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Research Paper

Revegetation affects soil denitrifying communities in a riparian ecotone

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ABSTRACT

Denitrification is one of the most important processes in the nitrogen (N) cycle due to its permanently removing excess N from ecosystems into the atmosphere. In practice, revegetation has employed to facilitate the process for preventing nitrogen from terrestrial into aquatic ecosystems, in particular in the terrestrial-aquatic ecotone (i.e., riparian zone). However, how revegetation drives the shift in the denitrifying bacterial community and consequently alters denitrification is still unclear. In this study, we investigated soil denitrifiers in three vegetation types with respective dominant species of trees, shrubs and herbs in the water-level-fluctuate-zone in the Three Gorges Reservoir, China. We hypothesized that revegetation affected the composition of denitrifiers. Results revealed that the functional gene composition in herb samples was well separated from that in tree samples, which was dependent on the interactions between plant traits (i.e., species number and diversity, root C:N ratio) and environmental factors (i.e., soil temperature and pH). Herb soils has more abundance of *nirS* and *nirK* genes and *nirS* gene diversity due to their higher species number, soil pH, soil organic C, TN, soil C:N ratio and root C:N ratio compared with the shrub and tree soils. Vegetation types did not significantly affect soil denitrification rate, which could be largely explained by the combined effects of plant attributes (species number, root organic C and root N), and soil pH. Our results have demonstrated that herb plantations could increase the abundance of soil denitrifiers through altering both the quantity and quality of SOC and soil pH.

1. Introduction

Microbial denitrification plays an important role in riparian ecosystems by permanently removing excess nitrogen (N) from the system as dinitrogen gas (N_2) (Sirivedhin and Gray, 2006; Guo et al., 2013), which can account for approximately 82% of the total nitrate (NO_3^- –N) loss in riparian zone (Bedard-Haughn et al., 2006; Kreiling et al., 2011). This N transformation process is of global importance due to its contribution to N_2O accumulation in the atmosphere (Ravishankara et al., 2009). The N_2O is a potent greenhouse gas with a global warming potential about 298 times greater than that of carbon dioxide (Nakicenovic and Swart, 2000; Liu et al., 2013). Given the adverse environmental impact of excess N, reasonable management of the riparian N-cycle is highly desirable.

Soil microorganisms are key drivers in almost all biogeochemical processes including denitrification, and their abundance and composition would be affected by changes in environmental factors (Fuhrman, 2009; Liu et al., 2013). Previous studies have indicated that denitrification rate can be influenced by resource availability (i.e., soil organic C and N availability) (Klemedtsson et al., 2005), and environmental factors (i.e., soil temperature, moisture and pH) by regulating the structure and function of the denitrifying community (Bateman and Baggs, 2005). In turn, the alterations in the composition of microbial communities also reflect the effects of environmental change (Wallenstein et al., 2006; Liu et al., 2013). Although the relationship between environmental factors and denitrification is well documented, the mechanisms governing the interaction of the denitrifying community, resource availability, environmental factors, and the biological aspects of denitrification are poorly understood and even controversial (Taroncher-Oldenburg et al., 2003; Wakelin et al., 2007).

Denitrification involves four enzymatically catalyzed reductions of NO_3^- to nitrite (NO_2^-), nitrite oxide (NO), N_2O and finally N_2 (Philippot, 2002; Ellen et al., 2006). These processes consist of multiple genes encoding four metalloenzymes, including dissimilatory NO_3^- reductase, NO_2^- reductase, NO reductase, and N_2O reductase (Canfield et al., 2010). The denitrifiers are distinguished from

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other NO_3^--respiring bacteria by the reduction of NO_2^- to NO. This reaction is the rate-limiting step in denitrification and is catalyzed by the cytochrome cd1 encoded by *nirS* or Cu-containing enzyme encoded by *nirK* under oxygen-limited condition (Zumft, 1997). Both *nirS* and *nirK* genes are most widely used as molecular markers in the studies of denitrifying communities (Braker et al., 1998; Henry et al., 2004). However, the detailed community composition and abundances of denitrifiers in riparian zone are still largely unknown (Wang et al., 2014).

Revegetation is one of the important factors that drives the diversity and function of the riparian ecosystem and has been extensively studied (Tall et al., 2011; Audet et al., 2014). Revegetation is widely used as an effective measure to restore and protect the riparian ecosystem (Lu et al., 2010; Kreiling et al., 2011). In general, revegetation alters soil C inputs into soils and then regulates soil N transformation (Booth et al., 2005; Cheng et al., 2010), and reduces soil N concentrations by absorbing mineral N for plant growth (Kreiling et al., 2011). Moreover, revegetation can cause a shift in bacterial and fungal communities in riparian soil (Ye et al., 2013). Nevertheless, the understanding of the impact of revegetation in riparian zone on the interactive links of denitrifying communities, soil environmental factors, and vegetation attributes remain incomplete.

With the full functioning of the Three Gorges Dam project, the water level of the reservoir fluctuates from 145 m above sea level in summer (May to September) to 175 m in winter (October–April), forming the water-level fluctuation zone (WLFZ) with a total area of 350 km^2 in the Three Gorges Reservoir (Zhong and Qi, 2008), and causing various ecological implications including losses of previous vegetation (New and Xie, 2008; Ye et al., 2015). Revegetation has been carried out for recent years to restore and protect the riparian ecosystem. Species including herb (*Cynodon dactylon* and *Hemarthria sibirica*), shrub (*Hibiscus syriacus*, *Morus alba,* and *Salix variegate*), and tree (*Salix chaenomeloides* and *Taxodium distichum*) were selected for revegetation since they can tolerate summer exposure and winter inundation (Lu et al., 2010). However, little information is available about the impacts of revegetation on soil denitrifying communities.

In this study, we investigated vegetation attributes (vegetation species number, diversity, total coverage and above-ground biomass, root biomass, and root N), key soil properties, soil denitrification rates and the abundance of denitrification genes under different vegetation types (herb, shrub and tree) in the WLFZ of the Three Gorges Reservoir. It was hypothesized that there were significant differences in the composition of denitrifier community, the N substrate availability and soil environmental factors among difference vegetation types. To test this hypothesis, we specifically focused on (1) how different vegetation types have potentially impacted soil inorganic N concentrations, environmental factors and denitrifier community; and (2) how the associated changes in soil properties (i.e., soil organic C, soil C:N ratio, soil temperature and moisture, and pH) and plant traits (i.e., species number and diversity, root C:N ratio) determine soil *nirS* and *nirK* communities.

2. Materials and methods

2.1. Site description

Samplings were carried out at the Zhongxian Revegetation station (30°26′N 108°11′E) in Chongqing, located in the Three Gorges Reservoir, China (Ye et al., 2012). The local climate belongs to subtropical monsoon, with an annual mean temperature of 16.5–19 °C and monthly average temperature of 3.4–7.2 °C in January and 28–30 °C in July. The annual mean precipitation is 886–1614 mm, 80% of which falls between April and October (Ye et al., 2011, 2015). The soil belongs to purple soil (Regosols in FAO taxonomy, Entisol in USDA taxonomy) with composition of 29% sand, 49% silt and 22% clay in the top 20 cm. Before revegetation, the average pH soil in the study region is 6.22 ± 0.09 and average soil moisture and bulk density were 19.99 ± 0.71% and 2.31 ± 0.03 g cm^{-3}, respectively. Soil NO_3^--N and NH_4^+-N concentrations were 30.76 ± 3.14 mg kg^{-1} and 7.10 ± 0.47 mg kg^{-1}, respectively. And soil organic C and total N were 6.21 ± 0.43 g kg^{-1} and 0.59 ± 0.03 g kg^{-1}, respectively (Ye et al., 2012). The water level of the study region fluctuates from 145 m in summer to 175 m in winter.

Before submergence, vegetation in the WLFZ of the Three Gorges Reservoir was dominated by *Setaria viridis*, *Digitaria ciliaris*, *Leptochloa Chinensis*, *Cynodon dactylon*, *Hemarthria altissima*, *Capillipedium Assimile*, *Ficus tikoua*, *Pterocarya stenoptera*, and *Vitex negundo* (Lu et al., 2010; Ye et al., 2013). After the prolonged submergence since 2008, the plant community is dominated by annual plants such as *Echinochloa crusgalli* and *Bidens tripartita,* and perennials including *C. dactylon* and a few alien invasive plants, such as *Eupatorium adenophorum and Alternanthera philoxeroides* (Zhong and Qi, 2008). Revegetation has been carried out to restore the riparian ecosystem along the elevation from 155 m to 175 m since March 2008. The plant community at elevations of 155–165 m is dominated by herbs including *C. dactylon* and *Hemathria sibirica*, with a mean coverage of 95%. The plant community at elevations of 165–172 m consists primarily of shrub species of *Hibiscus syriacus*, *Morus alba* and *Salix variegate*, with a density of eight stems per square meters. The plant community at elevations of 172–175 m is dominated by tree species including *Salix chaenomeloides* and *Taxodium distichum*, with the mean planting density of four stems per square meters (Ye et al., 2012, 2013).

2.2. Field measurements

Field surveys were conducted in August, 2014, when the reservoir's water level was 145 m and the sampling sites were exposed to the air after flooding. Three stands of each vegetation type (50 m × 1000 m), including tree, shrub, and herb were delineated in this study. Within each stand, we randomly set up 3 plots (5 m × 5 m each) for each vegetation type to investigate the plant community. Plant species in the quadrats were identified according to Van der Meijden (2005). The coverage of each species was estimated according to the Braun-Blanquet method (Braun-Blanquet, 1932). Plant diversity was determined by Shannon-Wiener heterogeneity index (H) (Shannon and Weaver, 1949). Three sub-plots (1 m × 1 m each) were randomly selected in each plot (5 m × 5 m) to measure soil denitrification rate.

The net denitrification rates were measured using the *in situ* acetylene blocking technique (Tiedje et al., 1989). Although the technique can inhibit gross nitrification and potentially underestimate denitrification when coupled to nitrification, it still seems appropriate for the experimental design (Groffman et al., 2006). The PVC tubes, 5 cm in diameter and 100 cm in height, were inserted 20 cm deep into ground at each sub-plot. Acetylene gas was injected into the chambers until 10% (v/v) of the headspace of the chamber was occupied by the gas. Then, headspace gas samples were collected every 20 min for 2 h. Accumulated nitrous oxide concentrations in the gas samples were analyzed using a gas chromatograph (Hewlett Packard 5890). For calculating the denitrification flux, only the slope which showed a linear increase in nitrous oxide concentrations with time was selected (Song et al., 2010; Ye et al., 2015). At each sub-plot, five top soil samples (0–20 cm) were collected and the samples were bulked to form a composite sample. A total of 27 soil samples were kept in an icebox when transporting to laboratory and preserved at −80 °C before DNA extraction. The roots were collected using a root corer with a