, especially when turnover is slow or incomplete (Kankaala et al. 2007). During the summer-stratified period, a considerable fraction of hypolimnetic CH$_4$ can be oxidized just below the oxycline (Bastviken et al. 2008; Saarela et al. 2020). Given the uncertainty in the proportion of hypolimnetic GHG emissions released to the atmosphere vs. oxidized/taken up in the water column, hypolimnetic GHG concentrations measured throughout the summer stratified period have emerged as a useful and robust metric to assess the role of the whole ecosystem in the global C cycle (Bastviken et al. 2008; Encinas Fernández et al. 2014; Vachon et al. 2019) and provide important insights into the potential GWP of reservoirs and lakes with oxic vs. anoxic hypolimnia.

Our current understanding of the effects of anoxia on potential GWP from lakes and reservoirs are largely derived from observational or modeling studies and remain untested experimentally at the whole-ecosystem scale. Ecosystem-scale studies and experiments are necessary to understand how multiple, seasonally correlated environmental drivers (i.e., temperature, oxygen, organic C supply) control hypolimnetic GHGs, especially under future climate and land use change (Barley and Meeuwig, 2017). Studies assessing the effects of oxic vs. anoxic hypolimnia on GHG concentrations or emissions largely rely on comparisons between the prestratified oxic period and the summer-stratified anoxic period (López Bellido et al. 2009; Encinas Fernández et al. 2014; Vachon et al. 2019) or ecosystem models (Bartosiewicz et al. 2019).
While these studies represent an important step in understanding how GHGs will respond to changing hypolimnetic DO, they are unable to account for the effects of seasonally variable environmental parameters such as temperature, nutrient loading, allochthonous C loading, and autochthonous C production, among others.

We used whole-ecosystem experiments to manipulate hypolimnetic DO in a temperate reservoir over three summers to study how changing oxygen conditions alter hypolimnetic GHGs (CO₂, CH₄) and GWPs. We compared hypolimnetic GHGs and maximum hypolimnetic GWPs in the experimentally oxygenated reservoir with an upstream reference reservoir to examine how increasing hypolimnetic anoxia will alter the role of lakes and reservoirs in the global C cycle.

**Methods**

**Study sites and sampling methods**

We studied the effects of DO on hypolimnetic GHG concentrations and GWP in Falling Creek Reservoir (FCR; 37.30°N, 79.84°W) and Beaverdam Reservoir (BVR; 37.31°N, 79.81°W; Fig. 1A). FCR is a shallow ($Z_{\text{max}} = 9.3$ m), eutrophic reservoir located in Vinton, Virginia. FCR is dimictic and thermally stratified from April to October (McClure et al. 2018). The reference reservoir, BVR, has similar morphometry ($Z_{\text{max}} = 12$ m), stratification, water chemistry, and land use history (Gerling et al. 2016). BVR is located 3 km upstream and provides the primary inflow for FCR via a 1.7-km-long stream (Gerling et al. 2016). Both reservoirs have low-alkalinity and are owned and operated by the Western Virginia Water Authority as drinking water supplies and were constructed in the late 1800s (Gerling et al. 2016).

**Hypolimnetic oxygenation system (HOx)**

FCR has a side-stream hypolimnetic oxygenation system (HOx; Fig. 1B) that was installed to prevent hypolimnetic anoxia without altering thermal stratification or water temperature (Gerling et al. 2014). HOx systems are increasingly used to prevent water quality impairment associated with anoxia (Preece et al. 2019). The HOx was operated during the summer-stratified periods of 2016, 2017, and until 30 July in 2018 to maintain a well-oxygenated hypolimnion in FCR (Table SI.1). BVR also exhibits hypolimnetic anoxia but does not have a HOx system.

**Temperature, dissolved oxygen, and dissolved GHGs**

We collected water temperature, DO, and GHG profiles at ~1 week intervals from FCR and BVR during the ice-free period in 2016, 2017, and 2018 (Carey et al. 2019, 2020). Temperature and DO profiles were collected using an SBE 19plus V2 CTD and an SBE 43 Dissolved Oxygen Sensor (SeaBird Electronics, Bellevue, Washington). The CTD has a
scan rate of 4 Hz and a profile descent rate of < 0.2 m s\(^{-1}\). CTD casts were used to determine the date of fall turnover for each year and reservoir. Fall turnover was defined as the first day in autumn when temperatures at 1 and 8 m were the same (McClure et al. 2018). To assess the strength of thermal stratification, buoyancy frequency was calculated using CTD temperature profiles and LakeAnalyzer software (Read et al. 2011) in Matlab 2019a.

Water samples for dissolved CH\(_4\) and CO\(_2\) concentrations were collected with a 4-L Van Dorn sampler (Wildlife Supply, Yulee, Florida) routinely from the hypolimnion of FCR (8, 9 m) and BVR (9 m) at the deepest site (Fig. 1A; Figs. SI.1, SI.2). Water samples from each depth were transferred into two replicate 20-mL vials while preventing air exposure, then kept on ice. Samples were analyzed on a Gas Chromatograph with a Flame Ionization Detector (GC-FID) within ~ 24 h following McClure et al. (2018). We did not include CH\(_4\) ebullition in our estimates of hypolimnetic dissolved CH\(_4\) as we assumed there were negligible amounts of bubble dissolution in the hypolimnion of these shallow reservoirs (McGinnis et al. 2006).

**Maximum hypolimnetic GWP**

Volume-weighted (VW) hypolimnetic CO\(_2\) and CH\(_4\) were calculated using GHG replicate samples collected in the hypolimnion of each reservoir and scaled to the volume of each ~ 1 m depth-layer (8, 9 m for FCR; 9 m for BVR; Gerling et al. 2016; Carey et al. 2018; McClure et al. 2018; Tables SI.2, SI.3). We conservatively estimated the top of the hypolimnion as below the seasonally persistent thermocline and oxycline, even during periods of thermocline variability or formation of metalimnetic oxygen minima in FCR (6.5 m in FCR, 7 m in BVR; Fig. 2; Fig. SI.3). VW hypolimnetic concentrations were calculated for each GHG replicate then averaged.

Additionally, we note that water samples for dissolved GHGs were collected in BVR at 11 m sporadically throughout the study period; however, because samples were not consistently collected at this depth; we did not include them in subsequent calculations. We examined if omitting samples at 11 m caused us to underestimate VW hypolimnetic CH\(_4\) and CO\(_2\) concentrations by comparing calculated VW hypolimnetic concentrations using both 9 and 11 m as compared to just samples from 9 m on the days when we did have
data from both depths. Based on the median difference between the VW hypolimnetic concentrations on \( n = 44 \) d, we likely underestimated CH\(_4\) and CO\(_2\) in BVR by \( \sim 15\% \) and \( \sim 1\% \), respectively, over time. Thus, our results for BVR’s VW hypolimnetic CH\(_4\) concentrations and GWP are conservative.

Maximum hypolimnetic GWP, as CO\(_2\) equivalents, were calculated separately for each summer-stratified period and reservoir. First, the maximum CH\(_4\) and CO\(_2\) VW hypolimnetic concentrations were identified for each reservoir and year (Table S1.4). While we recognize that the maximum GHG concentrations were likely underestimates, the sampling frequency among years and reservoirs was consistent and thus these estimates provide a robust metric for comparison. The maximum VW hypolimnetic CO\(_2\) and CH\(_4\) concentrations were then converted to GWP, as CO\(_2\) equivalents. Second, CH\(_4\) was converted to mass units, multiplied by 34 to reflect the GWP of CH\(_4\) over a 100-yr horizon, and then added to the maximum and converted CO\(_2\) (GWP = 1; Myhre et al. 2013) to give the hypolimnetic GWP. Hypolimnetic GWP was calculated for each GHG replicate, then averaged.

**Results**

**Water column dissolved oxygen concentrations**

Hypolimnetic DO was > 8 mg L\(^{-1}\) for FCR during summers 2016 and 2017 while the hypolimnion in BVR was anoxic (< 1 mg L\(^{-1}\)) throughout the stratified period for all summers (Fig. 2). In summer 2018, the HOx in FCR was deactivated on 30 July, resulting in anoxic bottom waters from August through turnover. HOx operation did not alter thermal stratification in FCR, which was generally similar to BVR (Fig. S1.3). Buoyancy frequency was generally similar between FCR and BVR across years (Fig. S1.4).

---

**Fig 3.** Volume-weighted (VW) hypolimnetic (Hypo) dissolved CH\(_4\) concentration ([CH\(_4\); \( \mu \)mol L\(^{-1}\)]) for experimentally oxygenated FCR (blue lines) and reference BVR (orange lines) in (A) 2016, (B) 2017, and (C) 2018. Dashed vertical lines indicate the date of fall turnover for each reservoir. For 2018, the vertical solid line indicates when the HOx system in FCR was deactivated. Error bars represent the standard deviations between replicates.
Hypolimnetic GHG concentrations

There was significantly higher CH₄ in the hypolimnion of anoxic BVR vs. oxic FCR (Fig. 3): among years, concentrations in BVR were 15–800× higher and generally 2 orders of magnitude greater than in FCR. During summer 2018, there was an increase in CH₄ in the hypolimnion of FCR in August after the HOx was deactivated. Both reservoirs exhibited similar patterns in summer hypolimnetic CH₄ among years: for example, concentrations were lowest in 2017 and greatest in 2018. Despite the large differences in CH₄, hypolimnetic CO₂ was on the same order of magnitude for both reservoirs (Fig. 4). In 2018, hypolimnetic CO₂ concentrations in FCR increased throughout the summer, resulting in substantially higher hypolimnetic CO₂ prior to fall turnover.

We note a delay in CH₄ accumulation in the hypolimnia following onset of anoxia (2016–2018 for BVR; 2018 for FCR; Fig. 3). In BVR, hypolimnetic anoxia usually occurred in early May (Fig. 2), yet hypolimnetic CH₄ concentrations did not substantially increase until early June (CH₄ > 10 mg L⁻¹; Fig. 3). In FCR, there was rapid onset of anoxia after 30 July 2018 when the HOx system in FCR was deactivated, yet a similar delay of ~4 weeks in increasing hypolimnetic CH₄ (Figs. 2, 3).

Maximum hypolimnetic GWP

Maximum hypolimnetic GWP calculated for the summer stratified period was an order of magnitude greater for reference BVR than experimentally oxygenated FCR in all 3 yr (Fig. 5). The difference in maximum hypolimnetic GWP between reservoirs was driven by substantially higher maximum concentrations of hypolimnetic CH₄ in BVR (Fig. 5). In comparison, maximum hypolimnetic CO₂ was slightly higher in FCR for 2016 and 2017 and substantially higher in 2018, as compared to BVR (Fig. 5A). Maximum hypolimnetic GWP was

![Figure 4](image-url)

**Fig 4.** Volume-weighted (VW) hypolimnetic (Hypo) dissolved CO₂ concentration ([CO₂]) for experimentally oxygenated FCR (blue lines) and reference BVR (orange line) in (A) 2016, (B) 2017, and (C) 2018. Dashed vertical lines indicate the date of fall turnover for each reservoir. In 2018, the vertical solid line indicates when the HOx in FCR was deactivated. Error bars represent the standard deviation between replicates.
highest for FCR during 2018 due to the lack of sustained HOx operation (Fig. 5B).

Discussion

Hypolimnetic DO controls GHG concentrations

There were substantially lower CH4 concentrations in the oxic hypolimnion of the experimentally oxygenated reservoir (FCR) as compared to the anoxic hypolimnion of the reference reservoir (BVR) over all 3 yr, resulting in lower maximum hypolimnetic GWP in FCR (Figs. 3, 5). Hypolimnetic CO2, however, was on the same order of magnitude for both reservoirs (Fig. 4), indicating that CH4 was the main driver of differences in maximum hypolimnetic GWP between reservoirs. This has important implications for future C cycling in lakes and reservoirs as the extent, duration, and prevalence of anoxic hypolimnia are expected to increase (Butcher et al. 2015; Jenny et al. 2016).

Our study builds on previous modeling and monitoring studies which suggest that hypolimnetic DO is likely the dominant control on GHGs in lakes and reservoirs (Huttunen et al. 2001; Bastviken et al. 2008; Encinas Fernández et al. 2014; Vachon et al. 2019). Our multi-year whole-ecosystem experimental approach demonstrates that hypolimnetic anoxia resulted in elevated hypolimnetic CH4 concentrations and high maximum hypolimnetic GWP. Conversely, oxic conditions in the hypolimnion of FCR suppressed the production and accumulation of CH4, significantly reducing the maximum hypolimnetic GWP. Hypolimnetic CO2 concentrations, however, were comparable between both the anoxic and oxic hypolimnia despite contrasting redox conditions. Our results are similar to Huttunen et al. (2001), who reported comparable CO2 concentrations between oxic and anoxic hypolimnia.

Our data demonstrate that increased hypolimnetic anoxia will increase hypolimnetic CH4 concentrations but suggest that other factors, such as organic C quality, alkalinity, anaerobic redox processes, and CH4 oxidation rates may also be important drivers of hypolimnetic CO2 concentrations in addition to anoxia.

There is considerable debate on the fate of hypolimnetic GHGs, particularly CH4. The amount of hypolimnetic CH4 emitted to the atmosphere upon fall turnover can vary up to 50%, with the remaining hypolimnetic CH4 oxidized in the water column (Kankaala et al. 2007; Bastviken et al. 2008; Encinas Fernández et al. 2014; Vachon et al. 2019). While it is beyond the scope of this study to assess the fate of hypolimnetic CH4, we acknowledge this debate and highlight its importance for future work. Consequently, we suggest that maximum hypolimnetic GWP be viewed as a metric of maximum GWP, rather than of hypolimnetic emissions, allowing for direct comparisons of hypolimnetic GHGs across years and reservoirs.

Additional environmental controls on GHG concentrations

In addition to increasing the prevalence and duration of hypolimnetic anoxia, climate and land use change are predicted to influence hypolimnetic GHG concentrations by changing bottom-water temperatures, thermal stratification, and allochthonous and autochthonous organic C loading and production (Bartosiewicz et al. 2019; Beaulieu et al. 2019). Previous modeling work suggests that future CH4 and CO2 dynamics will be primarily driven by increased duration of anoxia and secondarily by changing hypolimnetic temperature (Bartosiewicz et al. 2019). To test this hypothesis, we explored variation in hypolimnetic temperature, primarily at
the sediment–water interface (SWI) (Fig. S1.5). Overall, BVR had substantially lower SWI temperature (by ~2.7–4.0°C) than FCR. However, BVR still had higher maximum hypolimnetic GWP as a result of elevated CH₄ (Figs. 3, 5), due to BVR’s anoxic hypolimnion. Consequently, our results support Bartosiewicz et al. (2019) in that hypolimnetic DO was a more important driver of hypolimnetic CH₄ concentrations in our reservoirs as compared to cooler temperatures.

In summer 2018, SWI temperature was ~1.5°C lower within both reservoirs as compared to previous years, yet both FCR and BVR had higher GHG concentrations, suggesting that additional factors, such as organic C quantity and quality, may also be important drivers of hypolimnetic GHGs. For example, there was a substantial phytoplankton bloom in BVR in 2018 which may have fueled the increased production of CH₄ in the anoxic hypolimnion during the late-summer, despite cooler hypolimnetic temperatures (Fig. S1.6). There is a strong correlation between primary production and CH₄ concentrations across freshwater ecosystems (West et al., 2016), indicating that increased primary production due to land-use change may fuel increases in hypolimnetic CH₄ concentrations in the future (Beaulieu et al. 2019). While our study demonstrates the dominant control on hypolimnetic GHG accumulation and maximum hypolimnetic GWP is DO, additional work is needed to determine how other variables (e.g., temperature, organic C quantity and quality) and their interactions will influence hypolimnetic GHG concentrations under future conditions.

While whole-ecosystem experiments are needed to understand how multiple environmental drivers may impact C cycling under variable conditions (Carey et al. 2018), we recognize caveats with this approach. For example, given sampling constraints and variability in turnover dates across years and reservoirs, we did not fully capture GHGs for the entire stratified period. Specifically, turnover in BVR in 2018 did not occur until 29 October, yet the last GHG sample was collected on 19 October. Additionally, our definition of fall turnover may not accurately describe when accumulated hypolimnetic GHG concentrations are greatest, though we note that our observed hypolimnetic GHG concentrations fall within the range of other lakes and reservoirs (Huttunen et al. 2001; Kankaala et al. 2007; Bastviken et al. 2008; López Bellido et al. 2009; Encinas Fernández et al. 2014; Vachon et al. 2019). While these two reservoirs are located adjacent to each other and thus have similar land use histories and meteorological conditions, as well as generally similar phytoplankton (Fig. S1.6) and dissolved organic C concentrations (Fig. S1.7), suggesting similar organic matter dynamics, every ecosystem is inherently unique and may have variable responses to hypolimnetic DO. Finally, we also note that the hypolimnion, as defined in this study (> 6.5 m in FCR; > 7 m in BVR), represents ~ 5% and ~ 11% of the total reservoir volume for FCR and BVR, respectively. Thus, while the hypolimnion likely plays a disproportionate role in driving the GHG budgets of these two ecosystems (Bartosiewicz et al. 2019; Vachon et al. 2019), this remains unquantified.

**Role of lakes and reservoirs as hotspots of GHG production**

This study demonstrates the importance of oxygen in controlling hypolimnetic GHGs and GWP by simultaneously assessing hypolimnetic CH₄ and CO₂ in two adjacent, eutrophic, temperate reservoirs with contrasting hypolimnetic DO. Hypolimnetic CH₄ was substantially greater in anoxic hypolimnion as compared to oxic hypolimnion both across the summer-stratified season and on shorter timescales (i.e., FCR in late summer 2018). Conversely, we found hypolimnetic CO₂ concentrations for oxic and anoxic hypolimnion were on the same order of magnitude. Moreover, we observed a delay in hypolimnetic CH₄ accumulation relative to CO₂ following anoxia onset, which suggests that in addition to affecting GWP of lakes and reservoirs, alternate redox pathways that precede methanogenesis and produce CO₂ (e.g., denitrification; manganese, iron, or sulfate reduction) may affect GHG production and accumulation. Additionally, high concentrations of CO₂ observed under anoxic conditions may be evidence of high rates of anaerobic methane oxidation, though additional research is needed to determine the specific pathway (Reed et al. 2017).

The whole-ecosystem oxygenation system in FCR offers a unique opportunity to assess in situ how predicted changes in anoxia may alter future GHGs in waterbodies. Data from the National Inventory of Dams (USACE 2013) indicate that FCR and BVR are not unique reservoirs, suggesting that our results are likely applicable to other waterbodies. For example, the surface areas of most reservoirs in the United States are <1 km² and 9% are > 100 yr old, like FCR and BVR. Consequently, there are hundreds of older reservoirs across the United States accumulating vast stores of organic C that can be potentially mineralized to CH₄ and CO₂. Our results indicate that if hypolimnetic anoxia continues to increase, there will be substantially higher hypolimnetic CH₄ and maximum GWP from lakes and reservoirs in the future, which has important implications for the global C cycle.

**References**

Balmer, M., and J. Downing. 2011. Carbon dioxide concentrations in eutrophic lakes: Undersaturation implies atmospheric uptake. Inland Waters 1: 125–132. https://doi.org/10.5268/IW-1.2.366.

Barley, S. C., and J. J. Meeuwig. 2017. The power and the pitfalls of large-scale, unreplicated natural experiments. Ecosystems 20: 331–339. https://doi.org/10.1007/s10021-016-0028-5.

Bartosiewicz, M., A. Przytulska, J. Lapierre, I. Laurion, M. F. Lehmann, and R. Maranger. 2019. Hot tops, cold bottoms: Synergistic climate warming and shielding effects increase...
carbon burial in lakes. Limnol. Oceanogr. **4**: 132–144. https://doi.org/10.1002/lo2.10117.

Bastviken, D., L. Persson, G. Odham, and L. Tranvik. 2004. Degradation of dissolved organic matter in oxic and anoxic lake water. Limnol. Oceanogr. **49**: 109–116. https://doi.org/10.4319/lo.2004.49.1.0109.

Bastviken, D., J. J. Cole, M. L. Pace, and M. C. Van de Bogert. 2008. Fates of methane from different lake habitats: Connecting whole-lake budgets and CH₄ emissions. J. Geophys. Res. **113**: 1–13. https://doi.org/10.1029/2007JG000608.

Bastviken, D., L. J. Tranvik, J. A. Downing, P. M. Crill, and A. Enrich-Prast. 2011. Freshwater methane emissions offset the continental carbon sink. Science **331**: 50–50. https://doi.org/10.1126/science.1196808.

Beaulieu, J. J., T. DelSonstro, and J. A. Downing. 2019. Eutrophication will increase methane emissions from lakes and impoundments during the 21st century. Nat. Commun. **10**: 1375. https://doi.org/10.1038/s41467-019-09100-5.

Butcher, J. B., D. Nover, T. E. Johnson, and C. M. Clark. 2015. Sensitivity of lake thermal and mixing dynamics to climate change. Clim. Change **129**: 295–305. https://doi.org/10.1007/s10584-015-1326-1.

Carey, C. C., J. P. Doubek, R. P. McClure, and P. C. Hanson. 2018. Oxygen dynamics control the burial of organic carbon in a eutrophic reservoir: Oxygen dynamics control OC burial. Limnol. Oceanogr. **3**: 293–301. https://doi.org/10.1002/lo2.10057.

Carey C. C., A. S. Lewis, R. P. McClure, A. B. Gerling, J. P. Doubek, S. Chen, M. E. Lofton, K. D. Hamre. 2019. Time series of high-frequency profiles of depth, temperature, dissolved oxygen, conductivity, specific conductivity, chlorophyll a, turbidity, pH, oxidation-reduction potential, photosynthetic active radiation, and descent rate for Beaverdam Reservoir, Carvins Cove Reservoir, Falling Creek Reservoir, Gatewood Reservoir, and Spring Hollow Reservoir in Southwestern Virginia, USA during 2016–2019. Environmental Data Initiative, [accessed 2020 January 17]. Available from https://doi.org/10.6073/pasta/1fc7d2a5c699c6a651793d6ba06d375ae2

Carey, C. C., R. P. McClure, B. R. Niederlehner, M. E. Lofton, and A. G. Hounshell. 2020. Time series of dissolved methane and carbon dioxide concentrations for Falling Creek Reservoir and Beaverdam Reservoir in southwestern Virginia, USA during 2016–2018. Environmental Data Initiative, [accessed 2020 June 25]. Available from https://doi.org/10.6073/pasta/c5d0a145a9e5a166daa08b10d6d44

Cole, J. J., and others. 2007. Plumbing the global carbon cycle: Integrating inland waters into the terrestrial carbon budget. Ecosystems **10**: 172–185. https://doi.org/10.1007/s10021-006-9013-8.

Cole, J. J., D. L. Bade, D. Bastviken, M. L. Pace, and M. Van de Bogert. 2010. Multiple approaches to estimating air-water gas exchange in small lakes: Gas exchange in lakes. Limnol. Oceanogr.: Methods **8**: 285–293. https://doi.org/10.4319/iom.2010.8.285.

Deemer, B. R., and others. 2016. Greenhouse gas emissions from reservoir water surfaces: A new global synthesis. BioScience **66**: 949–964. https://doi.org/10.1093/biosci/biw117.

Downing, J. A., and others, 2006. The global abundance and size distribution of lakes, ponds, and impoundments. Limnol. Oceanogr. **51**: 2388–2397. https://doi.org/10.4319/lo.2006.51.5.2388

Encinas Fernández, J., F. Peeters, and H. Hofmann. 2014. Importance of the autumn overturn and anoxic conditions in the hypolimnion for the annual methane emissions from a temperate lake. Environ. Sci. Technol. **48**: 7297–7304. https://doi.org/10.1021/es4056164.

Engel, F., S. Drakare, and G. A. Weyhenmeyer. 2019. Environmental conditions for phytoplankton influenced carbon dynamics in boreal lakes. Aquat. Sci. **81**: 35. https://doi.org/10.1007/s00227-019-0631-6.

Gerling, A. B., R. G. Browne, P. A. Gantzer, M. H. Mobley, J. C. Little, and C. C. Carey. 2014. First report of the successful operation of a side stream supersaturation hypolimnetic oxygenation system in a eutrophic, shallow reservoir. Water Research **67**: 129–143. https://doi.org/10.1016/j.watres.2014.09.002.

Gerling, A. B., Z. W. Munger, J. P. Doubek, K. D. Hamre, P. A. Gantzer, J. C. Little, and C. C. Carey. 2016. Whole-catchment manipulations of internal and external loading reveal the sensitivity of a century-old reservoir to hypoxia. Ecosystems **19**: 555–571. https://doi.org/10.1007/s10021-015-9951-0.

Hulthe, G., S. Hulth, and P. O. J. Hall. 1998. Effect of oxygen on degradation rate of refractory and labile organic matter in continental margin sediments. Geochim. Cosmochim. Acta **62**: 1319–1328. https://doi.org/10.1016/S0016-7037(98)00044-1.

Huttunen, J. T., T. Hammar, J. Alm, J. Silvola, and P. J. Martikainen. 2001. Greenhouse gases in non-oxygenated and artificially oxygenated eutrophied lakes during winter stratification. J. Environ. Qual. **30**: 387–394. https://doi.org/10.2134/jeq2001.302387x.

Jenny, J. P., P. Francus, A. Normandeau, F. Lapointe, M. E. PerGa, A. Ojala, A. Schimmelmann, and B. Zolitschka. 2016. Global spread of hypoxia in freshwater ecosystems during the last three centuries is caused by rising local human pressure. Glob. Change Biol. **22**: 1481–1489. https://doi.org/10.1111/gcb.13193.

Kankaala, P., S. Taipale, H. Nykänen, and R. I. Jones. 2007. Oxidation, efflux, and isotopic fractionation of methane during autumnal turnover in a polyhumic, boreal lake. J. Geophys. Res. **112**: G02033. https://doi.org/10.1029/2006JG000336.

López Bellido, J., T. Tulonen, P. Kankaala, and A. Ojala. 2009. CO₂ and CH₄ fluxes during spring and autumn mixing periods in a boreal lake (Pääjärvi, southern Finland).
J. Geophys. Res. 114: G04007. https://doi.org/10.1029/2009JG000923.

McClure, R. P., K. D. Hamre, B. R. Niederlehner, Z. W. Munger, S. Chen, M. E. Lofton, M. E. Schreiber, and C. C. Carey. 2018. Metalimnetic oxygen minima alter the vertical profiles of carbon dioxide and methane in a managed freshwater reservoir. Sci. Total Environ. 636: 610–620. https://doi.org/10.1016/j.scitotenv.2018.04.255.

McGinnis, D. F., J. Greinert, Y. Artemov, S. E. Beaubien, and A. Wüest. 2006. Fate of rising methane bubbles in stratified waters: How much methane reaches the atmosphere? J. Geophys. Res. 111: C09007. https://doi.org/10.1029/2005JC003183.

Myhre, G., and others. 2013. Anthropogenic and natural radiative forcing. In T. F. Stocker, and others [eds.], Climate change 2013: The physical science basis. Contribution of working group I to the fifth assessment report of the intergovernmental panel on climate change. Cambridge, UK: Cambridge Univ. Press.

Preece, E. P., B. C. Moore, M. M. Skinner, A. Child, and S. Dent. 2019. A review of the biological and chemical effects of hypolimnetic oxygenation. Lake Reserv. Manage. 35: 229–246. https://doi.org/10.1080/10402381.2019.1580325.

Read, J. S., D. P. Hamilton, I. D. Jones, K. Muraoka, L. A. Winslow, R. Kroiss, C. H. Wu, and E. Gaiser. 2011. Derivation of lake mixing and stratification indices from high-resolution lake buoy data. Environ. Model. Softw. 26: 1325–1336. https://doi.org/10.1016/j.envsoft.2011.05.006.

Reed, D. C., B. R. Deemer, S. van Grinsven, and J. A. Harrison. 2017. Are elusive anaerobic pathways key methane sinks in eutrophic lakes and reservoirs? Biogeochemistry 134: 29–39. https://doi.org/10.1007/s10533-017-0356-3.

Saarela, T., A. J. Rissanen, A. Ojala, J. Pumpanan, S. L. Aalto, M. Tiirola, T. Vesala, and H. Jäntti. 2020. CH4 oxidation in a boreal lake during the development of hypolimnetic hypoxia. Aquat. Sci. 82: 19. https://doi.org/10.1007/s00027-019-0690-8.

Stumm, W., and J. J. Morgan. 1996, Aquatic chemistry: Chemical equilibria and rates in natural waters, 3rd Edition. Wiley.

Tranvik, L. J., J. J. Cole, and Y. T. Prairie. 2018. The study of carbon in inland waters—from isolated ecosystems to players in the global carbon cycle: The study of carbon in inland waters. Limnol. Oceanogr. 3: 41–48. https://doi.org/10.1002/lol2.10068.

USACE (United States Army Corps of Engineers). 2013. National inventory of Dams (NID). [accessed 2016 January 5]. Available from https://nid.sec.usace.army.mil/ords/f?p=105:22:9830782489026::NO

Vachon, D., T. Langenegger, D. Donis, and D. F. McGinnis. 2019. Influence of water column stratification and mixing patterns on the fate of methane produced in deep sediments of a small eutrophic lake. Limnol. Oceanogr. 64: 2114–2128. https://doi.org/10.1002/lno.11172.

West WE, Creamer KP, Jones SE. 2016. Productivity and depth regulate lake contributions to atmospheric methane: Lake productivity fuels methane emissions. Limnol. Oceanogr. 61: S51–S61. http://doi.org/10.1002/lno.10247.

Wik, M., B. F. Thornton, D. Bastviken, J. Uhlbäck, and P. M. Crill. 2016. Biased sampling of methane release from northern lakes: A problem for extrapolation. Geophys. Res. Lett. 43: 1256–1262. https://doi.org/10.1002/2015GL066501.

Acknowledgments

We thank Bobbie Niederlehner, Kathryn Krueger, Zackary Munger, Jonathan Doubek, and many undergraduate helpers for their assistance with field collection and laboratory analysis. Additionally, we thank the Western Virginia Water Authority (WWA), especially Cheryl Brewer and Jamie Morris, for long-term access to field sites and logistical support. Madeline Schreiber provided helpful feedback on the manuscript and the FCR Carbon team helped catalyze many ideas related to this work. We also thank two anonymous reviewers and the managing editor for providing helpful feedback to improve the manuscript. We gratefully acknowledge funding from U.S. National Science Foundation grants CNS-1737424, DEB-1702506, DEB-1753639, DEB-1926050, and DBI-1933016; the Virginia Tech Institute for Critical Technology and Applied Science; and Fralin Life Sciences Institute at Virginia Tech.

Submitted 26 February 2020
Revised 07 August 2020
Accepted 05 October 2020