Denitrification and the controlling factors in Yunnan Plateau Lakes (China): Exploring the role of enhanced internal nitrogen cycling by algal blooms

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ABSTRACT

Denitrification plays an important role in nitrogen (N) removal in freshwater ecosystems. This internal process regulates the fluctuations of N concentration, especially for lakes with high nutrients concentrations and long residence time. Lakes in Yunnan plateau (southwestern China) provide typical cases, while studies in this region have been rare. Therefore, we studied denitrification of two lakes (Lake Dianchi in hypereutrophic state and Lake Erhai in mesotrophic) in this region. We used acetylene inhibition technique to quantify potential denitrification rate (PDR) of these lakes in April and August, 2015 and 2016. PDR of the sediments ranged 0–1.21 μmol/(N·m^2·hr), and that of overlying water ranged 0–0.24 μmol/(N·L·hr). Then, we used Least Angle Regression to determine the controlling factors for denitrification. Nutrients controlled PDR from two aspects: providing essential nitrogen sources; and affecting the richness and metabolism of denitrifying bacteria. In April, both aspects limited PDR; while only nitrogen sources limited PDR in August, due to depleted nitrate and enhanced denitrifying bacteria activity. Ammonia was most significant to denitrification, indicating that nitrate from nitrification transported to the bottom of well-mixed lake provide major N source by denitrification. The high PDR and low nitrate concentrate in August were evidence of an enhanced internal N cycling by algal blooms.

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Introduction

Eutrophication has become an increasingly serious problem globally due to anthropogenic nutrient input, especially excessive inputs of nitrogen (N) and phosphorous (P) (Gao et al., 2015; O'Neil et al., 2012; Paerl and Paul, 2012; Smith et al., 1999; Yao et al., 2017). In order to control eutrophication, the common practice is reduction of external N and P inputs. However, external load reduction does not always lead to significant lowering of N and P concentrations in water column as we expect. Increasing evidence support that, internal cycling processes of the lake are a major cause that prevents the remediation of eutrophic lakes (Carpenter, 2005; McCarthy et al., 2016; Wu et al., 2017). While internal cycling of P has been studied relatively thorough, much of the details about internal N cycling remain poorly characterized due to its complexity. Denitrification is one of the major processes of N cycling. It is also a dominant N sink and an important regulator of N concentration for lakes (Burgin and Hamilton, 2007; Seitzinger, 1988). Therefore, its rate, variability among different regions, controlling factors, and interactions with other internal cycling processes are vital aspects for us to reveal.

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N concentration is an important regulator of phytoplankton biomass, especially in hypereutrophic lakes and when algae blooms (Harke et al., 2016; Havens, 1995; Paerl et al., 2016; Scott and McCarthy, 2010). Apart from total phytoplankton biomass, N concentration could also influence the dominance of cyanobacteria, and the relative abundance of toxic strains of cyanobacteria (Harke et al., 2016; Smith, 1983; Vrede et al., 2009). Moreover, the control of N concentration is important not only for lakes themselves, but also for the downstream rivers and ocean that are often N limited (Elser et al., 2007; Paerl et al., 2011b, 2016). Denitrification is recognized as an important pathway that is capable of removing N and eliminating N concentrations. It transfers nitrate to N_2, and removes N from the ecosystem permanently (Hayatsu et al., 2008; Saunders and Kalff, 2001). It could remove 30%–50% of external N loads for some lakes, which makes it a dominant N sink for lakes (Burgin and Hamilton, 2007; Gardner et al., 1987; Harrison et al., 2008). Since denitrification is capable of removing considerable mass of N, it is of great significance to the well-being of a freshwater ecosystem and could help with the remediation of eutrophication.

However, as an internal process, denitrification is relatively hard to manipulate (Liu et al., 2014). We need to know the basic facts of denitrification, including its rates and controlling factors, if we want to enhance N removal through it. Previous studies uncovered a group of factors that could exert influences on denitrification, including nitrate concentration, pH, temperature, oxygen, organic matter and so on (Knowles, 1982; Svensson, 1998; Wallenstein et al., 2006; Zhong et al., 2010b). The most limiting ones could vary from lakes to lakes. Nevertheless, lakes that share similarities, like their morphology and external climate, might also share similarities in their patterns of internal processes including denitrification (Kosten et al., 2009; Piñaochoa and Álvarezcobelas, 2006). For example, in a stratified lake, hypolimnetic nitrate concentration is low because the stratification prevented the transportation of nitrate from epilimnion. As a result, the controlling factor for denitrification of stratified lakes is often nitrate (Bellinger et al., 2014; Tomaszek, 1995). While for a nitrate-rich lake where concentration of organics is low, the denitrifying process is mostly limited by organic concentration (Madsen, 1979; Svensson, 1998). When compared at larger scales, depth, trophic states, geological morphology of lakes, and land use within the watershed control denitrification more significantly (Bellinger et al., 2014; Enrich-Prast et al., 2015; Myrstener et al., 2016; Piñaochoa and Álvarezcobelas, 2006).

Lakes in Yunnan plateau constitute a typical group of lake. It is characterized by semi-enclosure, which means the inflows are much higher than the outflows (Yang et al., 2004). This made nutrients easy to accumulate within the lake, and the internal processes, including denitrification, especially important to this kind of lake (Zhang et al., 2016). Their semi-enclosure, along with high population density and high nutrient recharge, makes lakes in this region highly susceptible to eutrophication (Yang et al., 2004). With that being said, it is obvious that denitrification is an extremely important process for N removal and lowering of N concentration for lakes within this region.

Previous studies pointed out the importance of characteristics and morphology of lake basins, which could have a higher-hierarchical control over other factors like climate or temperature (Kosten et al., 2009; Olsen et al., 2015; Piñaochoa and Álvarezcobelas, 2006). However, studies on certain morphologies, like semi-enclosed lakes, are less characterized. For our study sites, Lake Dianchi and Lake Erhai are two typical lakes of this kind. Although denitrification is very important for them, studies on this subject have been rare. Liu et al. (2015) investigated potential denitrification rates (PDRs) of 20 lakes in Yunnan and Guizhou plateau including Lake Dianchi and Lake Erhai, but they did not consider the seasonal variation and controlling factors for denitrification. We would further elucidate the elements of denitrification, including its rate, variation and controlling factors for the two plateau lakes.

Apart from these features that set this region apart, we also want to uncover the interactions of internal processes by our cases. Processes of the entire N cycling are closely intertwined. Alterations in internal environments and other N cycling processes would influence denitrification (Ni and Wang, 2015; Nizzoli et al., 2010; Wetzel, 2001). Algae is known to be the key regulator for internal cycling processes (Cottingham et al., 2015; Zhu et al., 2013). While effects of algal bloom on denitrification and N cycling is complicated, and somehow contradictory according to previous studies (Conley et al., 2009; Howarth et al., 2011; Turner et al., 2008). Uptake and sedimentation of phytoplankton contribute to low N concentration during late summer, which can lower denitrification rate (Chan and Campbell, 1980; Christensen et al., 1990; Han et al., 2014). While on the other hand, algal bloom leads to hypoxia, which increases denitrification rate (Nizzoli et al., 2010; Turner et al., 2008; Wang et al., 2015). So, the influence of algal bloom on denitrification still needs further investigation.

Lake Dianchi and Lake Erhai experience annual algae bloom, leading to persistent hypoxia or even anoxia. This made the two lakes an appropriate place for our study. We hypothesized that denitrification is an important N sink for the two plateau lakes, and the algae bloom could alter the denitrification rate. To test our hypothesis, we tried to answer the following questions: (a) How much N could be removed by denitrification in plateau lakes? (b) What are the controlling factors for denitrification in plateau lakes and how these factors control denitrification? (c) The interactions between denitrification and other N cycling processes. Specifically, we quantified PDR by acetylene inhibition. Besides, we determined many other environmental properties, including temperature, total nitrogen (TN), total phosphorous (TP), ammonia, nitrate, organics, denitrification genes and so on. Based on these data, we determined the controlling factors of denitrification by LARS, explored the seasonal variations and tried to illustrate interactions of N cycling processes.

1. Materials and methods

1.1. Study area

Lake Dianchi and Lake Erhai are two lakes located in Yunnan plateau, southwestern China (Appendix A Fig. S1). Their semi-enclosure morphology made them susceptible to eutrophication (Yang et al., 2004). Due to increasing population density and heavy nutrients recharge within the watershed, last

decades have witnessed significant degradation of water quality in Lake Dianchi and Lake Erhai (Gao et al., 2015; Liu et al., 2012).

Lake Dianchi (latitude 24°28′–25°28′ N, longitude 102°30′–103°00′ E, altitude 1886 m, average depth 5.4 m, and maximal depth 9.9 m) is the largest lake in Yunnan Province, with a surface area of 300 km². It has been polluted heavily since the 1980s due to the tremendous population and economic growth. It is currently one of the three most polluted lakes in China. Lake Erhai (latitude 25°36′–25°58′ N, longitude 100°06′–100°18′ E, altitude 1972 m) is the second largest lake in Yunnan Province. The surface area of Lake Erhai is 249.8 km², with an average depth of 10.5 m. Located in the most developed regions of Yunnan Province, Lake Erhai also receives high pollutants from point and non-point sources. The last two decades have seen a significant deterioration of Lake Erhai.

Great efforts (7.7 billion dollars over the past 20 years) have been investigated to improve the water quality of the two lakes, but the results are not as good as expected, especially for the reduction of N. TP concentration of Lake Dianchi has decreased from over 0.3 mg/L to approximately 0.1 mg/L, while TN fluctuated around 2 mg/L and didn't decrease significantly (Appendix A Fig. S2). For Lake Erhai, both TN and TP increased over the past years. TN increased from about 0.3 to 0.6 mg/L, and TP from 0.025 to 0.03 mg/L. TN increased much faster than TP (Appendix A Fig. S2). Excessive N has become a more serious concern for the two lakes. Besides control of external N load, the influence of internal N cycling processes should also be evaluated.

1.2. Sampling and analyses

1.2.1. Sampling

Eight and six sampling sites were selected for Lake Dianchi and Lake Erhai, respectively (Appendix A Fig. S1 and Table S1). Considering the spatial heterogeneity, sampling sites distributed relatively uniform over the entire water surface. Samples of surface sediment and overlying water were collected from each site in April and August 2015 and 2016. We chose to sample in April and August because they represent dry season and rainy season, respectively. For Lake Dianchi, algae began to flourish in April, attained maximum population size, and began to decline in August (Appendix A Fig. S3). The biomass of algae increased from 3.00 to 6.99 mg/L, the dominant species switched from green algae to cyanobacteria (Appendix A Fig. S4), and the algal diversity decreased. For Lake Erhai, the seasonal growth followed the same pattern, which began in April and declined in August (Appendix A Fig. S3). We studied the PDR during those two months to investigate the possible influence of algal blooms on internal N cycling.

Sediment and water samples were collected using a columnar sampler (Kajak, Denmark). After the collection, sediments and overlying water were transformed and stored in polyethylene bottles. Samples were collected in triplicate at each site. And extra samples were collected for use of chemical analysis. Some water quality variables were detected on site with a portable water quality monitor (AP-2000, China), including water temperature, pH, dissolved oxygen (DO), and oxidation–reduction potential (ORP). After collection, samples were transported to the laboratory as soon as possible. Samples

for chemical analysis were stored at −20°C, and samples for determination of PDRs were stored at approximately 4°C and shaded to maintain the biological activity.

1.2.2. Analyses of sample characteristics
Chemical analyses of water included detection of nitrate (NO_3), ammonia (NH_4), TN, TP, and DIP concentrations. Chemical analyses of surface sediment included detection of nitrate, ammonia, TN, TP, dissolved inorganic phosphorous (DIP), dissolved organic nitrogen (DON), total organic carbon (TOC), dissolved organic carbon (DOC), and humus (including humic acid and fulvic acid) concentrations. These indicators were chosen based on previous literature that identified limiting factors for denitrification in other regions. In order to make our analysis more comprehensive, we tried to include as many indicators as possible in our study. Details of detection method for each indicator were listed in Appendix A Table S2.

Quantitative Polymerase Chain Reaction (q-PCR) was used to quantify the nitrite reductase gene *nirS* in sediments, which estimates the richness of denitrification bacteria. Details of q-PCR were also listed in Appendix A Table S2.

1.2.3. Measurements of potential denitrification and N_2O production
We used acetylene inhibition technique to measure PDR (Yoshinari et al., 1977). Our incubation procedures followed Sørensen (1978) and Tiedje et al. (1989), with some modifications due to laboratory and lake conditions. The experimental processes were as follows: (1) As soon as the samples were transported to the lab, pre-cultivation began. Sediment and overlying water were first added together to sealed bottles and cultivated in a dark environment at proper temperatures (average on site temperature of each sample) for about 24 hr to reestablish the actual on-site environment. The height of sediment in the bottle was about 5 cm, and the water filled the rest of the bottle to avoid extra oxygen. (2)After pre-cultivation, the overlying water was transferred into another bottle to detect PDRs of sediment and the overlying water separately. Each bottle was purged with nitrogen before being sealed to ensure anaerobic conditions. (3)Then 20% (V/V) of the nitrogen in the headspace was replaced by acetylene, which inhibits the last step of denitrification and makes N_2O the final product of denitrification. Another group of samples without acetylene was cultivated to detect N_2O production rate. (4) After 8-hr cultivation, N_2O concentrations were detected by a Gas Chromatograph (Agilent 7890A, USA) equipped with an electron-capture detector (temperature of inlet, detector, and oven were 200°C, 320°C, 230°C, respectively, flow rate 1 mL/min), and the concentration was calculated by gas standards. The PDR and N_2O production rate could be calculated by N_2O concentration.

The potential PDRs were calculated by final N_2O concentration according to the following equations:

$$DR_S = \frac{V_g c_{N_2O}}{A} \tag{1}$$

$$DR_w = \frac{V_g c_{N_2O}}{V_l} \tag{2}$$

where DR_S and DR_w are the PDRs of the sediment and overlying water, respectively; V_g is the volume of gas in the

cultivating bottle; c_{N_2O} is the concentration of N_2O in the bottle after cultivation; A is the cross-sectional area of the bottle; and V_1 is the volume of water in the bottle. We calculated the portion of N_2O distributed in headspace and sample, and find that the dissolved portion was negligible, so made some simplification (more detailed equations and calculations can be found in Yao et al. (2016)).

1.3. Data analyses

1.3.1. Determination of controlling factors: least angle regression (LARS)

Methods used to determine controlling factors, traditionally, are Pearson correlation or linear regression based on ordinary least squares (OLS). However, considering the complex influence of many environmental factors on the PDR, and the internal correlations between the environmental factors that would make the results less reliable, it would be inappropriate to determine the effect of environmental factors by these simple methods. Also, these methods are not able to rank the relative importance of each factor. To avoid influence of internal correlation of environmental factors, and to find out which factor had the most significant influence on PDR, we used Least Angle Regression (LARS), a stabilized stepwise regression (Efron et al., 2004; Uraibi et al., 2017; Zhao et al., 2017).

LARS can stabilize the coefficient of predictors when there is collinearity and it produces a lower prediction and estimation error of the response variable than traditional methods (Uraibi et al., 2017; Zhao et al., 2017). It builds up estimated regression model in successive steps, which begins from all coefficients of the predictors equal to zero, and adds one predictor each step. For each step, the predictor chosen is supposed to be the one most correlated with the response. In other words, the order of predictors being added to the model represents their relative importance to the response (Efron et al., 2004).

Predictors are added to the model step by step. At first, the error of the estimated model could be reduced with predictors added, for these predictors truly explain the response. However, after certain steps, the error in the estimated model increases with predictors added, because these predictors are not so relevant and only add random errors to the model. The mean squared errors (MSEs) of each step are given by cross validation. The step with the lowest MSE, which is the most fitted model, should be chosen, and the corresponding predictors of this step are the most significant factors to the response.

For this study, arguments (x_{ij} in the equations) were temperature, nutrient concentrations in the sediment (NO_3, NH_4, TN, TP, DIP, DON, TOC, DOC, humus), nutrients in the overlying water (NO_3, NH_4, TN, TP, DIP, TOC), and two type variables, "lake" (Lake Dianchi and Lake Erhai) and "season" (April and August) (type variables, including "lake" and "season" here, were represented by 0 and 1), which gives a total of 18 variables (represented by j in the argument matrix, $j = 18$). Response variables, a.k.a. y_i, were PDRs of sediment and overlying water. Data for the two lakes were analyzed together. All variables contained data from 14 sites of the two lakes, and four times sampling and detection in total ($i = 56$). We applied LARS to each season, separately; and both seasons together. The most significant factors given by LARS for each month were controlling factor for denitrification in the corresponding season. Comparison of results for the two seasons, and the result of analyzing two seasons together, we meant to find out the drivers of seasonal variation.

Location and scale transformations were performed to ensure that the covariates had a mean value of zero and unit length to avoid the effects of different magnitudes of variables. LARS analyses were performed using the "lars" package (Efron et al., 2004) in R software.

1.3.2. Quantifying of internal cycling rate

To quantify cycling rate, we used residence time in hydrodynamics as a reference (Monsen and Monismith, 2002), we defined cycling rate of nitrate as the inverse of its resident time. If denitrification was the only removal process, then cycling rate (CR) could be expressed as:

$$CR = \frac{DR}{NO_3^-} \tag{3}$$

where DR represents PDR and NO_3^- represented nitrate concentrations in water. This equation excluded the influence of other N removal processes like phytoplankton uptake, which lead to underestimation of CR. However, this simplification emphasized the focus on denitrification and enabled a quantifiable comparison.

1.3.3. Spatial interpolation

We used inverse distance weighted interpolation to calculate the total N removal by denitrification. N removed by denitrification in sediment and overlying water was added together, and we assumed that denitrification was limited to the bottom 1 m in overlying water, where the oxygen concentration is low enough for denitrification. The interpolation was performed in ArcGIS.

2. Results and discussion

2.1. PDRs, variations and its contribution to N removal in Lake Dianchi and Lake Erhai

The PDR in sediments of Lake Dianchi ranged from 0.07 to 0.54 μmol/(N·m²·hr), with a mean value of 0.18 μmol/(N·m²·hr) in April, and it ranged from 0.23 to 1.21 μmol/(N·m²·hr), with a mean value of 0.43 μmol/(N·m²·hr) in August. Compared to Lake Dianchi, PDRs in Lake Erhai were much lower, it could drop below detection limit with a highest of 0.009 μmol/(N·m²·hr) in April, and slightly increased to 0.02–0.16 μmol/(N·m²·hr) in August (Table 1).

For overlying water of Lake Dianchi, PDRs ranged from 0.0004 to 0.0025 μmol/(N·m²·hr), with a mean value of 0.0011 μmol/(N·m²·hr) in April, and increased significantly to 0.0063–0.2362 μmol/(N·m²·hr) in August. Similar pattern was found in Lake Erhai but with a lower range. Highest PDRs in April was 0.0016 μmol/(N·m²·hr), with a mean value of 0.0005 μmol/(N·m²·hr). While in August, it ranged from 0.0071 to 0.0610 μmol/(N·m²·hr) (Table 1).

Adding denitrification in sediments and the overlying water together, denitrification in Lake Dianchi removed 3.91 ton/(N·month) in April, and 161 ton/(N·month) in August. For Lake Erhai, denitrification removed 1.50 ton/(N·month) in

Table 1 – Potential denitrification rate (PDR) and estimated total N removed by denitrification for Lake Dianchi and Lake Erhai.

	PDR in sediments (μmol/(N·m²·hr))		PDR in overlying water (×10⁻³ μmol/(N·m²·hr))		Total N removed by denitrification (ton/(N·month))	
	April	August	April	August	April	August
Lake Dianchi	0.18 ± 0.12	0.43 ± 0.28	1.1 ± 0.54	52 ± 66	3.91	161
Lake Erhai	0.03 ± 0.02	0.05 ± 0.04	0.54 ± 0.60	19 ± 17	1.50	48.5

PDRs were expressed by mean ± standard deviation.

April and 48.5 ton/(N·month) in August (Table 1) (refer to Appendix A Fig. S6 for interpolation results). This significant increased PDR in August could reduce N concentrations in water column by approximately 0.13 mg/L for Lake Dianchi, and 0.02 mg/L for Lake Erhai.

Except the variation of PDR in different period, PDR in Lake Dianchi was higher than in Lake Erhai (Fig. 1). If we compare denitrification rate in the sediment in both lakes (Fig. 1a and c), and denitrification rate in overlying water in both lakes (Fig. 1b and d), we will find the rates of Lake Dianchi were well above that of Lake Erhai for both. Thus, we can safely draw

conclusion that PDRs in Lake Dianchi were higher than Lake Erhai, which conforms with most literatures (Myrstener et al., 2016; Piñaochoa and Álvarezcobelas, 2006; Saunders and Kalff, 2001).

2.2. Controlling factors of denitrification in the two plateau lakes

According to our results of LARS (Table 2, Fig. 2, refer to Appendix A Figs. S7–S12 for details of analyzing process), "lake" and "season" were significant factors for PDRs in
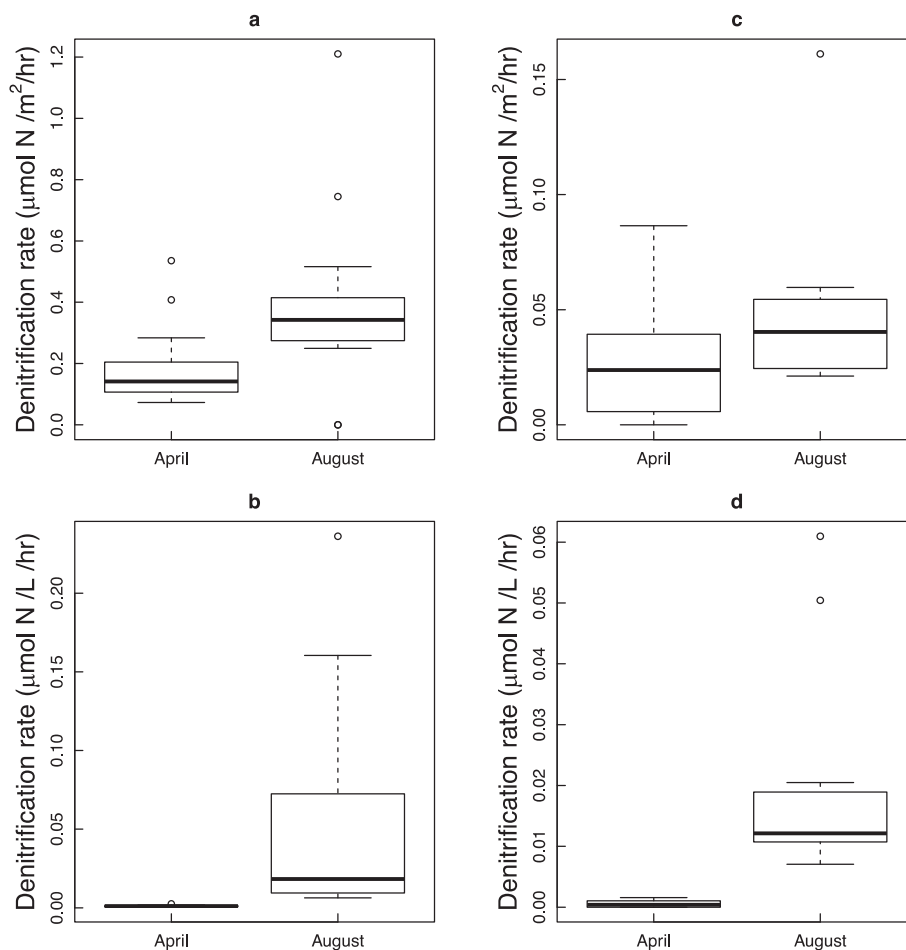
Fig. 1 – Variations in potential denitrification rate. (a) Potential denitrification rate in sediments of Lake Dianchi; (b) potential denitrification rate in overlying water of Lake Dianchi. (c) potential denitrification rate in sediments of Lake Erhai; (d) potential denitrification rate in overlying water of Lake Erhai.

**Table 2 – The controlling factors as determined by LARS analyses.**

	Season	Most relevant factors to PDR			
Both	Sediment	Ammonia	Lake	TOC	TN
	Overlying water	Ammonia	Season	Humus	TN
April	Sediment	TP	Lake	DIP	
	Overlying water	DON	Nitrate	DOC	TOC
August	Sediment	Ammonia	Lake	Nitrate	TN
	Overlying water	Nitrate	Ammonia	TN	

Three datasets, including separated data for each period and the entire data of both seasons, were analyzed. The results reflected the controlling factors under different temporal scales. The order of the factors listed represents their order of significance.

sediment and overlying water, respectively. This was consistent with our findings of variation in PDR (Fig. 1). In April, the most important factors for sediment denitrification were TP, "lake", and DIP. For overlying water, the most important factors were DON, nitrate, DOC, and TOC. In August, the most important factors for PDRs in sediments were ammonia, "lake", nitrate, and TN, and the most important factors for overlying water were nitrate, ammonia, and TN.

The controlling factors for August included different forms of N, ammonia, nitrate, and TN. It is noticeable that nitrate, as the substrate that could be used by denitrification directly, is not as important as ammonia and organic matter to PDR as controlling factors. Moreover, PDRs were much higher in August, regardless of the fact that nitrate concentrations were minor, some even below detection limit (Fig. 3). Considering the above two facts, we conclude that nitrification might provide important N source for denitrification.

Although the importance of coupled nitrification and denitrification has long been recognized (Jenkins and Kemp, 1984), the relationship between PDR and nitrate were more often observed in field studies (Chan and Campbell, 1980;
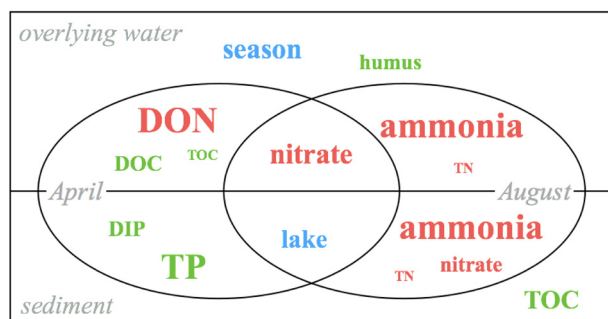
Fig. 2 – Controlling Factors of Denitrification. Factors in two circles were significant factors for April and August separately, and factors in the outer rectangle were significant for the combined data. The upper part represents overlying water and the lower part represents sediments. Different colors represent different types of factors: blue for type variables, red for N sources, and green for factors that were related to biological activity. The size of each factor illustrates its significant.

Chen et al., 2010; Kemp and Dodds, 2002; Wall et al., 2005). Generally, nitrification occurs at the surface where dissolved oxygen is sufficient for the reaction, which is also the case for Lake Dianchi (Chen et al., 2010; Rysgaard, 1993; Wetzel, 2001). While denitrification occurs mainly at the water–sediment interface and the surface layer of the sediment. Some studies found that denitrification is limited by hypolimnetic nitrate availability in summer, when the transportation of nitrate is blocked by stratification (Hamersley et al., 2009; Hasegawa and Okino, 2004; Sørensen et al., 1979; Zhong et al., 2010a). But for shallow lakes with small temperature variations like Lake Dianchi and Lake Erhai, stratifications were unstable, and could be easily destroyed by perturbations. Chances are large for nitrate produced at the surface be transported to the bottom and be used by denitrification. As a result, although nitrate concentrations were low in August, nitrification could supply N for denitrification.

Although temperature was often identified as an important reason for seasonal variation, we found it less important in our cases. Small variance of temperature in Yunnan plateau should be the main reason for this phenomenon. Previous studies that were generally conducted in temperate zones where the temperature varied greatly with seasons (Kemp and Dodds, 2002; Myrstener et al., 2016; Saunders and Kalff, 2001). The high variance in temperature could induce significant influence on denitrification rate. While in our cases, the temperature maintained at a relative high level, and varied within a small range. Especially for sediment and overlying water at the bottom, temperature variance was rather small. For Lake Dianchi, temperature varied from 17.5 to 25.0°C, and for Lake Erhai, from 15 to 24.5°C, both lakes varied within 10°C. With such small temperature variance, it makes sense that temperature was not identified as the primary controlling factors for denitrification in this region.

2.3. Enhanced denitrification as a result of enhanced internal N cycling by algae bloom

2.3.1. Algae bloom enhanced denitrification and N cycling rate
We quantified cycling rate of nitrate by Eq. (3). When the effect of decreased nitrate concentration and increased PDR was multiplied, the enhancement of cycling rate was extremely significant (Fig. 4). In April, the average residence time of nitrate was about 1438 days, but in August, it was only 14.37 days, which is a cycling rate about 100 times faster than in April.

PDR increased significantly in August compared to that of April, as a result of the algal bloom. August is about the start of decline phase of phytoplankton. Dead cells of phytoplankton settled to the sediment at decline phase. This sedimentation increased organics concentration (Fig. 3, TOC in the sediment was increased). Increased organics promoted organic nitrogen mineralization (Fig. 3, DON in the sediment was increased significantly), and ammonia was the product of mineralization being released into the overlying water (Fig. 3, ammonia in the overlying water was increased) (Xu et al., 2006). Enhanced mineralization and nitrification not only provided nitrate, but also lead to low oxygen concentration in the bottom. Both contributed to higher PDR. As the rates of mineralization, nitrification, denitrification, and sediment release, which were the main processes of internal N cycling, all increased, we
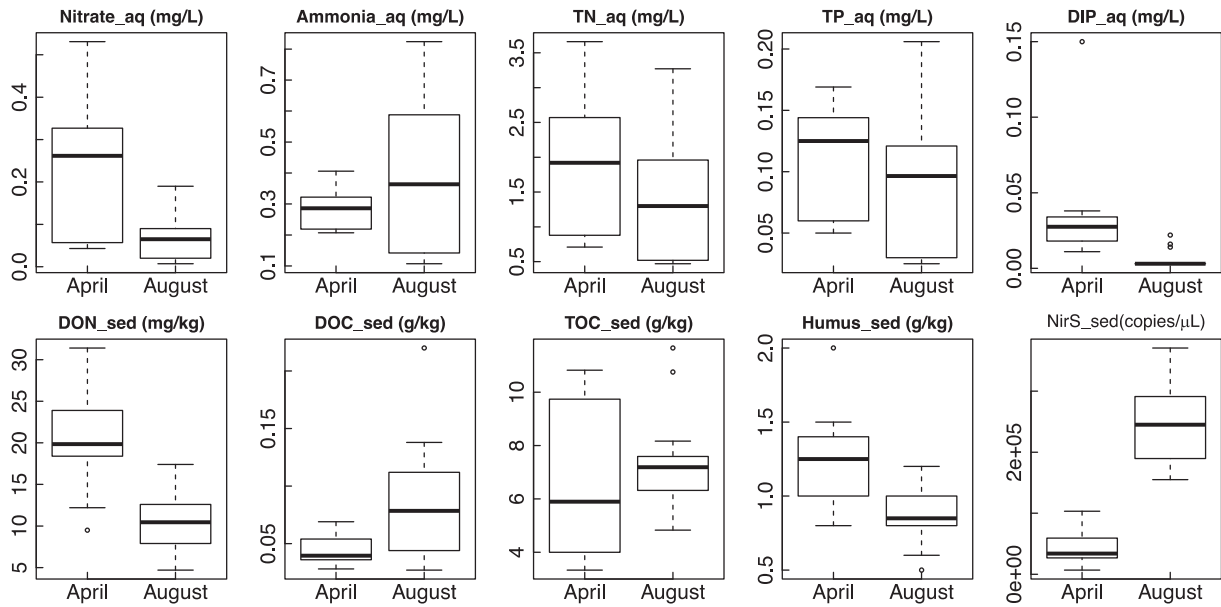
Fig. 3 – Variation in nutrient concentrations and abundance of denitrification gene _Nir S_. Only nutrients that were determined to be significant in denitrification are shown. "_aq" represented concentrations in the water, and "_sed" represented concentrations in the sediment. "Apr" represented April, and "Aug" represented August.

concluded that there was a higher internal cycling rate. High N cycling rate means a quick transformation between different species of N, and ensured the availability of nitrate for denitrification.

The low nitrate concentration of overlying water (Fig. 3) and significantly enhanced PDR indicated that, of all the internal N cycling processes, denitrification was enhanced most. Nutrient concentrations in the water column is actually a balance of related cycling processes. For nitrate, the concentration mainly depends on nitrification, algal uptake and denitrification (Paerl et al., 2011a; Tam and Wong, 1996; Wetzel, 2001). Algal uptake may be the reason for low nitrate concentrations at the growing phase. But since algae begun to decline in August, uptake of

nitrate was weak, and could not be the main reason for low nitrate concentration. Then, it became reasonable that the reason for low nitrate concentration was that N removal by denitrification exceed nitrification. As we discussed above, N mineralization and nitrification were also enhanced, but still did not compensate for the nitrate consumed by denitrification. This also indicated that denitrification exceeded nitrification at the end of algal blooms. Contrary to the general belief that in shallow, well-mixed lakes, nitrification should exceed denitrification. Because the relative high dissolved oxygen should be more favorable to aerobic reactions, thus, more favorable to nitrification than denitrification (Jensen et al., 1992; Li and Katsev, 2014; Windolf et al., 1996). While in Lake Dianchi and
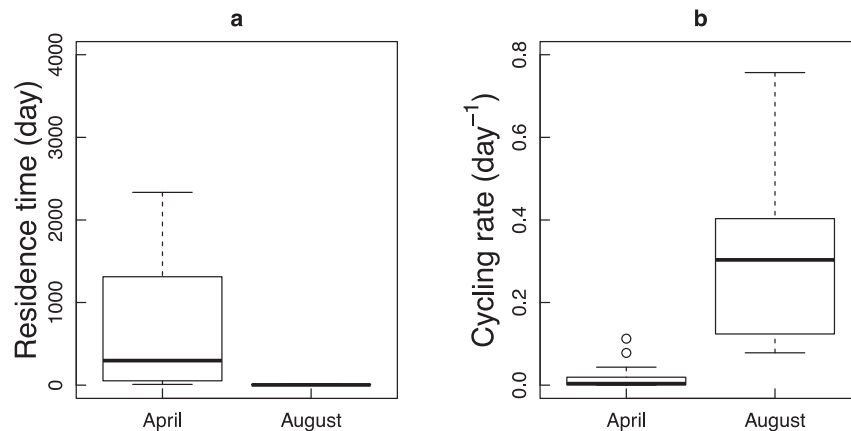
Fig. 4 – Residence time and cycling rate of nitrates when considering denitrification alone. (a) residence time, (b) cycling rate. As cycling rate of denitrification has been defined as the inverse of residence time of its reactants, longer residence time and lower cycling rate occurred in April, but in August, residence time was short and cycling rate was fast.

Lake Erhai, denitrification took advantage at the decline phase of algal blooms, due to the favorable environment (low DO and rich organics) that was created by these blooms.

Although algae were thought to be an important regulator of internal nutrients cycling due to the substrate provided by algal sedimentation (Han et al., 2014; Jensen et al., 1992), few studies have addressed the enhanced denitrification by algae blooms. Competition between algae uptake and denitrification for nitrate can reduce the PDR by decreasing nitrate availability (Chan and Campbell, 1980). Christensen et al. (1990) estimated that exponential growth of benthic microalgae reduced denitrification activity by up to 85% in a nitrate-rich stream. However, these findings do not conflict with ours, because the negative effects that result from algae growth do not apply to the decline phase where decomposition dominates. For the growing period, algae absorb nutrients and release oxygen, but for the decline phase, decomposition, nutrient release, and oxygen consumption dominate, which creates a favorable environment for denitrification. So, the influence of algae blooms on PDR differs at different stages.

Excessive N loads stimulate algae blooms. In turn, the algae bloom could enhance denitrification that removes N at the end. This constitutes a negative feedback that could reduce N to some extends. However, this negative feedback is not able to compensate for excessive input. Experiments and model studies conducted in shallow, eutrophic lakes have proved that N removal due to denitrification, though important, is not sufficient to control algae blooms (Asaeda and Van Bon, 1997; Paerl et al., 2011a); control of the external N load is still required.

2.3.2. The mechanism for enhanced denitrification by algal bloom

The controlling factors of the two months revealed how algal bloom altered denitrification. In August, the significant factors are different forms of N, except for the type variable "lake", including ammonia, nitrate, and TN. In April, N sources was also a controlling factor, reflected by the significance of nitrate and DON. But the controlling factors for April were not limited to N sources. Organic matters, represented by DOC and TOC, also showed their significance. Organic matters influence denitrification from two aspects. First, organics can provide necessary substrate for bacteria growth, which influences the richness of denitrifying bacteria. Second, decomposition of organics consumes oxygen. Denitrification, as an anaerobic metabolism pathway, requires low oxygen concentration. So, high organic concentration will create a favorable environment for denitrification (Knowles, 1982).

Two P related factors, TP and DIP, were also significant to denitrification in April. Higher phosphorus leads to a lower N: P ratio, which affects the metabolism of denitrification bacteria directly. And lower N:P ratio also results in greater competition between denitrification bacteria and algae. Algae is more competitive; thus, growth of denitrifying bacteria was inhibited, and denitrification was affected indirectly (Piñaochoa and Álvarezcobelas, 2006).

So, TOC, DOC, TP and DIP, the above four factors influence denitrification through the growth and metabolism of denitrifying bacteria. As a result, controlling factors of denitrification in April could be categorized into two groups: nitrogen sources, including DON and nitrate; and factors that influence bacteria activity, including DOC, TOC, TP, and DIP (Fig. 2).

Comparing the controlling factors of the two seasons, both nitrogen and biological activity limited denitrification in April, but nitrogen source was the only limiting factor in August (Fig. 2). The increased abundance of denitrification bacteria and the decreased nitrate concentration explained this variation in controlling factors. Nitrate concentration in August was much lower, some even below the detection limit in Lake Dianchi (Fig. 3). Nitrate is the direct nitrogen source for denitrification. Therefore, PDR should be limited with nitrate depleted. On the other hand, the q-PCR results of denitrification genes showed that the abundance of denitrifying gene *nir* S in August was much higher than in April (Fig. 3), which means that the richness of denitrifying bacteria increased significantly in August. This result supported our idea that organics enhanced the growth of denitrifying bacteria, and further enhanced denitrification. With biological activity of denitrification bacteria enhanced, and nitrate concentration decreased, biological activity no longer limited denitrification significantly, and nitrogen source became the only controlling factor in August.

3. Conclusions

PDR in Lake Dianchi and Lake Erhai displayed significant seasonal variation. PDRs were much higher in August (decline phase of algal blooms) than in April (start of algal growth), especially in overlying water. PDR in Lake Dianchi was much higher than Lake Erhai, due to the eutrophic state.

Ammonia concentrations affected PDR more than nitrate, especially in August, which proved that nitrate from nitrification was an important nitrogen source in plateau lakes. For shallow, unstratified lakes, nitrate from nitrification at the water surface could be transported easily to the bottom and provide major N source for denitrification.

The controlling factors could be grouped into two categories, nitrogen sources and biological activity related. Nitrogen sources included nitrate, ammonia, DON, and TN. Biological activity was reflected by related nutrients, which included TOC, DOC, TP, and DIP. In April, both nitrogen source and biological activity limited denitrification, but in August, with nitrate concentration decreased and biological activity enhanced, nitrogen source was the only limiting factor.

Algal blooms enhanced the PDR during its decline phase, because it provided rich organics that could be mineralized into ammonia, which provided nitrate for denitrification through nitrification, and created a favorable anaerobic environment for denitrification. Moreover, the overall internal N cycling rate of the lake was also enhanced.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jes.2018.05.028.

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