



## Off-flavor compounds from decaying cyanobacterial blooms of Lake Taihu

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### Abstract

The effect of cyanobacterial bloom decay on water quality and the complete degradation of cyanobacterial blooms in a short period were examined by an enclosure experiment in Gonghu Bay of Lake Taihu, China. Water quality parameters as well as taste and odor compounds during the breakdown of cyanobacterial blooms were measured. Results showed that the decay of cyanobacterial blooms caused anoxic water conditions, decreased pH, and increased nutrient loading to the lake water. The highest concentrations of dimethyl sulfide (DMS), dimethyl trisulfide (DMTS), and  $\beta$ -cyclocitral were observed in the anoxic water, at 62331.8, 12413.3, and 1374.9 ng/L, respectively. 2-Methylisoborneol was dominant during the live growth phase of cyanobacterial blooms, whereas DMS and DMTS were dominant during the decomposition phase. Dissolved oxygen, pH, and chlorophyll *a* were negatively correlated with DMS, DMTS, and  $\beta$ -cyclocitral, whereas total phosphorus, total nitrogen, and ammonium ( $\text{NH}_4^+\text{-N}$ ) were positively correlated with DMS, DMTS,  $\beta$ -cyclocitral, and  $\beta$ -ionone. The experimental results suggested that preventing the anaerobic decomposition of cyanobacterial blooms is an important strategy against the recurrence of a malodor crisis in Lake Taihu.

**Key words:** cyanobacterial blooms; decay; taste and odor compounds; Lake Taihu; water crisis

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### Introduction

Cyanobacterial blooms are becoming a widespread problem in the aquatic environment because of eutrophication (Paerl et al., 2001; Lehman, 2007). One of the primary consequences of bloom occurrence is the release of odorous compounds. These odorous compounds can dramatically impact water supplies, decrease the esthetic quality and consumer acceptability of drinking water, and increase the costs of water treatment. Thus, identification and quantification of these odorous compounds are necessary. Previous studies have mainly focused on the production of taste and odor compounds (e.g., geosmin, 2-methylisoborneol (MIB),  $\beta$ -ionone,  $\beta$ -cyclocitral) during the growth of isolated cyanobacteria species (Watson, 2003; Zaitlin and Watson, 2006; Smith, 2008). Relatively little attention has been given to the off-flavor compounds released during the subsequent decomposition of cyanobacterial blooms.

Lake Taihu is the third largest freshwater lake in China, with a surface area of 2338 km<sup>2</sup>, mean depth of 1.9 m, and maximum depth of 2.6 m. Lake Taihu becomes eutrophic with cyanobacterial blooms every summer (Qin et al.,

2007). The southeastern monsoons drive cyanobacterial blooms to the northern and western parts of the lake during this season; thus, dense cyanobacterial blooms with malodor can be observed near the shoreline (Qin et al., 2007). Lake Taihu is also an important source of drinking water for several cities. The taste and odors of water in Lake Taihu have triggered great public concerns since a serious drinking water crisis occurred in Wuxi City between May and early June in 2007. The deteriorated water emitted a strong septic and marshy odor, forcing two million citizens to resort to bottled drinking water (Yang et al., 2008; Zhang et al., 2010). Therefore, understanding taste and odor production during cyanobacterial decomposition is essential to taste and odor compound-related water quality control and prevention in the future. The current study conducted a field enclosure experiment near the shore of Gonghu Bay to determine the water quality variables and taste and odor compound dynamics during the decay of dense cyanobacterial blooms. Relationships between water quality variables and taste and odor compounds were also analyzed.

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## 1 Materials and methods

### 1.1 Experimental design and sampling

Gonghu Bay is located at the northeastern portion of Lake Taihu, and suffers wind-driven dense surface accumulation of cyanobacterial blooms every summer. Two square enclosures ( $2\text{ m} \times 2\text{ m}$  in area) for replicate experiments were constructed with waterproof materials in a near-shore area of the Gonghu Bay from 23 August to 18 September in 2009, where dense cyanobacterial blooms accumulated (**Fig. 1**). The enclosures were unsealed from sediment at the bottom and maintained by four rigid horizontal hoops. Water depth was around 1.5 m. The enclosure wall was extended 20 cm above the mean water level to prevent waves and rising water from entering. Water samples from the enclosures were collected 0.5 m below the water surface during the study period. Cyanobacterial blooms in the enclosures were alive and green after the enclosures were constructed. The enclosures reduced circulation and enhanced cyanobacterial decomposition. The heavy cyanobacterial blooms in the enclosures had substantially decomposed by the first sampling time, the third day after the enclosures were constructed (25 August; **Fig. 1a**). Heavy rains poured on day 11 (2 September), when the water temperature dropped greatly. The color of the cyanobacteria changed from white to green, and its growth became the dominant process (**Fig. 1b**).

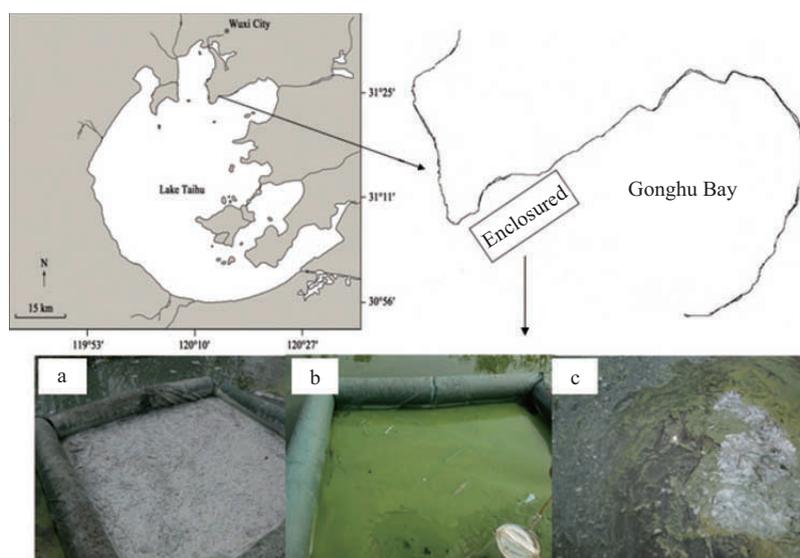
Dissolved oxygen (DO) and pH were measured with a YSI 6600 multi-parameter water quality sonde (YSI Incorporated, Yellow Springs, USA). Duplicate water samples were collected in 1 L narrow-necked PE bottles at a depth of 0.5 m below the surface, leaving no headspace. The collected samples were stored immediately in a portable refrigerator ( $4^{\circ}\text{C}$ ), and then transported to the laboratory.

After arrival at the laboratory, all samples for off-flavor analysis were stored at  $-20^{\circ}\text{C}$  and analyzed within 24 hr. Nutrient analyses of ammonium ( $\text{NH}_4^+\text{-N}$ ), total phosphorus (TP), and total nitrogen (TN) were performed within 24 hr of sample collection, according to the standard methods of China's National Environmental Protection Agency. Water samples were filtered through a glass microfibre filter (GF/C, Whatman, Brentford, UK) and filters were extracted overnight at  $4^{\circ}\text{C}$  with 95% ethanol for chlorophyll *a* (chl-*a*) determination. After the extracted samples were centrifuged, absorption was determined by a spectrophotometer (LAMBDA 1050, Perkin Elmer, USA). All concentrations reported in this study were the averages of duplicate sample measurements.

### 1.2 Detection for dissolved and particulate taste and odor compounds

A 300-mL water sample was filtered through  $0.45\ \mu\text{m}$  filter paper (GF/C, Whatman, Brentford, UK) to separate the dissolved and particulate fractions of the lake water. The filtrate with the dissolved fraction was transferred into 40 mL amber vials with Teflon-fitted caps, leaving no headspace, and then immediately stored at  $4^{\circ}\text{C}$  until analysis within 24 hr.

The filter residue with the particulate fraction was frozen and thawed again. The mash was then rinsed into a 250 mL round-bottom flask with 100 mL of MilliQ-water, and sodium chloride was added to give a final concentration of 20% (W/V), stirred with a micro stirrer. Extraction was performed with an APEX Ordinary Pressure Microwave-Assisted Extraction System (PreeKem Scientific Instruments Co., Ltd., China). The temperature was set at  $80^{\circ}\text{C}$ , and the nitrogen (carrier gas) flow was set at 80 to 100 mL/min. The microwave was activated



**Fig. 1** Sketch of Lake Taihu and the changes in appearance of cyanobacterial blooms in enclosures: (a) anaerobic decayed cyanobacterial blooms in enclosures on day three; (b) the color of cyanobacterial blooms changed to green and the live growth phase dominated after the rain; and (c) the cyanobacterial bloom decomposition phase dominated again due to increasing water temperature.

for 15 min, during which distillates from the re-suspended residue migrated to the condenser, which was cooled with liquid nitrogen to avoid thermal decomposition, and then dissolved with 50 mL MilliQ-water. The elution was also transferred into 40 mL amber vials with Teflon-fitted caps, stored at 4°C immediately, and analyzed within 24 hr. All prepared samples were subjected to purge and trap concentration coupled with gas chromatography-mass spectrometry for quantitative determination. Qualitative and quantitative analyses of taste and odor compounds in the water samples were conducted following the method from our previous study (Deng et al., 2010).

The standards of the taste and odor compounds, including geosmin, MIB,  $\beta$ -cyclocitral,  $\beta$ -ionone, dimethyl trisulfide (DMTS), and dimethyl sulfide (DMS), were obtained from Sigma-Aldrich (Buchs, Switzerland) in the highest purity available. Instruments used for the taste and odor compound analysis included a gas chromatograph (Shimadzu, Japan), a mass spectrometer (Shimadzu-QP2010 plus, Japan), a purge and trap concentrator (O.I. Analytical 4660, USA), and an autosampler (O.I. Analytical 4551, USA). The optimized experimental parameters of purge and trap in this study include trap (#10, Tenax/silica gel/carbon molecular sieve), injection volume of 25 mL, purge tube temperature of 45°C, purge time of 11 min, purge flow rate of 45 mL/min, desorb preheat temperature of 185°C, and desorb temperature of 190°C (2 min).

A capillary column (30 m  $\times$  0.25 mm  $\times$  0.25  $\mu$ m, Rxi®-17) was used to separate the taste and odor compounds. The gas chromatography injector temperature was set at 270°C, with a split ratio of 10:1. The oven temperature program was as follows: initially held at 40°C for 2 min, increased to 100°C at 30°C/min, increased to 135°C at 5°C/min, increased to 250°C at 30°C/min, and maintained at 250°C for 5 min. The helium carrier gas flow rate was 3 mL/min. The following parameters were used for the selected ion monitoring mode:  $m/z$  62 and 47 for DMS;  $m/z$  126, 79, and 111 for DMTS;  $m/z$  95, 108, and 135 for MIB;  $m/z$  137, 152, and 123 for  $\beta$ -cyclocitral;  $m/z$  112, 125, and 149 for geosmin; and  $m/z$  177, 135, and 91 for  $\beta$ -ionone. Stock solutions were prepared in methanol. External standard calibration was the quantification option used. A calibration curve was built with standard mixture samples at concentration levels of 1 to 500 ng/L.

### 1.3 Pearson correlation and redundancy analysis

Relationships between water quality variables and taste and odor compounds were developed using Pearson correlation analysis. The analysis was conducted using SPSS 16.0 for Windows (SPSS Inc., Chicago, USA). Significant correlation was assumed when  $p < 0.05$  in either positive or negative correlations. A redundancy analysis was performed using CANOCO 4.5 for Windows to identify the key water quality variables that affect taste and odor concentrations (Ter Braak and Šmilauer, 2002).

## 2 Results

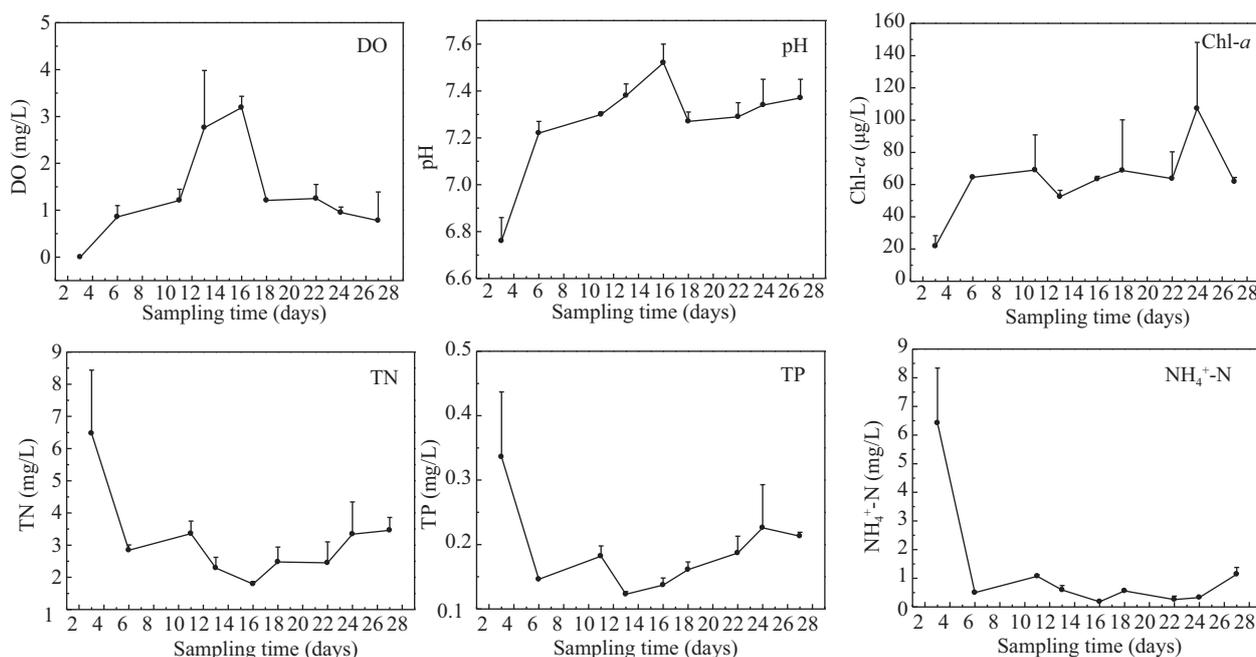
### 2.1 Changes in water quality variables

Based on the appearance of cyanobacterial blooms and chl-*a* concentrations in the enclosures, the entire experiment can be roughly divided into three phases: The lowest chl-*a* concentration was observed at 21.7  $\mu$ g/L on day 3, when heavy cyanobacterial blooms in the enclosures were substantially decayed (first phase). Chl-*a* began to increase and reached a (high) maximum level of 107.2  $\mu$ g/L on day 24, which may be mainly attributed to the drop in water temperature around day 6, when the color of the cyanobacterial blooms changed to green and the blooms were dominated with a live growth phase (second phase). Afterward, water temperature gradually increased, the chl-*a* concentration considerably declined, and a decomposition phase again dominated the cyanobacterial blooms (third phase). Moreover, a typical anoxic environment was formed in the enclosures on day 3. DO concentration increased and peaked on day 16 (3.2 mg/L), but significantly declined to approximately 1 mg/L afterward. The variation trends of TP, TN, and  $\text{NH}_4^+\text{-N}$  concentrations were similar, with the highest concentrations concurrently observed on day 3: 0.34, 6.47, and 6.42 mg/L, respectively. The concentrations then considerably declined, with a minimum of 0.12, 1.79, and 0.18 mg/L, respectively, observed in the second phase; but increased markedly in the third phase. pH values were 6.8 to 7.5, with the minimum measured on day 3 (Fig. 2).

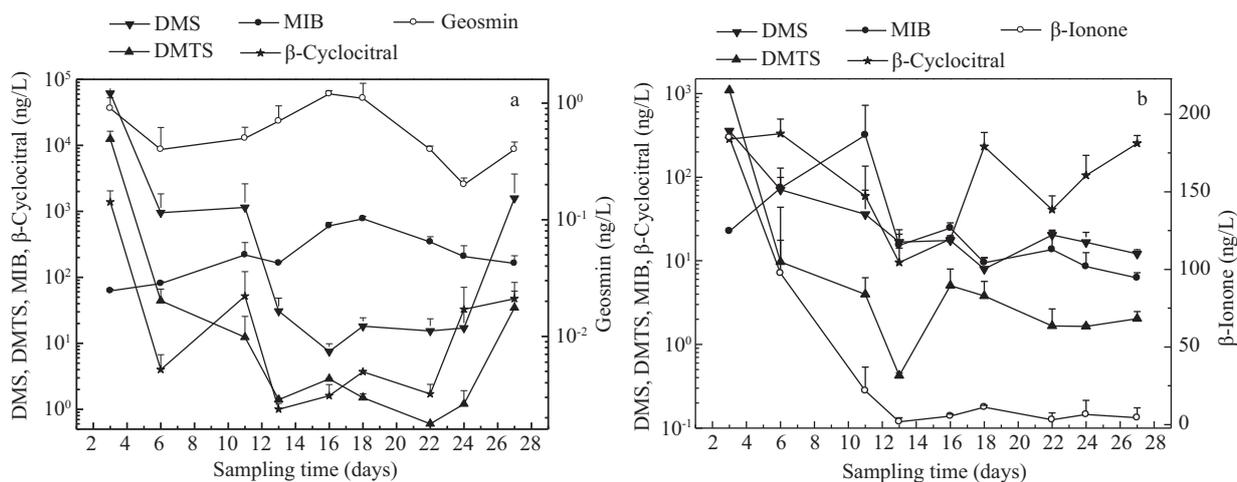
### 2.2 Fluctuations of dissolved and particulate taste and odor compounds

Dissolved DMS, DMTS, and  $\beta$ -cyclocitral clearly followed a similar variable trend. The concentrations of DMS, DMTS and  $\beta$ -cyclocitral concurrently reached an extremely high level on day 3 (first phase), with a maximum of 62,331.8, 12,413.3 and 1374.9 ng/L, respectively. However, these concentrations considerably declined in the second phase, with a minimum of 7.5, 0.1, and 1.0 ng/L, respectively, and remained low until day 22, then increased significantly in the third phase. Variations of dissolved MIB concentrations followed a reverse pattern, experiencing an upward trend starting on day 3 and peaking on day 18 (maximum of 772.7 ng/L), but then decreased markedly in the third phase. Dissolved geosmin exhibited an extremely low concentration on every sampling occasion, near our detection limit (about 0.1 ng/L; Fig. 3a).

The ranges of particulate DMS, DMTS, and  $\beta$ -cyclocitral were 8.0–356.8 ng/L, 0.4–1093.9 ng/L, and 9.5–284.3 ng/L, respectively; the maximum levels were concurrently observed on day 3, and the minimum on day 13 and day 18. The concentration of particulate MIB ranged from 6.2 to 318.7 ng/L and reached a peak on day 11.  $\beta$ -Ionone could only be detected in the particulate



**Fig. 2** Changes in DO, pH, Chl-*a*, TN, TP, and  $\text{NH}_4^+\text{-N}$  in the enclosures during the experiment.



**Fig. 3** Variations of dissolved (a) and particulate (b) taste and odor compounds in enclosures during the experiment. DMS: methyl sulfide; DMTS: dimethyl trisulfide; MIB: 2-methylisoborneol.

fractions; the maximum (185.0 ng/L) also occurred on day 3, and then dropped quickly and remained at a low level (< 10 ng/L). Geosmin was not found in the particle-bound fraction (Fig. 3b).

### 2.3 Relative ratio of different taste and odor compounds

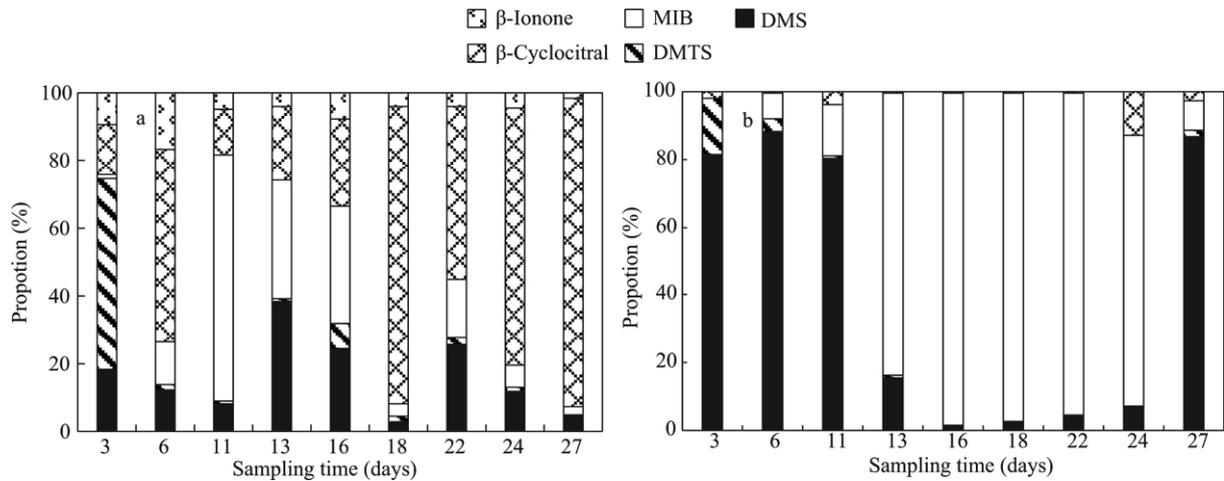
$\beta$ -Cyclocitral was the most abundant of the particulate taste and odor compounds, showing the largest proportion of > 90% on day 27.  $\beta$ -Ionone, MIB, DMS, and DMTS were also monitored during the entire period, with the proportions varying at different sampling times (Fig. 4a). DMS and MIB were the most abundant of the dissolved taste and odor compounds, showing a relative ratio of > 80% from day 3 to day 11 and from day 13 to day 24,

respectively. At the last sampling time, DMS increased and became dominant again (> 80%; Fig. 4b).

### 2.4 Relationship between water quality variables and taste and odor compounds

Strong positive correlations were observed among DMS, DMTS,  $\beta$ -cyclocitral, and  $\beta$ -ionone. No significant relationships were found between MIB, geosmin, and the water quality variables measured in the present study. DMS, DMTS,  $\beta$ -cyclocitral, and  $\beta$ -ionone were positively correlated with the nutrients, but negatively correlated with pH, DO, and chl-*a* (Table 1).

Results of the redundancy analysis illustrated that the water quality variables measured in the present study explained 90% of the variations of taste and odor compounds.

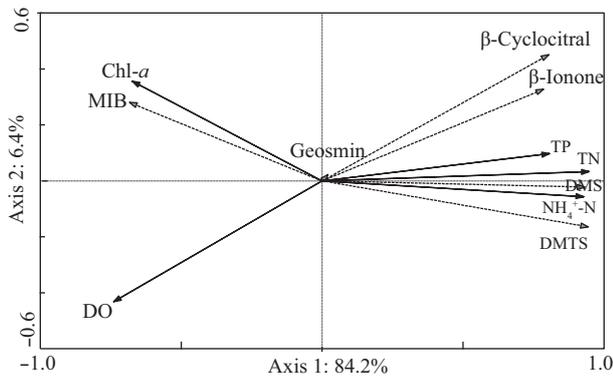


**Fig. 4** Relative ratio of particulate (a) and dissolved (b) taste and odor compounds in the enclosures during the experiment.

**Table 1** Correlations among water quality variables and taste and odor compounds (dissolved+particulate)

	DMS	DMTS	MIB	Geosmin	$\beta$ -cyclocitral	$\beta$ -ionone
pH	-0.917**	-0.915**	-	-	-0.942**	-0.913**
DO	-0.523**	-0.511**	-	-	-	-
Chl- <i>a</i>	-0.718*	-0.715*	-	-	-0.685*	-
TN	0.919**	0.911**	-	-	0.938**	0.827**
TP	0.852**	0.848**	-	-	0.860**	0.679*
NH <sub>4</sub> <sup>+</sup> -N	0.990**	0.986**	-	-	0.976**	0.866**
DMS	1	1.000**	-	-	0.977**	0.883**
DMTS	1.000**	1	-	-	0.974**	0.879**
MIB	-	-	1	-	-	-
Geosmin	-	-	-	1	-	-
$\beta$ -Cyclocitral	0.977**	0.974**	-	-	1	0.921**
$\beta$ -Ionone	0.883**	0.879**	-	-	0.921**	1

\*\*  $p < 0.01$ , \*  $p < 0.05$ ; -: means no significant correlations.



**Fig. 5** Redundancy analysis biplot showing variable concentrations of taste and odor compounds (dissolved+particulate) in relation to water quality variables.

TN explained most of the variations of the taste and odor compounds, with a proportion of 80.8% (**Fig. 5**).

### 3 Discussion

In this study, experiments were designed to investigate the impact of cyanobacterial decay in the water column. Changes in physiochemical parameters indicated that wa-

ter quality deteriorated, with low DO, low pH, and high nutrients. Preliminary experiments showed that the chl-*a* of the experimental area was 169.4  $\mu\text{g/L}$ . Rapid decay of cyanobacteria directly decreased cyanobacterial densities and chl-*a*, and depleted oxygen throughout the decomposition process (de Figueiredo et al., 2006); thus, the oxygen remaining in the water to support other plankton in the vicinity may not be sufficient (Jones, 1987). Microbes consumed the organic matter accumulated in the dense cyanobacteria, and carbon dioxide produced during the microbial respiration can decrease the pH of the lake water (Cai et al., 2011; Castaldelli et al., 2003). Moreover, the present study demonstrated that the death and decay of cyanobacterial blooms were accompanied by drastic increases in nitrogen and phosphorus. NH<sub>4</sub><sup>+</sup>-N increased tenfold to twentyfold when DO was depleted quickly to zero. These increases agree with some previous studies, which indicated that cyanobacteria cell lysis resulted in a large release of nutrients (Ginzburg et al., 1998a, 1998b; Ye et al., 2010). Nutrients released during the decomposition of cyanobacterial blooms represent a potential source of internal nutrient loading to lakes. The results of the present study yield insights into the impact of cyanobacterial decay on the nutrient cycling of lakes.

Water in the enclosures was black and malodorous. DMS and DMTS were found in both the oxic and anoxic water samples. However, concentrations were much higher in the anoxic water samples, and were almost 31 and 1241 times their threshold levels, respectively (Deng et al., 2010). Therefore, DMS and DMTS were the dominant taste and odor compounds and contributed most to the septic odor in the anoxic decay phase of the cyanobacterial blooms. Previous reports suggested that volatile organic sulfur compounds are produced by the biodegradation of sulfur-containing organic matter (Caron and Kramer, 1994; Lomans et al., 1997). Massive cyanobacterial blooms in our sampling area resulted in the accumulation of a large amount of organic matter, and DMS and DMTS production can be stimulated during the decomposition of cyanobacterial blooms. The highest concentrations of DMS and DMTS observed in the decay phase of cyanobacterial blooms may have been caused by the fact that the bacteria synthesized volatile organic sulfur compounds from dead algae more efficiently than from living algae cells (Watson et al., 2008).

$\beta$ -Cyclocitral, which was identified as the product of *Microcystis* spp., can be released during the growth of *Microcystis* and the disruption of cell integrity (Watson, 2003; Watson et al., 2008). In the present study, high concentrations of  $\beta$ -cyclocitral were observed in the anoxic water samples, far exceeding its odor threshold concentration (Deng et al., 2010). The highest concentrations of  $\beta$ -cyclocitral, observed in the anoxic decay phase of the cyanobacterial blooms, may be due to large amounts of  $\beta$ -cyclocitral generated by cell damage or death of cyanobacteria (Watson et al., 2008). Previous reports indicated that  $\beta$ -cyclocitral induces the decomposition of cyanobacterial blooms (Ozaki et al., 2008), thereby enhancing DMS and DMTS release from decomposed cyanobacterial blooms. Therefore, strong correlation was found in the relationships between DMS, DMTS, and  $\beta$ -cyclocitral when dense cyanobacterial blooms decayed. Consistent with Peter et al. (2009),  $\beta$ -ionone was found only in the particle-bound samples. This finding can be attributed to the water-insoluble character of  $\beta$ -ionone: dissolved fractions were likely short-lived in the water, with rapid loss by volatilization (Watson, 2003).

Geosmin and MIB, two of the odorous metabolites from cyanobacteria, are also produced by terrestrial actinomycetes, fungi, and other micro-organisms (Shapiro et al., 1990; Suffet et al., 1996; Smith et al., 2008). In contrast to the aforementioned taste and odor compounds, geosmin and MIB were dominant in the live growth phase of cyanobacterial blooms, indicating that both were responsible for the earthy, musty odor in the water during this phase.

In this study, correlation analysis revealed that changes in DO, pH, chl-*a*, TN, TP, and  $\text{NH}_4^+$ -N had potential effects on the concentrations of taste and odor compounds.

These findings are consistent with some previous studies (Shapiro et al., 1990; Downing et al., 1999; Smith, 2003), underlining the regulating factors of taste and odor compounds. These chemical parameter changes were mainly induced by cyanobacterial breakdown; thus, the relationships were consistent in the dense cyanobacterial blooms areas, especially when dense cyanobacterial blooms decayed. The results presented in the current study aim to stimulate future research to develop predictive models for taste and odor compound production based on water quality variables to better predict objectionable taste and odor in water supplies.

## 4 Conclusions

The present study demonstrated that the decay of cyanobacterial blooms caused an anoxic water condition, increased nutrient loading, and released high concentrations of volatile organic sulfur compounds, such as DMS and DMTS, to the lake water. Concentrations of taste and odor compounds in the water column were significantly correlated with changes in pH, DO, TN, TP, and  $\text{NH}_4^+$ -N. Thus, preventing anaerobic decomposition of cyanobacterial blooms is an important strategy against the recurrence of a malodor crisis in Lake Taihu.

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