

LETTER

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

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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

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Data Availability Statement: The greenhouse gas data (Carey et al. 2020; <https://portal.edirepository.org/nis/mapbrowse?packageid=edi.551.1>) in this manuscript are archived in the Environmental Data Initiative (EDI) repository and accessible via their online portal (<https://environmentaldatainitiative.org/>). The temperature and oxygen depth profiles (Carey et al. 2019) are also archived in the EDI repository.

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carbon dioxide concentrations, leading to greater hypolimnetic global warming potential of anoxic hypolimnia. Our study indicates that the global warming potential of hypolimnetic greenhouse gas concentrations may increase as the prevalence of hypolimnetic anoxia increases due to global change.

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, CO₂; methane, CH₄), 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 CO₂ and CH₄ 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 CO₂ and CH₄ diffusive emissions are the production and subsequent concentrations of dissolved CO₂ and CH₄ 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 CO₂ and CH₄ 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 CH₄ concentrations will increase with greater hypolimnetic anoxia (Encinas Fernández et al. 2014; Vachon et al. 2019). Diffusive CH₄ fluxes measured during fall turnover support this prediction. Large fluxes of CH₄ are observed from ecosystems with anoxic hypolimnia (Encinas Fernández et al. 2014), with smaller CH₄ fluxes from oxic hypolimnia (López Bellido et al. 2009), suggesting greater accumulation of hypolimnetic CH₄ under anoxic vs. oxic conditions over a summer stratified-period. Similarly, modeling studies have shown higher CH₄ in the hypolimnia of lakes in response to anoxia (Bartosiewicz et al. 2019). Given that CH₄ is a 34× more potent greenhouse gas than CO₂ 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 CO₂ concentrations as a result of increasing hypolimnetic anoxia are less certain. It is predicted that hypolimnetic CO₂ 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 CO₂ concentrations for both experimentally oxygenated and anoxic hypolimnia (Huttunen et al., 2001), suggesting that CO₂ 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 (CH₄: Bastviken et al. 2008; Vachon et al. 2019; Saarela et al. 2020) or taken up by phytoplankton (CO₂: Balmer and Downing, 2011; Engel et al. 2019). Encinas Fernández et al. (2014) estimated that up to ~ 50% of the total hypolimnetic CH₄ stored in anoxic hypolimnia is directly released to the atmosphere during turnover. Other studies have estimated the release of hypolimnetic CH₄ 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 CH₄ 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 (CO_2 , CH_4) 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

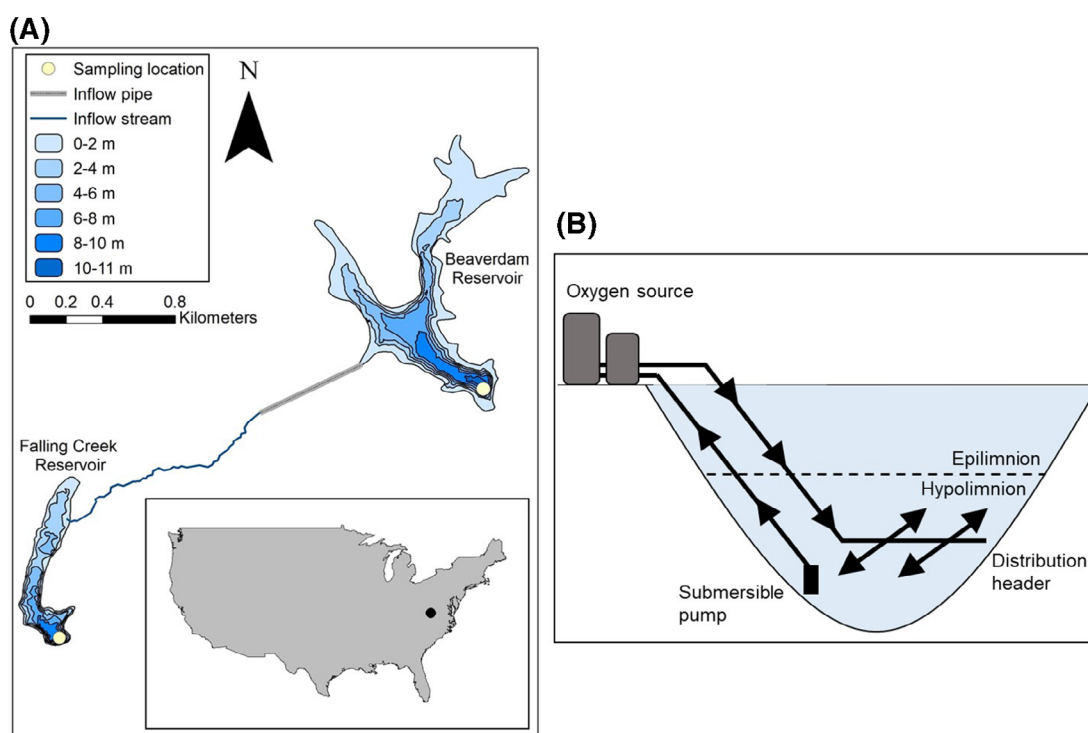


Fig 1. (A) Map of Falling Creek (FCR) and Beaverdam (BVR) Reservoirs located in Southwest, Virginia (see map inset; adapted from McClure et al. 2018). The yellow dots in each reservoir mark the location of sampling at the deepest point of each reservoir. (B) Schematic of the hypolimnetic oxygenation (HOx) system constructed in the hypolimnion of FCR (adapted from Gerling et al. 2014).

scan rate of 4 Hz and a profile descent rate of $< 0.2 \text{ m s}^{-1}$. 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 $\sim 24 \text{ h}$ 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 $\sim 1 \text{ m}$ 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

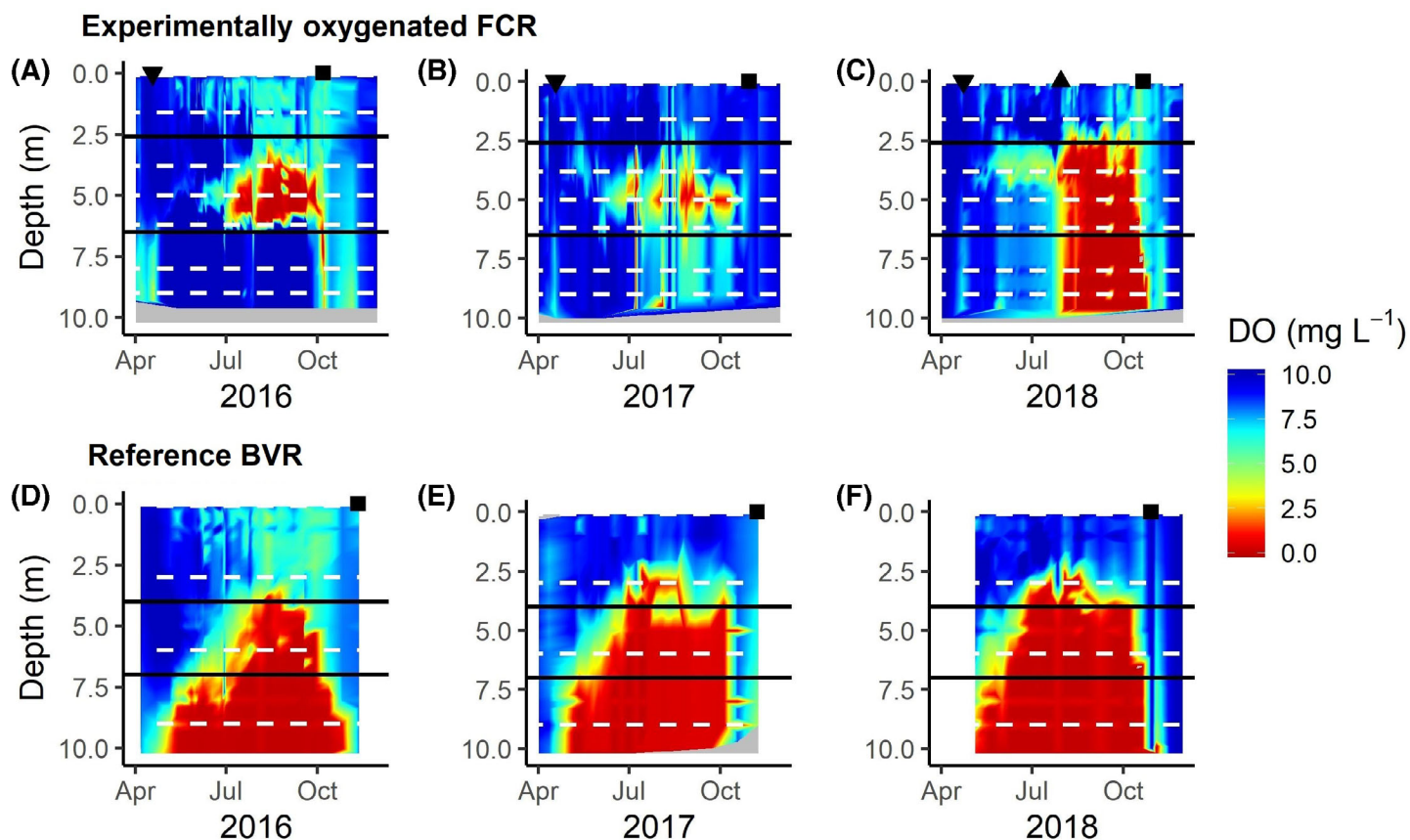


Fig 2. Dissolved oxygen heatmaps for FCR in (A) 2016, (B) 2017, and (C) 2018 and for BVR in (D) 2016, (E) 2017, and (F) 2018. Black triangles indicate when the oxygenation system was turned on (triangle down) and off (triangle up) in FCR during 2018. The black square indicates the date of fall turnover for each reservoir and year. White dashed lines indicate depths of sample collection for greenhouse gas (CO_2 and CH_4) concentrations. Solid black lines indicate the boundaries used to distinguish the epilimnion, metalimnion, and hypolimnion.

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 GWPs, 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 SI.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 $> 8 \text{ mg L}^{-1}$ for FCR during summers 2016 and 2017 while the hypolimnion in BVR was anoxic ($< 1 \text{ mg L}^{-1}$) 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. SI.3). Buoyancy frequency was generally similar between FCR and BVR across years (Fig. SI.4).

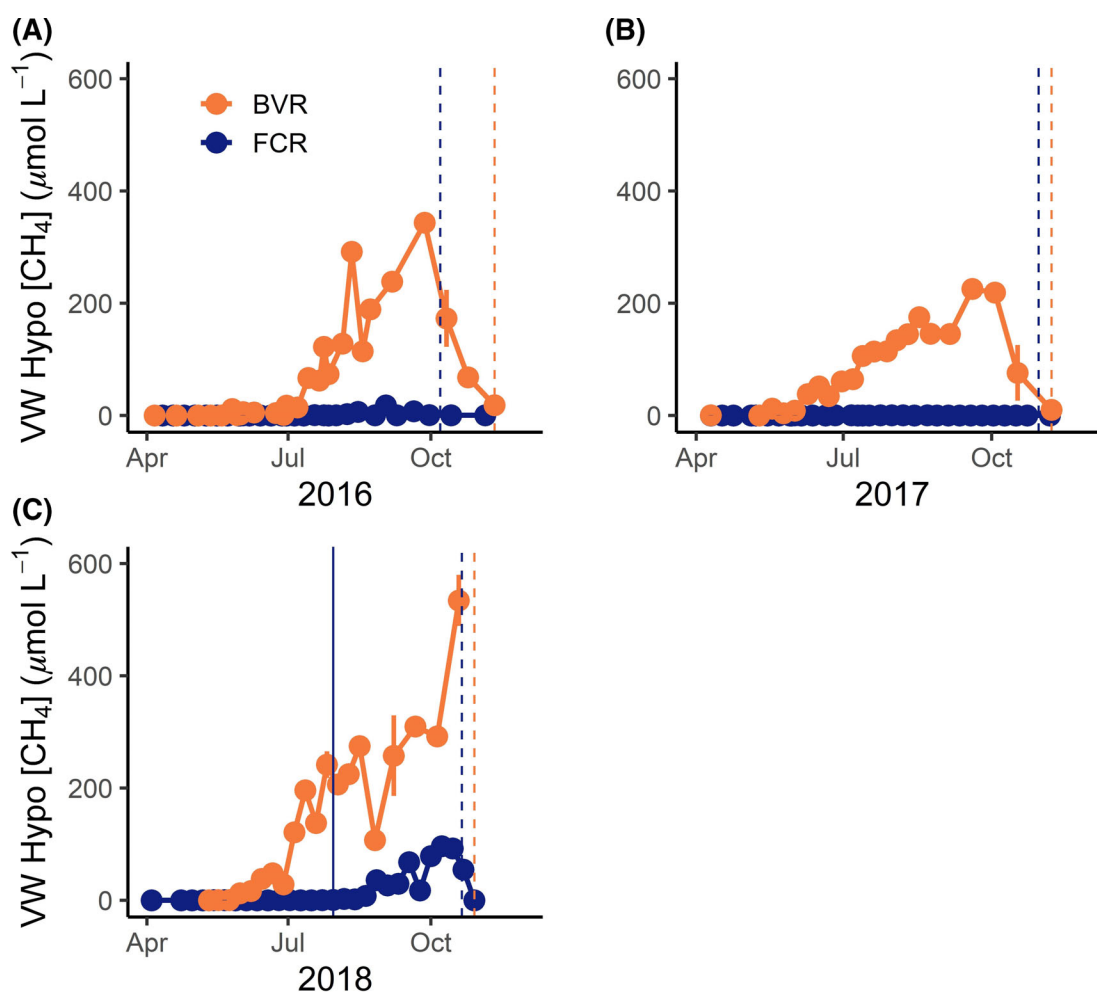


Fig 3. Volume-weighted (VW) hypolimnetic (Hypo) dissolved CH_4 concentration ($[\text{CH}_4]$) for experimentally oxygenated FCR (blue lines) and reference BVR (orange lines) in (A) 2016, (B) 2017, and (C) 2018. Dashed vertical lines indicate the date of fall turnover for each reservoir. For 2018, the 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 CH_4 in the hypolimnion of anoxic BVR vs. oxic FCR (Fig. 3); among years, concentrations in BVR were 15–800 \times higher and generally 2 orders of magnitude greater than in FCR. During summer 2018, there was an increase in CH_4 in the hypolimnion of FCR in August after the HOx was deactivated. Both reservoirs exhibited similar patterns in summer hypolimnetic CH_4 among years: for example, concentrations were lowest in 2017 and greatest in 2018. Despite the large differences in CH_4 , hypolimnetic CO_2 was on the same order of magnitude for both reservoirs (Fig. 4). In 2018, hypolimnetic CO_2 concentrations in FCR increased throughout the summer, resulting in substantially higher hypolimnetic CO_2 prior to fall turnover.

We note a delay in CH_4 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 CH_4 concentrations did not substantially increase until early June ($\text{CH}_4 > 10 \text{ mg L}^{-1}$; 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 CH_4 (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 CH_4 in BVR (Fig. 5). In comparison, maximum hypolimnetic CO_2 was slightly higher in FCR for 2016 and 2017 and substantially higher in 2018, as compared to BVR (Fig. 5A). Maximum hypolimnetic GWP was

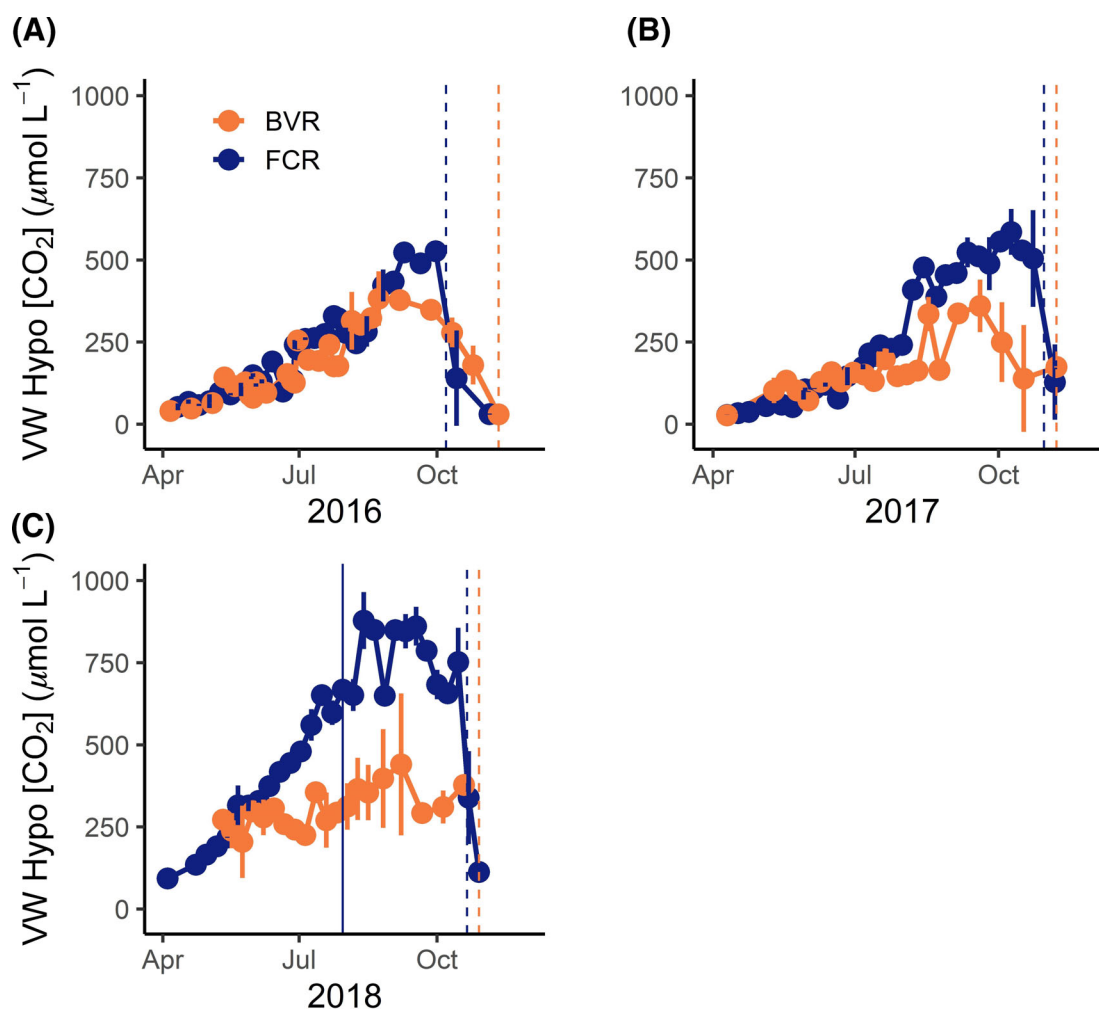


Fig 4. Volume-weighted (VW) hypolimnetic (Hypo) dissolved CO_2 concentration ($[\text{CO}_2]$) for experimentally oxygenated FCR (blue lines) and reference BVR (orange line) in (A) 2016, (B) 2017, and (C) 2018. Dashed vertical lines indicate the date of fall turnover for each reservoir. In 2018, the vertical solid line indicates when the HOx in FCR was deactivated. Error bars represent the standard deviation between replicates.

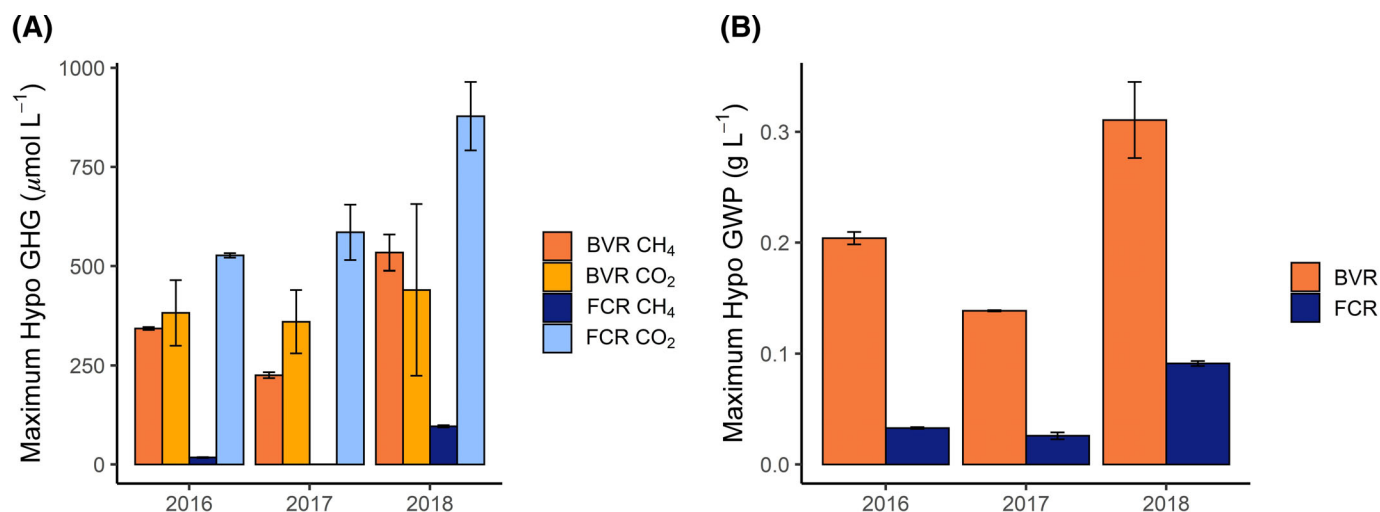


Fig 5. (A) Maximum hypolimnetic GHG concentrations for each summer stratified period for both CH₄ and CO₂ in BVR and FCR, by year. (B) Maximum hypolimnetic GWP for FCR (experimentally oxygenated) and BVR (reference). Error bars represent the standard deviation as calculated from replicate samples.

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. SI.5). Overall, BVR had substantially lower SWI temperature (by $\sim 2.7\text{--}4.0^\circ\text{C}$) than FCR. However, BVR still had higher maximum hypolimnetic GWP as a result of elevated CH_4 (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 CH_4 concentrations in our reservoirs as compared to cooler temperatures.

In summer 2018, SWI temperature was $\sim 1.5^\circ\text{C}$ 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 CH_4 in the anoxic hypolimnion during the late-summer, despite cooler hypolimnetic temperatures (Fig. SI.6). There is a strong correlation between primary production and CH_4 concentrations across freshwater ecosystems (West et al., 2016), indicating that increased primary production due to land-use change may fuel increases in hypolimnetic CH_4 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. SI.6) and dissolved organic C concentrations (Fig. SI.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 $\sim 5\%$ and $\sim 11\%$ 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 CH_4 and CO_2 in two adjacent, eutrophic, temperate reservoirs with contrasting hypolimnetic DO. Hypolimnetic CH_4 was substantially greater in anoxic hypolimnia as compared to oxic hypolimnia both across the summer-stratified season and on shorter timescales (i.e., FCR in late summer 2018). Conversely, we found hypolimnetic CO_2 concentrations for oxic and anoxic hypolimnia were on the same order of magnitude. Moreover, we observed a delay in hypolimnetic CH_4 accumulation relative to CO_2 following anoxia onset, which suggests that in addition to affecting GWP of lakes and reservoirs, alternate redox pathways that precede methanogenesis and produce CO_2 (e.g., denitrification; manganese, iron, or sulfate reduction) may affect GHG production and accumulation. Additionally, high concentrations of CO_2 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 CH_4 and CO_2 . Our results indicate that if hypolimnetic anoxia continues to increase, there will be substantially higher hypolimnetic CH_4 and maximum GWP from lakes and reservoirs in the future, which has important implications for the global C cycle.

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