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RESEARCH ARTICLE

Contrasting patterns of 2-methylisoborneol (MIB) vs. geosmin across depth in a drinking water reservoir are mediated by cyanobacteria and actinobacteria

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Abstract

Taste and odor episodes caused by off-flavor secondary metabolites, such as 2-methylisoborneol (MIB) and geosmin, pose one of the greatest challenges for drinking water utilities around the world. The prevalence of these compounds is predicted to increase in the future as a function of nutrient enrichment and elevated temperatures of surface drinking water sources. We conducted a manipulative field experiment in a drinking water reservoir to elucidate patterns for two taste and odor compounds, MIB and geosmin, as well as two taxa known to produce these compounds, phytoplankton (more specifically, cyanobacteria) and actinobacteria, across different depths in response to nutrient enrichment with two common dissolved nitrogen forms, organic urea or inorganic nitrate. In general, we found that MIB levels increased by greater than 250% with nutrient enrichment mediated by increased phytoplankton biomass. However, the effect of the fertilization treatments on MIB decreased with depth with a 35% reduction at 7 m versus 1.5 m. In contrast, geosmin levels reached a maximum at the lowest measured depth (7 m), were unaffected by the fertilization treatments, and followed a similar pattern to the abundance of actinobacteria. Thus, our data suggest that the positive response of phytoplankton (e.g., cyanobacteria, such as *Oscillatoria* species) to the fertilization treatments is likely responsible for increased MIB, while geosmin concentrations may be a function of actinobacteria-mediated decomposition in the hypolimnion in our study system.

Keywords Actinomycetes · Blue-green algae · Eutrophication · Harmful algal blooms (HABs) · Nutrient enrichment · Pollution · *Streptomyces* · Taste and odor episodes

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Introduction

Taste and odor events cause some of the greatest challenges for drinking water utilities around the world and are expected to increase in frequency and intensity as surface waters respond to elevated temperatures and nutrient inputs (Kutovaya and Watson 2014; Watson et al. 2016; Zhang et al. 2017). Two common taste and odor compounds, 2-methylisoborneol (hereafter, MIB) and geosmin ((4*S*,4a*S*,8a*R*)-4,8a-dimethyl-1,2,3,4,5,6,7,-octahydronaphthalen-4a-ol), are attributed to two primary taxa: (1) phytoplankton (e.g., cyanobacteria, diatoms) and (2) actinobacteria (e.g., actinomycetes) (Wnorowski 1992; Sugiura et al. 1998; Chislock 2015; Su et al. 2015; Suurnakki et al. 2015; Clercin and Druschel 2019). Given the contrasting life histories of these two taxa, the observed relationship between depth and taste and odor compounds may help to elucidate the mechanisms responsible for off-flavor events. For example, taste and odor concentrations are predicted to be highest near the surface or mid-depth if phytoplankton

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(especially bloom-forming taxa, such as cyanobacteria) are abundant (Harris et al. 2016; Cai et al. 2017; Deng et al. 2019; Jia et al. 2019), while taste and odor concentrations will be highest near the sediments if actinobacteria are responsible for their production (Zwart et al. 2002). Benthic cyanobacteria capable of producing taste and odor compounds can further complicate these trends (Halstvedt et al. 2007; Su et al. 2017). Temperature-mediated stratification should amplify these patterns by minimizing mixing between the epilimnion and hypolimnion of a lentic waterbody (Su et al. 2015; Anuar et al. 2017; Gao et al. 2018). Thus, understanding how taste and odor compounds relate to the abundances of phytoplankton and actinobacteria across depth can be useful for ecosystem- and watershed-level management of these compounds, especially in the context of nutrient enrichment (Izaguirre and Taylor 2007).

Recent research has focused on cyanobacteria as a driver of MIB and geosmin levels in waterbodies (Jüttner and Watson 2007; Qi et al. 2020; Shen et al. 2020). Although past manipulative field experiments have demonstrated strong positive relationships among nutrients (both nitrogen and phosphorus addition), cyanobacterial biomass, and MIB (Olsen et al. 2016) and geosmin (Olsen et al. 2017), no field manipulation to our knowledge has explored how different nitrogen forms affect these interactions. Considering that inorganic nitrate and organic urea are commonly used fertilizers (United States Department of Agriculture, 2019) and are expected to vary in their utility for phytoplankton, including cyanobacteria, because of the energetic requirements to convert each into a usable form, both dissolved nitrogen forms were tested in this field experiment.

In contrast to cyanobacteria, predicting effects of nutrient enrichment on actinobacterial abundance and subsequent off-flavor episodes is difficult (Wood et al. 1985; Schrader et al. 2011; Schrader et al. 2013). Actinobacteria are ecologically important decomposers in lakes, particularly under hypoxic and anoxic conditions, and actinobacterial abundance is predicted to peak during the spring and fall in dimictic lakes (Allgaier and Grossart 2006) because of abundant available detritus and surface sediment disruptions caused by waterbody turnover. Nutrient enrichment may indirectly benefit actinobacteria through increased resource availability (e.g., algal and consumer detritus) over time.

Interestingly, MIB and geosmin levels were found to vary inversely in a recent manipulative field experiment (Olsen et al. 2017) suggesting that the mechanisms responsible for MIB and geosmin production may be very different and that management efforts to reduce either compound should consider effects on other taste and odor compounds. To elucidate the impact of eutrophication on interactions between phytoplankton and actinobacteria across depth on taste and odor compounds, we conducted a manipulative field experiment in a drinking water reservoir to determine how nutrient enrichment influences patterns in MIB, geosmin, phytoplankton biomass, and actinobacterial abundance. The three primary goals of this study were to understand (1) whether MIB and geosmin concentrations vary as a function of depth, and how these relationships are (2) affected by inorganic (nitrate) or organic (urea) nitrogen enrichment and (3) associated with taste and odor producing taxa, including cyanobacteria and actinobacteria.

Methods

Experiment and data

A manipulative enclosure experiment was conducted in a drinking water reservoir to determine the effects of three fertilization treatments ((1) control (no nutrients), (2) nitrate-based, and (3) urea-based fertilizer) on concentrations of phytoplankton (measured as chlorophyll-*a* and phytoplankton biovolume) and two common off-flavor compounds, MIB and geosmin, measured at each of four depths (1.5, 3, 5, and 7 m) across 3 weeks. The study lake is polymictic (maximum depth of 8 m) and eutrophic (total nitrogen (TN) = 300 μg l^{-1}; total phosphorus (TP) = 30 μg l^{-1}) due to seasonal runoff of nutrients (particularly urea-based fertilizers). A complete description of the experimental design and sampling activities are available in Supplementary Figure 1. Briefly, twelve enclosures (7.5 m deep, 1 m^2) were installed and filled on 26 July 2015, with two replicate "control" enclosures (unfertilized; ambient nutrient concentrations) and five replicates each for the nitrate-based and urea-based fertilizer treatments. Before nutrient manipulation began, four randomly selected enclosures were sampled on 28 July to determine baseline conditions across depth for (A) temperature (°C) and (B) irradiance (μmol/m2/sec) (Supplementary Figure 2). To control for variation due to N:P ratios (ambient N:P = 10 by weight), sodium phosphate was added to the "nitrate" and "urea" enclosures as well as the specific form of nitrogen. "Nitrate" and "urea" treatment enclosures were fertilized on 30 July with sodium phosphate to increase TP to 100 μg l^{-1} and with either sodium nitrate or urea to increase TN to 1000 μg l^{-1}. All enclosures were sampled on 6 and 20 August. A horizontal Van Dorn sampler was used to collect discrete depth samples (1.5, 3, 5, and 7 m) from each enclosure. In addition, depth-integrated water samples from the surface to 7-m depth were collected from each enclosure using a flexible tube sampler to understand water quality dynamics in the entire enclosure. Water samples were stored in acid-washed containers in a cooler on ice until they were processed in the lab. Gently mixed water samples were collected on 47-mm glass fiber filters (Pall A/E) with vacuum filtration, and filters were frozen at −20°C to estimate algal biomass as chlorophyll-*a* using fluorometry after a 24-h cold (4°C) extraction of seston from filters in 90% aqueous ethanol (Olsen et al. 2016). In addition,

whole water samples were collected in 20-ml glass vials, sealed with parafilm, and stored at 4°C for MIB and geosmin analyses using solid-phase microextraction (SPME) gas chromatography mass spectrometry after spiking each sample (12.5 ml) with 3 g of sodium chloride to promote the volatilization of target compounds (APHA 2012; Olsen et al. 2016). On the final sampling date, phytoplankton (including cyanobacteria and diatoms) species composition and biovolume were quantified and measured using inverted microscopy (× 100–400; 25 fields magnification^{-1}) of 1% Lugol's solution–preserved samples across depths (1.5, 5, and 7 m only) for two replicates per treatment (Olsen et al. 2016). For each sample, ten randomly selected individuals of each common genera were measured (e.g., length, width) with Nikon Image software to calculate average biovolume (μm^3 ml^{-1}) for each taxa that was converted to dry biomass (μg l^{-1}) by multiplying biovolume by 0.4 (Knoll et al. 2008). Cell densities were multiplied by average cell biomass to calculate species-specific biomass for each enclosure on each sampling day.

For actinobacteria, water samples collected at three depths (1.5, 5, and 7 m) on the final day of the experiment were stored at 4°C and used within 24 h of arrival to the lab. Each water sample was used directly from the sample bottle without dilution. The actinobacterial culture medium was prepared using the Actinomycete Isolation Agar (AIA, Difco^{TM}, BD) supplemented with 5% glycerol. The plates were prepared using the double agar technique with the top layer containing 55 μg ml^{-1} cycloheximide and inoculated with a water sample as described in Francy et al. (2014). For each water sample, 2 to 3 replicate plates were prepared and incubated at 28°C for 7–10 days until the growth of actinobacterial colonies was observed. The abundance of colonies for each plate was quantified via macro- and microscopic examination by selecting bacterial colonies with earthy odor and actinobacteria-specific morphological characteristics, such as aerial mycelium, an irregular and fuzzy edge, a chalky appearance, and a strong adherence to the growth medium (APHA 1999; AWWA 2004).

One enclosure (urea treatment) was damaged by a fishing lure during the first week of the experiment and was not included in statistical analyses.

Statistical analysis

Depth-integrated samples The effects of fertilization treatment on chlorophyll-*a*, geosmin, and MIB were analyzed using linear mixed effects models (treatment = fixed effect; enclosures nested in time = random effect) (Bates et al. 2015; Pinheiro et al. 2015). We also tested for a treatment × time interaction for each of the three response variables (treatment × time was not statistically significant for chlorophyll-*a* and geosmin). Data were checked for normality using quantile-quantile (QQ) plots and homogeneity of variance using residual plots. Chlorophyll-*a* and geosmin data were log-transformed to meet model assumptions. MIB was below the detection limit (~1 ng l^{-1}) for four out of eleven enclosures on 6 August. In contrast, MIB was present at detectable levels in all eleven enclosures on 20 August 2015. Therefore, we used a linear mixed effects model for left-censored data to analyze treatment effects for MIB over time (Vaida et al. 2007). As the treatment × time interaction was significant, we fit separate models for both later dates, 6 and 20 August, for MIB. We calculated a *Z*-score using our estimate and associated error for each parameter and compared this to the critical value for $\alpha = 0.05$ ($Z_{critical} = 1.96$) to test for statistical significance.

Discrete depth samples Linear mixed effects models were used to analyze the effects of fertilization treatment and depth on chlorophyll-*a*, geosmin, and MIB (treatment, depth, and treatment × depth interaction = fixed effects; enclosures = random effect). We first tested for a treatment × time interaction for each of the three response variables (treatment × time was statistically significant ($P < 0.05$) for all responses). Therefore, we fit separate models for each of the three response variables on 6 and 20 August 2015. We also used linear mixed effects models to analyze the effects of fertilization treatment and depth on the abundance of actinobacterial, cyanobacterial, and diatom biomass for data collected on 20 August 2015. Data were checked for normality and homogeneity using QQ- and residual plots. Depth, chlorophyll-*a*, geosmin, and abundance of actinobacteria, cyanobacteria, and diatoms data were log-transformed to meet model assumptions. MIB was below the detection limit (~1 ng l^{-1}) for eighteen of the forty-four enclosure/depth observations on 6 August. In contrast, MIB was present at detectable levels in all but one of forty-four enclosure/depth observations on 20 August. Therefore, we used a linear mixed effects model for left-censored data to analyze treatment effects for MIB for both dates (Vaida et al. 2007). We then calculated a *Z*-score using our estimate and associated error for each parameter and compared this to the critical value for $\alpha = 0.05$ ($Z_{critical} = 1.96$) to test for statistical significance. For each of the four response variables, we used Cohen's f^2 to compare effect sizes for fertilization vs. depth calculated using the conditional R^2 for the appropriate full and reduced linear mixed effects models (Nakagawa and Schielzeth 2013; Barton 2015). All statistical analyses were conducted using R.

Censored data Censored data for predictor and response variables are common in freshwater ecology, particularly when studying toxins, volatiles, and contaminants that are of concern are at very low environmental concentrations. In our experiment, geosmin data were uncensored; however, a subset of MIB concentrations were below analytical detection limits (~1 ng l^{-1}), particularly on 6 August (~41% of observations

for discrete-depth samples vs. ~2% of observations on 20 August for discrete-depth samples). We explicitly dealt with these censored observations by using linear mixed effects models for left-censored data. We were also interested in comparing model estimates of regression coefficients (betas) for models that take into account this censoring versus those that ignore it (i.e., linear mixed effects models). In general, beta estimates for both model types were similar for MIB data from 20 August when censored observations were less common (except for the depth effect). However, beta estimates were seriously affected (i.e., biased) due to censoring on 6 August (Table 1).

Results

Depth-integrated samples

Algal biomass as chlorophyll-*a* Fertilization significantly affected chlorophyll-*a* (nitrate: $T_8 = 4.69$, $P = 0.0016$; urea: $T_8 = 6.00$, $P = 0.0003$) with mean concentrations over 400% higher in the nitrate and urea treatments than the unfertilized control (ambient treatment) 1 week following fertilization (Fig. 1A). Interestingly, treatment effects of the two forms of dissolved nitrogen were not consistent over time (nitrate × time: $T_8 = 0.42$, $P = 0.69$; urea × time: $T_8 = 2.03$, $P = 0.076$). Mean chlorophyll-*a* 3 weeks after fertilization in the urea treatment was approximately 100% higher than in the nitrate treatment. However, mean chlorophyll-*a* in these two treatments was not significantly different over the course of the 3-week experiment ($T_9 = 0.87$, $P = 0.41$).

MIB The effect of fertilization treatment on MIB (Fig. 1B) was contingent on time ($Z_{\text{Nitrate} \times \text{Time}} = 9.28$; $Z_{\text{Urea} \times \text{Time}} = 10.36$; critical value = 1.96). On 6 August (1 week after treatment application), fertilization with nitrate and urea resulted in a marginally significant increase in MIB (relative to the ambient treatment) ($Z_{\text{Nitrate}} = 1.80$; $Z_{\text{Urea}} = 1.94$; critical value = 1.96). By 20 August (3 weeks after treatment application), fertilization with nitrate and urea resulted in a greater than 250% increase in MIB vs. the ambient treatment ($Z_{\text{Nitrate}} = 4.25$; $Z_{\text{Urea}} = 5.15$; critical value = 1.96). Mean MIB in the urea and nitrate treatments were similar ($Z = 1.34$; critical value = 1.96).

Table 1 Comparison of parameter estimates for MIB linear mixed effects models that take into left-censored data (i.e., below the detection limit) vs. those that ignore it on (A) 6 August and (B) 20 August 2015. Eighteen of forty-four observations were below the detection limit on 6 August while one of forty-four observations were below the detection limit on 20 August for discrete depth samples

	β_{Urea}	β_{Nitrate}	β_{Depth}
(A) 6 August			
Left censored mixed effects model:	2.59	2.88	−1.38
Mixed effects model:	1.16	1.33	−1.14
(B) 20 August			
Left censored mixed effects model:	15.52	12.87	−1.00
Mixed effects model:	14.97	12.32	−6.92

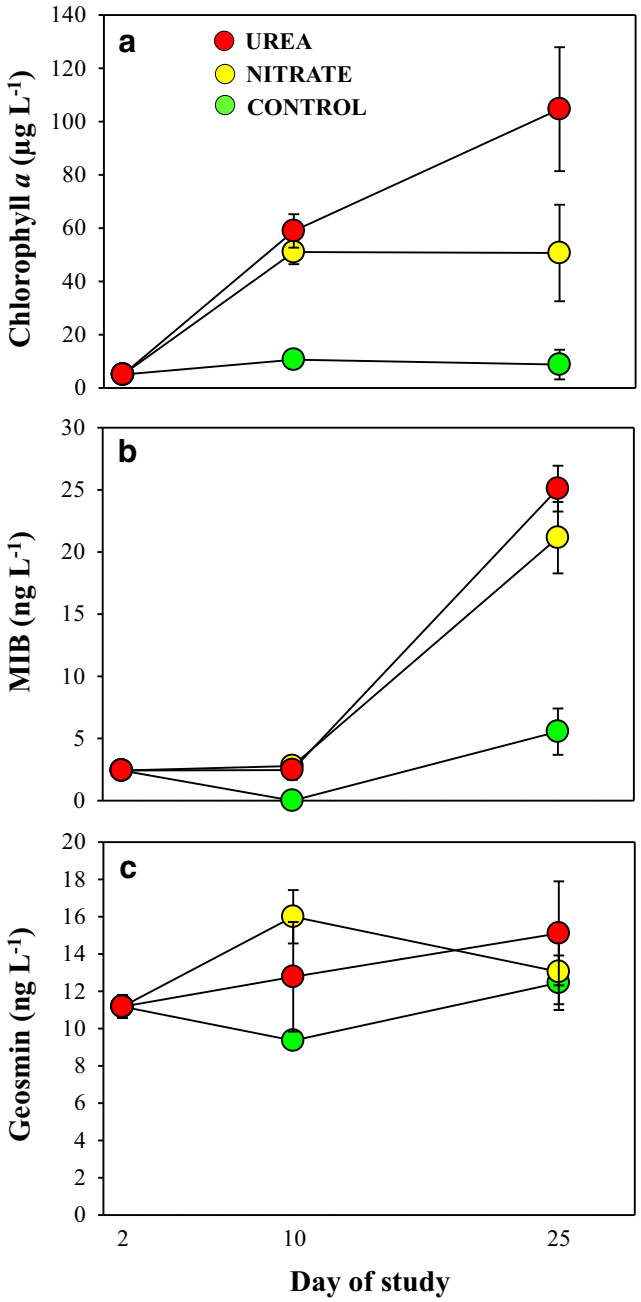

Fig. 1 Dynamics of (**a**) chlorophyll-*a*, (**b**) 2-methylisoborneol (MIB), and (**c**) geosmin collected from depth-integrated samples (surface to 7 m) over the course of the 3-week experiment. Data represent means ± one standard error. Red symbols denote the "urea" treatment, yellow symbols indicate the "nitrate" treatment, and green symbols indicate the "ambient" treatment

Geosmin The effect of fertilization treatment on geosmin (Fig. 1C) was not statistically significant (nitrate: $T_8 = 1.14$, $P = 0.29$; urea: $T_8 = 0.59$, $P = 0.57$) and did not depend on time (nitrate × time: $T_8 = -1.52$, $P = 0.17$; urea × time: $T_8 = -0.74$, $P = 0.48$).

Discrete depth samples

Treatment × time interactions were significant for chlorophyll-*a* under urea fertilization (urea × time: $T_{71} = 3.71$, $P = 0.0004$; nitrate × time: $T_{71} = 0.78$, $P = 0.44$) while nitrate fertilization significantly affected MIB (nitrate × time: $Z = 2.52$; urea × time: $Z = 1.68$; critical value = 1.96) and geosmin (nitrate × time: $T_{71} = -2.93$, $P = 0.0045$; urea × time: $T_{71} = -1.85$, $P = 0.068$) concentrations over time. Therefore, we fit two separate models (6 August 2015 and 20 August 2015) for each of the three responses. Only one model was fit for actinobacterial, cyanobacterial, and diatom abundance as samples were only collected on 20 August 2015 (i.e., 3 weeks after fertilization).

Algal biomass as chlorophyll-*a* (6 August 2015) The effect of fertilization treatment on chlorophyll-*a* was dependent on depth, as nitrate- and urea-based fertilization resulted in approximately 400% higher chlorophyll-*a* at near-surface depths (1.5 and 3 m) (relative to the ambient treatment), with the positive effect of fertilization rapidly decreasing with depth (depth × nitrate: $T_{30} = -2.15$, $P = 0.040$; depth × urea: $T_{30} = -2.95$, $P = 0.0062$). In contrast, chlorophyll-*a* did not decrease with depth in the ambient treatment (depth × ambient: $T_{30} = 2.15$, $P = 0.040$) (Fig. 2A). The relationship between depth and chlorophyll-*a* for the urea treatment was not significantly different than for the nitrate treatment ($T_{30} = -1.13$, $P = 0.27$). Depth had a larger effect on chlorophyll-*a* than the fertilization treatment on 6 August (Cohen's f^2: depth = 0.97; fertilization treatment = 0.36).

Algal biomass as chlorophyll-*a* (20 August 2015) Three weeks after treatment application, fertilization had resulted in a 425% increase in the nitrate-based ($T_8 = 2.50$, $P = 0.037$) and a 2100% increase in the urea-based treatments ($T_8 = 5.34$, $P = 0.0007$), relative to the ambient treatment (Fig. 2D). However, this effect was contingent on depth, as chlorophyll-*a* decreased by 77% at 7 m (relative to 1.5 m) in the urea treatment (depth × urea: $T_{30} = -3.11$, $P = 0.0041$). In contrast, chlorophyll-*a* increased by 75% at 7 m (relative to 1.5 m) in the control treatment; however, this effect was not statistically significant (depth × control: $T_{30} = 1.08$, $P = 0.29$). Chlorophyll-*a* was not significantly different across depths in the nitrate treatment (depth × nitrate: $T_{30} = -1.08$, $P = 0.29$). Effects of depth and treatment on chlorophyll-*a* were similar in magnitude on 20 August 2015 (Cohen's f^2: depth = 0.36; fertilization treatment = 0.34).

MIB (6 August 2015) Mean MIB was significantly higher in the fertilized treatments 1 week after application ($Z_{Nitrate} = 3.04$; $Z_{Urea} = 2.66$; critical value = 1.96). However, in general, MIB concentrations were low across all of the treatments (< 5 ng l^{-1}). MIB concentration did not differ across depths (Fig. 2B) ($Z_{Depth} = -1.38$; critical value = 1.96). Furthermore, the effect of fertilization treatment did not depend on depth ($Z_{Nitrate \times Depth} = 0.65$; $Z_{Urea \times Depth} = 0.071$; critical value = 1.96).

MIB (20 August 2015) Mean MIB in nitrate- and urea-fertilized treatments was approximately 300% higher than for the ambient treatment (Fig. 2F) ($Z_{Nitrate} = 4.93$; $Z_{Urea} = 5.75$; critical value = 1.96), with MIB significantly decreasing with depth (35% reduction at 7 vs. 1.5 m) ($Z_{depth} = -2.40$; critical value = 1.96). The depth × treatment interaction was not statistically significant ($Z_{Depth \times Nitrate} = 0.0013$; $Z_{Depth \times Urea} = -0.17$). Mean MIB in the urea treatment was not significantly different from the nitrate treatment ($Z = 1.28$; critical value = 1.96). The effect of fertilization treatment was larger in magnitude than the effect of depth (Cohen's f^2: fertilization treatment = 0.73; depth = 0.14).

Geosmin (6 August 2015) Mean geosmin in the nitrate treatment was higher than the ambient treatment (Fig. 2C) 1 week after treatment application ($T_{30} = 3.37$, $P = 0.0098$). In contrast, mean geosmin in the urea treatment was not significantly different from the ambient treatment ($T_{30} = 1.25$, $P = 0.25$). The effect of depth ($T_{32} = 0.020$, $P = 0.79$) and the interaction between fertilization treatment and depth (depth × nitrate: $T_{30} = 0.042$, $P = 0.97$; depth × urea: $T_{30} = -1.36$, $P = 0.19$) were not statistically significant.

Geosmin (20 August 2015) After 3 weeks, depth was the only factor that significantly affected geosmin ($T_{32} = 4.35$, $P = 0.0001$; Cohen's f^2 for depth = 0.56 vs 0.097 for treatment), with mean geosmin at 7 m 60% higher than at 1.5 m (Fig. 2F). The effect of depth was independent of fertilization treatment (depth × nitrate: $T_{30} = 0.12$, $P = 0.90$; depth × urea: $T_{30} = 0.81$, $P = 0.42$).

Cyanobacterial biomass (20 August 2015) Nitrate- and urea-based fertilization resulted in increased relative (Table 2) and absolute cyanobacterial biomass (Fig. 3A) across all depths (depth × nitrate: $T_{13} = 2.78$, $P = 0.02$; depth × urea: $T_{13} = 2.69$, $P = 0.02$). Cyanobacterial biomass tended to be higher in the urea- than the nitrate-based fertilization treatment. However, this difference was not statistically significant ($P = 0.74$). In general, cyanobacterial biomass declined with depth across all treatments ($T_{13} = -6.54$, $P = 0.0011$), with approximately 460% higher cyanobacterial biomass at 1.5 versus 7 m in the urea-based fertilization treatment (Fig. 3A). The effect of depth was larger in magnitude

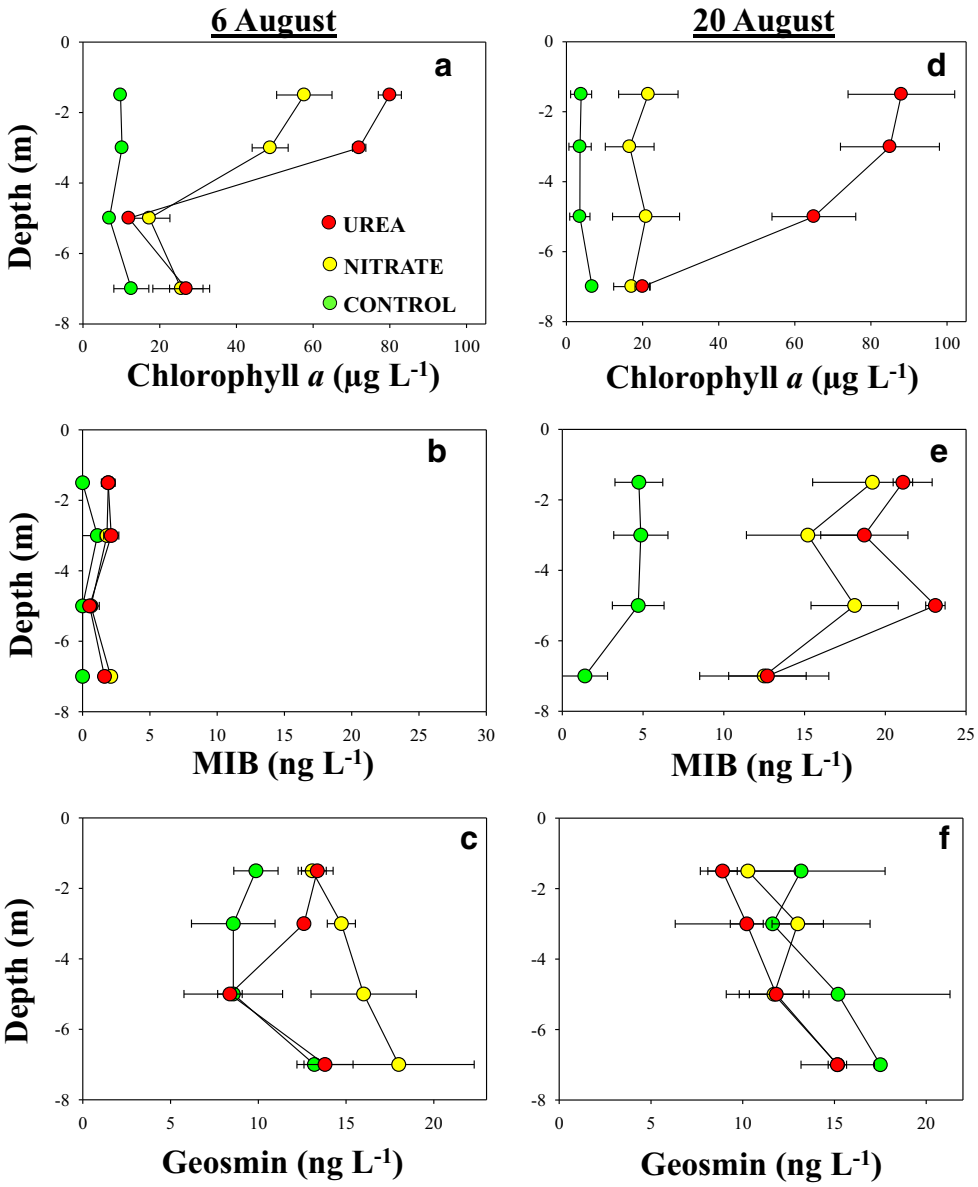

Fig. 2 Relationship between depth and chlorophyll-*a*, 2-methylisoborneol (MIB), geosmin one (6 August 2015, panels **a**–**c**) and 3 weeks (20 August 2015, panels **d**–**f**) after applying fertilization treatments. Red symbols indicate the "urea" treatment, yellow symbols indicate the "nitrate" treatment, and green symbols indicate the "ambient" treatment

than the effect of fertilization treatment (Cohen's f^2: depth = 0.99; fertilization treatment = 0.44).

Diatom biomass (20 August 2015) In general, diatom biomass was low (< 1 µg l^{-1}) across all treatments. Although absolute and relative diatom biomass tended to increase with depth (Table 2), especially in the nitrate treatment (Fig. 3B), the effects of fertilization treatment ($F_{2,5} = 0.33$, $P = 0.74$), depth ($T_{15} = 0.97$, $P = 0.35$), and their interaction were not statistically significant ($F_{2,13} = 0.82$, $P = 0.82$).

Actinobacterial abundance (20 August 2015) Actinobacterial abundance significantly increased with depth across all fertilization treatments (Fig. 3C) ($T_{21} = 4.46$; $P = 0.0002$). The effect of depth was independent of fertilization treatment (depth × nitrate: $T_{19} = 0.77$, $P = 0.45$; depth × urea: $T_{19} = 0.84$, $P = 0.41$). Although actinobacteria tended to be more abundant in the nitrate- and urea-fertilized treatments, this effect was not statistically significant (nitrate: $T_{19} = 1.11$, $P = 0.30$; urea: $T_{19} = 1.10$, $P = 0.30$). The effect of depth on actinobacterial abundance was larger in magnitude than the effect of treatment (Cohen's f^2: depth = 0.62; fertilization treatment = 0.018).

Discussion

The idea that nutrient pollution will increase the prevalence of taste and odor episodes in aquatic ecosystems is generally well-supported, based on a combination of laboratory experiments, observational studies, and an increasing body of field-based manipulative experiments (Olsen et al. 2016; Olsen et al. 2017). This research has largely focused on

Table 2 Mean relative abundance of phytoplankton taxa versus depth prior to establishing treatments ("Initial") and at the end of the experiment (August 20)

Depth (m)	(Cyanophyta (%)	(Bacillariophyta (%)	(Chlorophyta (%)	(Cryptophyta (%)	(Dinoflagellata (%)
(A) Pre-treatment					
1.5	30.6	64.5	3.0	1.1	0.8
5	20.8	74.7	2.9	0.8	0.8
7	8.2	88.5	1.8	1.5	0.0
(B) Final					
Control					
1.5	25.2	38.7	22.9	8.4	4.6
5	15.2	76.0	1.1	2.3	5.5
7	0.0	88.7	1.5	9.8	0.0
Nitrate					
1.5	90.9	5.2	3.1	0.8	0.0
5	78.0	14.5	2.6	1.2	3.7
7	66.4	22.8	8.9	2.0	0.0
Urea					
1.5	96.4	2.7	0.3	0.6	0.0
5	92.6	5.0	1.1	0.6	0.7
7	85.4	12.9	1.0	0.7	0.0

cyanobacteria as a driver of MIB and geosmin in waterbodies (Otten et al. 2016; Watson et al. 2016). In contrast, actinobacteria are ecologically important decomposers in lakes, particularly under hypoxic and anoxic conditions that are prevalent in eutrophic lakes during periods of stratification. We are not aware of any prior manipulative field experiments that have simultaneously examined the effects of nutrient enrichment on MIB and geosmin concentrations across depths (but see Durrer et al. 1999 and Jia et al. 2019). However, such effects may be potentially important in drinking water reservoirs as management efforts to reduce MIB (or geosmin) could inadvertently favor other taste and odor compounds.

Here, we demonstrate in a drinking water reservoir with a diverse starting assemblage of phytoplankton, including cyanobacteria, chlorophytes, cryptophytes, and dinoflagellates but dominated by diatoms (Table 2), a strong positive response of MIB to nutrient enrichment (Fig. 1), particularly at near-surface depths, mediated by cyanobacteria (Figs. 1, 2, and 3). In contrast, geosmin concentrations were unaffected by nutrient enrichment (Fig. 1) and reached a maximum in the anoxic hypolimnion where actinobacteria were abundant

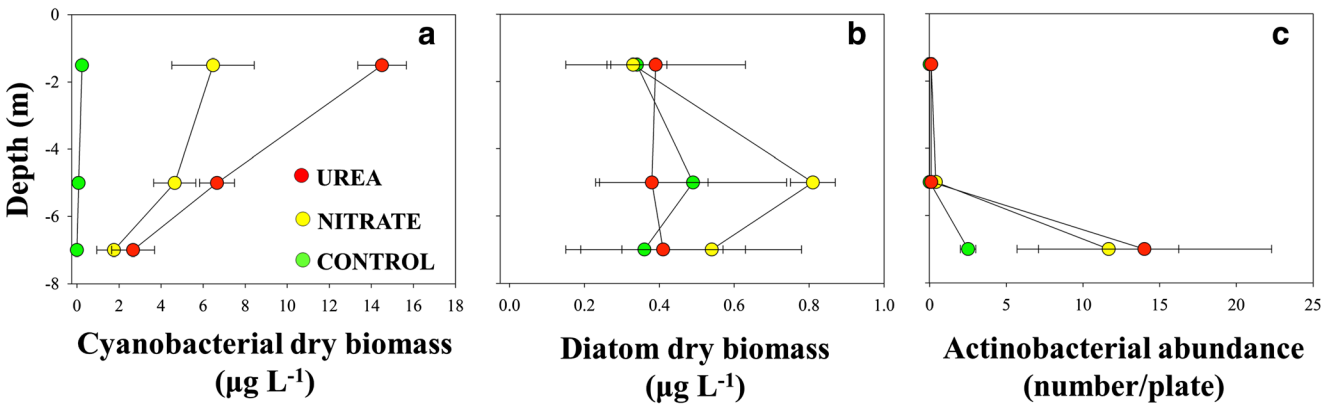

Fig. 3 Relationship between depth and (**a**) cyanobacterial dry biomass, (**b**) diatom dry biomass, and (**c**) actinobacterial abundance 3 weeks (20 August 2015) after applying fertilization treatments. Red symbols indicate the "urea" treatment, yellow symbols indicate the "nitrate" treatment, and green symbols indicate the "ambient" treatment

(Figs. 2 and 3). Interestingly, the effects of two dissolved nitrogen forms on phytoplankton and taste and odor concentrations were not consistent over time or depth (Figs. 1, 2, and 3). Phytoplankton responded rapidly (within 1 week) to nitrate and urea; however, the effect of nitrate stabilized for the final 2 weeks of the experiment while urea continued to promote phytoplankton (Figs. 1 and 2), especially cyanobacteria in the epilimnion (Fig. 3), until the end of the experiment. Diatoms dominated the control enclosures during the entire experiment although chlorophytes and cyanobacteria accounted for an increasing percent of phytoplankton biovolume by the end of the experiment (Table 2).

Although MIB responded slower to nitrogen fertilization than phytoplankton, by the end of the experiment, MIB was >400% higher than the control (Fig. 1). Such responses are not surprising if phytoplankton are primarily responsible for MIB production (Watson et al. 2016) and must first increase in abundance before producing secondary metabolites, such as MIB (Olsen et al. 2016; Olsen et al. 2017). Geosmin did not show strong responses to either nitrogen treatment (Fig. 1). Although past studies have shown high spatial and temporal heterogeneity in taste and odor compounds (Su et al. 2015; Harris et al. 2016), they have tended to be associated with phytoplankton capable of producing these compounds as we observed for MIB (Fig. 1).

Many drinking water reservoirs regularly stratify during warm periods thus creating ephemeral niches for aquatic organisms (Su et al. 2017). For example, many cyanobacteria commonly form surface scums when abundant and can outcompete other autotrophs for sunlight (Jia et al. 2019). Decomposers, such as actinobacteria, tend to be associated with sediment and are often found in hypoxic (to anoxic) environments due to intense heterotrophic respiration. As phytoplankton grow and die, they produce more resources for actinobacteria. Nutrient enrichment should promote these processes. In this study, large effects of nitrate or urea fertilization were highly dependent on depth. As predicted, both nitrogen forms promoted increased phytoplankton (especially cyanobacteria; Figs. 2 and 3) but these effects were primarily observed in the upper half of the water column. Urea continued to support high abundances of cyanobacteria until the end of the experiment (Fig. 3). MIB tended to be higher under urea enrichment relative to nitrate especially above the bottom of the water column (Fig. 3). The influence of depth on geosmin concentrations was apparent 1 week following nitrogen fertilization (Fig. 2) and may have been further promoted had the experiment lasted longer allowing for more organic matter to be deposited at the bottom of the enclosures. As expected, actinobacteria were much higher at the bottom of the water column across all three treatments but were promoted with both types of dissolved nitrogen (Fig. 3). Our data clearly show the complexities related to understanding which organisms are responsible for taste and odor (Clercin and Druschel 2019; Deng et al. 2019) and how nutrient pollution mediates these interactions (Shen et al. 2020). We encourage future experiments aimed at exploring the role of cyanobacteria and/or actinobacteria in the production of MIB and geosmin across space and time.

In the case of our study lake, we observed a concurrent decline in MIB with decreased water temperature and cyanobacterial abundance (i.e., *Oscillatoria* species) in the fall (October–December) of 2013 (Olsen et al. 2016). However, high levels of geosmin (>100 ng L^{-1}) remained, particularly at depths of 5–7 m where the primary raw water intake is located (Wilson, unpublished data). Consequently, the facility was unable to produce palatable drinking water. However, geosmin (and MIB) concentrations have subsequently been successfully reduced via repeated hypolimnetic releases/flushing during subsequent fall and spring seasons. Similar approaches may be promising in other eutrophic drinking water reservoirs plagued by actinobacteria-geosmin issues where manipulations of trophic state and ecosystem productivity are not feasible. In contrast, availability of alternate intake depth may be beneficial in reservoirs plagued by cyanobacterial blooms or taste and odor issues. While there clearly is no "silver bullet" in the management of taste and odor episodes, a holistic watershed-scale limnological approach can be beneficial. Drinking water facilities often have multiple intakes (at different depths) but can also consider adding additional infrastructure to provide flexibility for water production (e.g., multiple or adjustable intakes across depth), depending on the prevalence of MIB vs. geosmin. Such infrastructural improvements may be less costly over the long term than repeated use of oxidants and carbon products during water treatment as well as reduce harmful by-products of such treatment (Cook et al. 2001).

Future experiments that examine off-flavor, algal, and actinobacterial dynamics over longer periods of time and compare seasonal differences in the effects of nutrient enrichment will be informative. Longer-duration experiments may help to determine the importance of the hypothesized positive feedback between nutrient enrichment and actinobacterial abundance, mediated through enhanced algal growth and subsequent detrital accumulation. Furthermore, while the relationships that we observed may be representative for other stratified, eutrophic reservoirs in the summer, patterns will likely be completely different in the fall and spring within a reservoir during seasonal mixing and the winter (i.e., inverse stratification) (Watson 2003). These relationships may also vary longitudinally/spatially in reservoirs and as a function of stream inputs (Graham et al. 2010). Off-flavor-depth relationships may become particularly interesting when these stream inputs differ in ambient temperature from the main reservoir. In general, understanding effects due to hydrological variation in feeding streams (e.g., sediments, dissolved organic carbon, and nutrients) should be informative. Finally, incorporating sediment-water linkages (e.g., biofilms) in conjunction with detrital inputs will help to identify the mechanism(s) regulating geosmin, and possibly MIB, production in the hypolimnion (i.e., direct actinobacterial production vs. decomposition of off-flavor producing algal cells).

Conclusions

In conclusion, we found large effects of two forms of nitrogen fertilization, including urea and nitrate, on phytoplankton abundance, especially cyanobacteria. In addition, nitrogen fertilization promoted MIB production (>250% relative to unfertilized control enclosures). Consequently, there was a strong positive correlation between phytoplankton abundance and MIB for depth-integrated samples. The effect of nitrogen fertilization on geosmin was negligible. Interestingly, the effect of urea and nitrate fertilizations on MIB decreased with depth (35% reduction at 7 versus 1.5 meters) while geosmin concentrations were highest at the lowest measured depth (7 m) and tracked the pattern observed in actinobacterial abundance. Our results suggest that phytoplankton (e.g., cyanobacteria, such as *Oscillatoria* species) mediated MIB in response to nitrogen fertilization while actinobacteria mediated geosmin concentrations in response to hypolimnetic decomposition in the drinking water reservoir we studied.

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Data availability The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate This research followed the guidelines provided by Auburn University for ethical research. Consent to participate was not applicable for this study.

Consent for publication N/A

Competing interests The authors declare that they have no competing interests.

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