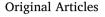
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# Does hydrological reconnection enhance nitrogen cycling rates in the lakeshore wetlands of a eutrophic lake?



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#### ABSTRACT

Lakeshore wetlands are thought to be hot spots for biogeochemical processes. However, many lakeshore wetlands have been hydrologically disconnected from the associated lake by levees that have altered the ecosystem services they provide. Lakeshore restoration projects have been undertaken to recover the hydrological connection; however, the effects of the various restoration practices on biogeochemical cycling remain unclear. Here, we compared variation in sediment nitrogen (N) cycling rates to assess the restoration benefits of N removal in a series of recently hydrologically restored lakeshore wetlands, including ponds and bottomlands, of Lake Dianchi, China. The results showed that nitrification rates were generally higher in the ponds, while denitrification rates were higher in the bottomlands. Hydrological reconnection stimulated the development of several sediment properties critical for N cycling rates in the ponds, including increases in sediment carbon (C) and N contents; however, bottomland reconnection increased sediment moisture and decreased sediment C and N contents likely due to erosion by wind-induced wave action. Correspondingly, hydrological reconnection significantly increased the sediment N cycling rates in ponds but decreased the sediment N cycling rates in bottomlands over time. Path analyses revealed that substrate characteristics, including moisture and C and N availability, were the critical drivers regulating wetland N cycling rates. These results imply that the restoration targets could not be met simply by hydrological reconnection. Future wetland restoration requires further understanding of the relationship between changes in sediment properties and biogeochemical processes.

#### 1. Introduction

Human activities have increased the input of reactive nitrogen (N) to lake ecosystems, causing eutrophication and other environmental problems. Sediment denitrification is widely recognized as a critical process that regulates N removal capacity (Seitzinger et al., 2006). In lakes and associated wetlands, the denitrification rates are generally high because denitrification is favored by anoxic or anaerobic conditions (Harrison et al., 2009; Lassaletta et al., 2012; Liu et al., 2018). Globally, the total denitrification in lakes is 31 Tg N yr<sup>-1</sup>, which is equivalent to the removal of approximately 12% of all land-based N sources (Seitzinger et al., 2006). Analyses of a global lake data set have shown that lakes can remove up to 90% of total N inputs from surrounding watersheds, thus, providing vital water quality benefits to downstream ecosystems (Finlay et al., 2013). Despite their significant importance, anthropogenic alterations have seriously degraded

freshwater lakes (Beeton, 2002; Liu et al., 2010). For example, impoldering activities have reduced the total lake area by 33% (5222 km<sup>2</sup>) in the middle and lower reaches of the Yangtze River, China (Cui et al., 2013). This alteration appears to have significantly restricted ecosystem services, including the efficiency of N removal.

To restore the ecosystem services of the degraded lake systems, massive wetland restoration projects have been implemented in China since the early 2000s. Many restoration projects include reestablishment of the hydrological connection between a lake and its historically associated wetlands by breaching levees or dikes. Such projects are expected to greatly improve the water quality of lakes by enhancing the efficiency of nutrient removal. In aquatic ecosystems, N removal capacity is associated with many physical and chemical factors such as temperature, water retention time and availability of nitrate, organic carbon (C), and oxygen (Bruesewitz et al., 2011; Nizzoli et al., 2018), as well as various biological factors such as the amount and types of plant

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roots and fauna present (Karjalainen et al., 2001; Forshay and Dodson, 2011; Veraart et al., 2011; Liu et al., 2015). Hydrological reconnection may significantly affect the physicochemical characteristics of wetlands, which in turn regulate nitrification and denitrification rates. Studies have demonstrated that denitrification rates are significantly higher in river-connected wetlands than in isolated wetlands (Racchetti et al., 2011). Hernandez & Mitsch (2006) have also found that the nitrous oxide (N<sub>2</sub>O) flux increases significantly after flooding in a riverine wetland. However, N removal has only received limited study in lakes with restored hydrological reconnection.

Lakeshore wetlands link aquatic ecosystems with the adjacent upland areas where water level fluctuations produce dynamic moisture and biogeochemical conditions, and these areas may therefore act as hot spots for biogeochemical processes, including denitrification. However, two main types of lakeshore wetlands, bottomlands and ponds, may respond differently to hydrological connectivity. Bottomlands often exhibit a gentle downward slope to the lake surface where temporal variations in water levels occur, periodically producing oxic and suboxic soils. The co-existence of aerobic and anaerobic conditions within the soil profile can support the occurrence of coupled nitrification-denitrification (Hefting et al., 2004). Accordingly, availability of dissolved organic matter may be a major controlling factor for bottomland denitrification rates (Pinay et al., 1993; Sheibley et al., 2006; Gift et al., 2010). Well-developed ponds can provide organic-rich sediments, but often, relatively stable water levels are maintained, resulting in anaerobic conditions. Anaerobic conditions inhibit the nitrification pathway from ammonium  $(NH_4^+)$  to nitrate  $(NO_3^-)$  and thus reduce N loss. Pond denitrification rates may be primarily regulated by NO3<sup>-</sup> availability. We therefore expected to find differences in N removal rates between these two wetland types.

In this study, we investigated nitrification and denitrification rates and associated environmental factors in flow-reconnected and unconnected lakeshore wetlands (bottomlands and ponds) of Lake Dianchi, southwestern China. We hypothesized that reconnected lakeshore wetlands would be characterized by high N removal capacity. Therefore, the primary objectives of this study were (1) to examine the influence of hydrological reconnection on sediment nitrification and denitrification rates of lakeshore wetlands and (2) to explore the direct and indirect effects of environmental factors on wetland nitrification and denitrification rates.

#### 2. Material and methods

#### 2.1. Study site

This study was carried out in Lake Dianchi ( $24^{\circ}29'-25^{\circ}28'N$ ,  $102^{\circ}29'-103^{\circ}01'E$ ), a eutrophic shallow lake located in Kunming in the central part of the Yunnan–Guizhou Plateau, China (Fig. 1). The lake has a surface area of  $310 \text{ km}^2$  and a shoreline of 150 km (Wang et al., 2014). The climate of Kunming is characterized by strong variations in seasonal precipitation with a monthly average precipitation of 18.5 mm from November to April and 149.3 mm from May to October.

Since the 1970s, construction of dams and concrete levees around Lake Dianchi has interrupted the natural connection between the lake and the surrounding wetlands that have been converted into aquaculture ponds and degraded bottomland habitat, resulting in a total decrease of approximately  $23 \text{ km}^2$  in the lake's surface area. Impoldering, in conjunction with increased wastewater discharge, has resulted in turbid lake water and the proliferation of blue-green algae. Over the past 50 years, the mean total phosphorus (TP) concentration increased by a factor of 27.5 and the mean total nitrogen (TN) concentration increased 10-fold, while the mean Secchi depth reduced from > 3 m in 1960 to 0.42 m in 2009 and submerged macrophyte cover decreased from 88% in 1961 to 2% in 2010 (Lu et al., 2012). To restore the aquatic environment, in 2009 the local government launched a project to reinstate the hydrological connection between the

lakeshore wetlands and the lake by breaching the levees. So far, a total area of  $7.416 \, \text{km}^2$  of lakeshore wetlands has been reconnected with Lake Dianchi.

#### 2.2. Field sampling

We selected 55 sampling sites around Lake Dianchi (Fig. 1) and categorized them into five groups according to wetland type and their hydrological connectivity with the lake: pond-unconnected (9 sites), pond-reconnected-3 yr (reconnected  $\leq$  3 years; 13 sites), pond-reconnected-5 yr (reconnected  $\geq$  5 years; 12 sites), bottomland-unflooded (unconnected with the lake; 7 sites) and bottomland-reflooded (reconnected disturbance, the actual number of sampling sites varied slightly. Thus, we carried out four sampling campaigns in April 2014 (37 sites), September 2014 (51 sites), April 2015 (49 sites) and September 2015 (44 sites).

At each pond site, three surface sediment samples (approximately the top 7 cm) were collected randomly using a Peterson grab sampler and then mixed and homogenized into a composite sample. Similarly, at each bottomland site, three intact sediment cores (approximately the top 10 cm) were randomly collected using a hand corer and subsequently mixed into a composite sample. Approximately 300 g of sediment sample was collected into a sealed bag and stored at 4 °C in a portable refrigerator. The mean water depths of ponds and bottomlands were 113 and 47 cm, respectively. Thus, a 500 mL composite surface water sample was collected at a depth of approximately 0.5 m in the ponds and at 0.2 m in the bottomlands before undertaking the sediment sampling.

#### 2.3. Measurements of nitrification and denitrification

The potential nitrification rate (PNR) was determined using the shaken-slurry method (Hou et al., 2013; Yao et al., 2018a). In brief, 10 g fresh sediment was weighed into a 250 mL sterile Erlenmeyer flask and mixed with 0.5 mL (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> (0.25 M) and 100 mL phosphate buffer solution (1 mM, pH 7.4). All flasks were incubated in an air bath thermostatic shaker (180 rpm, 25 °C) for 24 h. During the incubation period, 5 mL subsamples were taken from the slurry after 1, 4, 10, 16, and 24 h, and these were centrifuged at 4000 rpm for 5 min and filtered through GF/C filters (0.45 µm, Whatman, Maidstone, UK). The concentrations of NO<sub>3</sub><sup>-</sup> in the filtrates were measured using a Discrete Chemistry Analyzer (Autochem 1100, Xingrui Technology, China). PNR was calculated as the slope of the regression equation of NO<sub>3</sub><sup>-</sup> production and incubation time (Table S1).

The potential denitrification rate (PDR) and the unamended denitrification rate (UDR) were determined using the acetylene ( $C_2H_2$ ) inhibition method, which has been successfully used for a long time despite its shortcomings such as inhibiting nitrification (Smith and Tiedje, 1979; Groffman et al., 2006). PDR is a maximized evaluation of in situ denitrification with C and N addition, while UDR is a conservative evaluation without nutrient amendments (Xiong et al., 2017).

The PDR measurements were based on 50 g of fresh sediment mixed with 30 mL of incubation solution (0.18 g/L glucose, 0.1 g/L KNO<sub>3</sub>, 1 g/L chloramphenicol) in a 250 mL brown glass bottle (Xiong et al., 2015; Liu et al., 2016). To create anoxic conditions, the bottles were sealed with rubber stoppers and purged with 99.999%  $N_2$  for 2 min. Thirty mL of  $C_2H_2$  was added to each bottle with a syringe to inhibit reduction of  $N_2O$  to  $N_2$  during denitrification. All bottles were incubated in the thermostatic incubator (25 °C, in darkness) for 4 h. At the start and end of incubation, 10 mL of the headspace gas from each bottle was transferred into a gas cylinder with a syringe after vigorous and careful shaking. The concentration of  $N_2O$  was determined using a gas chromatograph (Agilent 7890, CA, USA) fitted with an electron capture detector. The measurement procedure for UDR was similar to that for PDR, but the fresh sediment was mixed with 30 mL of unfiltered in situ

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