Sediment nitrogen cycling rates and microbial abundance along a submerged vegetation gradient in a eutrophic lake

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HIGHLIGHTS

- N removal capacity and microbial abundance do not benefit from vegetation restoration.
- N cycling rates are largely determined by abiotic factors but not microbes and vegetation.
- Nitrification and denitrification rates were significantly different among seasons.

GRAPHICAL ABSTRACT
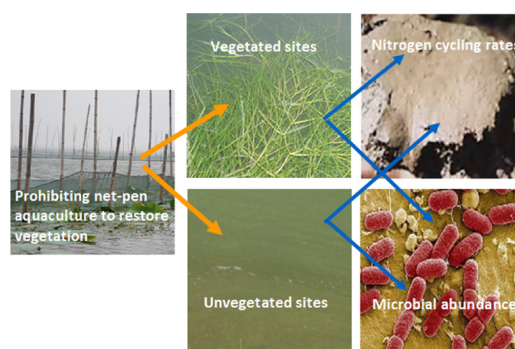
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ABSTRACT

Decline of submerged vegetation is one of the most serious ecological problems in eutrophic lakes worldwide. Although restoration of submerged vegetation is widely assumed to enhance ecological functions (e.g., nitrogen removal) and aquatic biodiversity, the evidence for this assumption is very limited. Here, we investigated the spatio-temporal patterns of sediment potential nitrification, unamended denitrification and N_2O production rates along a vegetation gradient in the Lake Honghu, where submerged vegetation was largely restored by prohibiting net-pen aquaculture. We also used five functional genes as markers to quantify the abundance of sediment nitrifying and denitrifying microorganisms. Results showed that unvegetated sediments supported greater nitrification rates than rhizosphere sediments of perennial or seasonal vegetation. However, the absence of submerged vegetation had no significant effect on denitrification and N_2O production rates. Additionally, the abundance of functional microorganisms in sediments was not significantly different among vegetation types. Season had a strong effect on both nitrogen cycling processes and microbial abundances. The highest nitrification rates were observed in September, while the highest denitrification rates occurred in December. The temporal variation of sediment nitrification, denitrification and N_2O production rates could be due to changes in water quality and sediment properties rather than submerged vegetation and microbial abundances. Our findings highlight that vegetation restoration in eutrophic lakes improves water quality but does not enhance sediment nitrogen removal rates and microbial abundances. Therefore, for reducing the N level in eutrophic lakes, major efforts should be made to control nutrients export from terrestrial ecosystems.

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1. Introduction

Due to increasing anthropogenic disturbances, many lakes worldwide have faced a number of environmental problems such as eutrophication, loss of aquatic biodiversity and toxic algal blooms (Vitousek et al., 2002; Liu et al., 2010). In shallow lakes, submerged plants are important primary producers and play a vital role in maintaining the clear water state (Scheffer et al., 2001). However, the species richness, cover and biomass of submerged vegetation in many lakes worldwide have declined due to eutrophication and fish farming (Li et al., 2002). The re-establishment of submerged vegetation is recognized as an efficient approach for improving ecological functions and aquatic biodiversity (Hilt et al., 2006; Bakker et al., 2013). However, there is limited knowledge of the influence of submerged vegetation restoration on sediment nitrogen (N) cycling processes and associated microorganisms, which are closely associated with the N removal efficiency and self-purification capacity of lake ecosystems (Forshay and Dodson, 2011).

The N removal in freshwater habitats is strongly associated with two microbial processes: nitrification and denitrification (McCrackin and Elser, 2010). Nitrification, the oxidation of ammonia (NH_4^+) to nitrite (NO_2^-) and subsequently to nitrate (NO_3^-), occurs only in the presence of oxygen (Vitousek et al., 2002). The first step of nitrification process is catalyzed by ammonia monooxygenase (*amoA*). Archaeal and bacterial *amoA* genes have been extensively used as molecular marker to quantify the abundance and diversity of ammonia-oxidizing archaea (AOA) and bacteria (AOB) in soils and sediments (Wankel et al., 2011). Denitrification, the successive reduction of NO_3^- to NO_2^-, nitric oxide (NO), nitrous oxide (N_2O) and dinitrogen gas (N_2), is favoured under anoxic or anaerobic conditions (Wallenstein et al., 2006). The reduction of NO_2^- to NO is the rate-limiting step, which is catalyzed by either a copper-containing enzyme encoded by the *nirK* gene or a cytochrome-cd1 enzyme encoded by *nirS* gene (Wallenstein et al., 2006). The conversion of N_2O to N_2 is catalyzed by N_2O reductase, which is encoded by the *nosZ* gene (Liu et al., 2013). The three functional genes are frequently used for studying denitrifier community in both aquatic and terrestrial ecosystems (Rich and Myrold, 2004).

It is long known that sediment N cycling processes in lakes are greatly influenced by water quality and sediment properties (Bruesewitz et al., 2011; Yao et al., 2016). Concentrations of N and carbon (C) are generally considered as the primary determinants of denitrification process, because NO_3^- acts as a terminal electron acceptor and organic C as an electron donor for denitrifying bacteria (Wallenstein et al., 2006). Some studies have also indicated that aquatic vegetation can significantly impact sediment nitrification and denitrification rates in freshwater environments (Forshay and Dodson, 2011; Veraart et al., 2011). Submerged plants may directly and indirectly influence the nitrification and denitrification processes through the modification of organic C inputs and sediment redox regimes via oxygen release from plant roots (Karjalainen et al., 2001; Liu et al., 2015b). A number of recent studies have examined the relationships between N-cycling processes and associated microbial communities, but their results are inconsistent (e.g., Wankel et al., 2011; Deslippe et al., 2014). For instance, Deslippe et al. (2014) reported that soil denitrification potential was positively correlated with the abundance of *nirS*–type denitrifier, whereas Dandie et al. (2011) did not find a significant relationship between denitrification rate and denitrifier abundance.

In this study, we seasonally determined sediment potential nitrification, unamended denitrification and N_2O production rates along a submerged vegetation gradient in the Lake Honghu of China, where submerged vegetation was largely restored by prohibiting net-pen aquaculture. We also used five functional genes as markers to quantify the abundance of sediment nitrifying and denitrifying microorganisms. We hypothesized that sediment N-cycling rates and microbial abundance would be significantly enhanced by submerged vegetation restoration. Therefore, the aims of our study were (1) to investigate the spatio-temporal patterns of sediment N-cycling rates and functional gene abundances in the Lake Honghu; (2) to evaluate the relative importance of local environmental factors and functional gene abundances in determining sediment N-cycling rates; and (3) to examine the relationships between functional gene abundances in lake sediments and local environmental factors.

2. Material and methods

2.1. Study area

The Lake Honghu (29°38′–29°59′N, 113°11′–113°28′E) is a subtropical shallow lake connected to the Yangtze River, located in the central China (Fig. 1). It is one of the largest freshwater lakes in the Yangtze River basin. However, after decades of intensive reclamation, the surface area of Lake Honghu has decreased substantially from 662 km² in the 1950s to approximately 307 km² in 2007 (Xiao and Liu, 2013). The lake has a mean water depth of 1.35 m and maximum water depth of 2.32 m. The dominant land use in the catchment of Lake Honghu is agriculture (mostly paddy fields), which covers approximately 70% of the land. Intensive aquaculture was introduced in Lake Honghu since 1980s and the total area of aquaculture reached 240 km² in 2004 (Xiao and Liu, 2013). Serious ecological problems such as eutrophication, water quality deterioration and submerged vegetation loss have emerged in the beginning of the 1990s (Li et al., 2002). For example, *Hydrilla verticillata*, the dominant submerged macrophyte in the Lake Honghu, covered an area of 209 km² in 1950s, 24 km² in 1980s and almost disappeared in 1990s (Song et al., 2016). To restore submerged vegetation and control water eutrophication, most of the culture enclosures in the lake were removed by local government in 2005. Consequently, the cover, biomass and species richness of submerged plant communities have increased considerably after 10 years of natural restoration (Song et al., 2016). For instance, the community of *Hydrilla verticillata* was partly restored and covered an area of 26 km² in 2010 and 47 km² in 2014. Nonetheless, submerged plant communities in some areas of Lake Honghu have still not been restored because of the absence of viable seeds and vegetative propagules in sediments (Xiao and Liu, 2013). Therefore, the unrestored areas can serve as the controls (space-for-time substitution) to evaluate the influence of vegetation restoration on ecological processes and biotic communities in lake sediments (Sundermann et al., 2011).

2.2. Field sampling

In March (spring), June (summer), September (autumn), and December (winter) of 2014, field sampling was conducted at 18 fixed sites in the Lake Honghu (Fig. 1). These sites were selected randomly and their longitude and latitude were recorded using a global positioning system. Three sediments (approximately the top 7 cm) were collected from a boat using a home-made grab sampler at each sampling site, and then mixed and homogenized to form a composite sample. If submerged vegetation was present in a sampling site, rhizosphere sediments were obtained from the whole root zone by shaking off sediments that were loosely adhering to the plant roots (Herrmann et al., 2009). In most cases, submerged plant communities at a lake site were dominated by two or more species whose roots were often intertwined and indistinguishable from one another. Therefore, to avoid the possible sampling bias, we did not distinguish the rhizosphere sediments from different submerged species in this study. Approximately 300 g of sediment was collected in a plastic bag and stored at 5 °C in a refrigerator. Moreover, about 10 g of sediment was collected at each site in a centrifuge tube and immediately frozen in liquid N_2. A 500-mL composite water sample was collected at a depth of approximately 0.5 m before collecting sediments to prevent sediments resuspension (Liu et al., 2015a).

At each sampling site, submerged plant communities were investigated using a plant grab (25 × 35 cm) with three replicates (Wang et al., 2014). The species richness was defined as the total number of