Soil Biology & Biochemistry 115 (2017) 137–147

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Hydrologic pulsing affects denitrification rates and denitrifier communities in a revegetated riparian ecotone

Chen Ye*, Xiaoli Cheng, Kerong Zhang, Ming Du, Quanfa Zhang*

Key Laboratory of Aquatic Botany and Watershed Ecology, Wuhan Botanical Garden, Chinese Academy of Sciences, Wuhan 430074, China

ARTICLE INFO

Article history:
Received 1 May 2017
Received in revised form
15 July 2017
Accepted 18 August 2017
Available online 24 August 2017

Keywords:
Hydrologic pulsing
Denitrification
Denitrifier community structure
Revegetation
Riparian zone

ABSTRACT

Hydrologic pulsing and revegetation strongly affect both denitrification rate and denitrifier communities in riparian zones, yet how they drive the shifts in the denitrifying bacterial community and consequently alter denitrification remains unclear. In this study, we investigated denitrification rate and denitrifier community structure and their abundance in different hydrological pulsing phases and vegetation types (tree, shrub, and herb) in the water-level-fluctuate-zone of the Three Gorges Reservoir, China. Results revealed that hydrologic pulsing greatly affected denitrification rate possibly by regulating the soil NO_3^--N concentration, with higher denitrification rate in the reflooding phase in tree soils than that in the saturated and drying phases. Hydrologic pulsing also significantly regulated the abundance and structure of denitrifier communities in herb soils by altering soil temperature, moisture, and NH_4^+-N. As expected, vegetation types affected the denitrifier communities by altering soil texture, pH, temperature, moisture, soil organic C, and C:N ratio, with higher abundance of *nirS* genes and higher diversity of *nirS* and *nirK* genes in herb soils. However, there was no significant relationship between denitrification rate and denitrifier communities, indicating that hydrologic pulsing regulated denitrification rate primarily by changing soil environmental factors rather than community composition or abundance of denitrifiers. Our results suggest that tree and herb plantations would potentially improve water quality in the riparian zone and adjacent rivers by increasing denitrification rate and abundance of denitrifiers, respectively.

1. Introduction

Hydrology is the most influential factor regulating the structures and functions of riparian zones (Mitsch and Gosselink, 2007). Hydrologic pulses have become more frequent and severe in riparian ecotones owing to uneven precipitation (Peralta et al., 2013), including four hydrological phases of a drying-rewetting cycle, i.e., inundated, saturated, drying, and reflooding (Song et al., 2010). Recently, increasing attention has been paid to the effects of hydrologic pulsing on riparian zones in terms of their biogeochemical processes and microbial communities (Song et al., 2010; Peralta et al., 2013; Chen et al., 2014).

Hydrological changes have a considerable effect on soil denitrification, one of the most important processes occurring in riparian zones (Song et al., 2010; Ye et al., 2015). Successive drying and flooding frequently result in observable changes in soil physics and chemistry and thus regulate soil denitrification (Cheng et al., 2007). In addition, vegetation types affect soil denitrification by changing soil properties, especially the input of organic matter to soil (Tall et al., 2011; Audet et al., 2014; Xiong et al., 2015). Although several studies have investigated the responses of denitrification to drying and rewetting cycles (Song et al., 2010; Chen et al., 2014), or to vegetation types (Cheng et al., 2010; Ye et al., 2015), how denitrification rate responds to hydrologic pulses under different vegetation types in riparian zones remain poorly understood.

Denitrification is generally mediated by denitrifier communities under anaerobic conditions (Philippot and Hallin, 2005); hence, the altered denitrification rate under hydrologic pulsing could be associated with changes in the denitrifier community structure (Chen et al., 2014). The structure and function of microbial communities are likely controlled by both transient proximal regulators (e.g., temperature, moisture, pH, soil organic C and N availability) and distal regulators (e.g., environmental disturbances) (Wallenstein et al., 2006; Morales et al., 2015). Hydrologic pulsing, acting as a physical stress and disturbance (Gordon et al., 2008),

* Corresponding authors.
E-mail addresses: yechen922@wbgcas.cn (C. Ye), qzhang@wbgcas.cn (Q. Zhang).

http://dx.doi.org/10.1016/j.soilbio.2017.08.018
0038-0717/

likely results in a drastic change in the composition and abundance of denitrifiers, which may cause subsequent changes in denitrification rate (Knorr et al., 2008). However, the role of the microbial community structure in regulating denitrification remains debatable, with several studies reporting weak or non-significant relationships between microbial community structure characteristics and denitrification rate (Dandie et al., 2011; Wu et al., 2012; Deslippe et al., 2014).

The denitrification process is mainly catalyzed by NO_2^- reductase, encoded by *nir* genes (*nirK* targets copper nitrite reductase and *nirS* targets cd1 nitrite reductase) (Zumft, 1997; Philippot, 2002; Ellen et al., 2006). The *nirS* and *nirK* genes are unique to denitrifiers and universal and thus are widely used as molecular markers in studies on denitrifier communities (Henry et al., 2004; Deslippe et al., 2014). Likewise, Song et al. (2010) reported that different types of riparian zones may exhibit diverse responses to hydrologic pulsing in terms of the structure and abundance of functional genes.

After the completion of the Three Gorges Dam (TGD) in 2008, water level fluctuated from 145 m above sea level in summer to 175 m in winter, and a total area of 350 km^2 water-level fluctuation zone (WLFZ) was formed in the Three Gorges Reservoir (TGR) (Zhong and Qi, 2008). The reversal of submergence time and prolonged inundation result in various ecological implications including losses of previous vegetation (New and Xie, 2008; Ye et al., 2015). Revegetation has been carried out at the Zhongxian Revegetation Station in the WLFZ of the TGR for 9 years to restore and protect the riparian ecosystem (Ye et al., 2012). The effects of vegetation on soil properties, soil N transformation including mineralization, nitrification, and denitrification, plant N uptake, N leaching, and denitrifier communities were investigated at the revegetation station from 2008 (Ye et al., 2012, 2015; 2017). The present study on the effects of hydrologic pulsing on denitrification and denitrifier communities under different vegetation types in the same site is a follow-up to those investigations.

Considering previous reports of variations in vegetation attributes, and differences in soil properties under different vegetation types likely to occur at our study site, we hypothesized that: 1) there are significant differences in the structure and abundance of denitrifier communities, denitrification rate among phases of hydrologic pulsing, and vegetation types; 2) the associated changes in environmental factors would determine soil denitrification rate and *nirS* and *nirK* communities; and 3) the structure and abundance of denitrifier communities are related to denitrification rate.

2. Materials and methods

2.1. Site description and experimental design

This study was conducted at the Zhongxian Revegetation Station (30°26′ N 108°11′ E) in Chongqing, located at the TGR, China (Fig. 1), which was described in detail by Ye et al. (2012). In brief, revegetation has been carried out to restore the riparian ecosystem along the elevation from 155 m to 175 m since March 2008. The dominant herb species include *Cynodon dactylon* and *Hemarthriasibirica* at elevations of 155–165 m; shrub species include *Hibiscus syriacus*, *Morus alba,* and *Salix variegate* at elevations of165–172 m; and tree species comprised *Salix chaenomeloides* and *Taxodium distichum* at elevations of 172–175 m (Ye et al., 2012). The region has a subtropical monsoon climate, with an annual mean temperature of 16.5–19 °C and monthly average temperatures of 3.4–7.2 °C in January and 28–30 °C in July. It receives an average annual rainfall of 886–1614 mm, 80% of which falls between April and October (Ye et al., 2011, 2015). The study region has purple soil (Regosols in FAO taxonomy, Entisol in USDA taxonomy) and water levels fluctuate from 145 m in summer to 175 m in winter.

To investigate the interaction of vegetation types and hydrologic pulses in the riparian ecosystem, the prescribed hydrologic pulsing experiment was established at six replicate plots (5 m × 5 m, 25 cm deep) under each vegetation type (herb, shrub, and tree) in June 2015, when the research site was exposed to the air after flooding. We analyzed soil properties, denitrification rate, and denitrifier communities during three different hydrological phases of a drying and rewetting cycle in each plot: saturated, drying, and reflooding. In early June 2015, after inundation of the reservoir, surface water level decreased naturally due to low precipitation for 1 week (from June 2 to 8, 2015); this was considered the saturated phase. Then, the water level continued to decline because of the high temperature and low rainfall for 2 weeks (from June 9 to 22, 2015), and all plots dried-up; this is the drying phase. After 2 weeks of drying, water from the river was pumped into the plots for the reflooding phase. It took 3 days to flood the plots and water level was maintained to approximately 5 cm above the soil for a week from June 26 to July 2, 2015.

2.2. Field measurements and sample collection

Field surveys were conducted in June 2015, when the sampling sites were exposed to air after flooding. The plant attributes for each experimental plot were investigated. 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). The aboveground biomass was obtained by harvesting all plants to the soil level. The roots were collected using a root corer with a diameter of 16 cm and length of 20 cm. Roots were washed by hand over a 0.5 mm sieve (Hefting et al., 2005).

Sampling was conducted on June 7, June 21, and July 1, 2015, for saturated, drying, and reflooding phases, respectively. For each hydrological phase, the net denitrification rate was measured using the *in situ* acetylene blocking technique (Tiedje et al., 1989). In each plot, three PVC tubes (5-cm diameter and 100-cm height) were inserted 20 cm deep into the 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. Thereafter, headspace gas samples were collected every 20 min for 2 h, to analyze the concentration of accumulated nitrous oxide using a gas chromatograph (Hewlett Packard 5890). For calculating the denitrification flux, only the slope that showed a linear increase in nitrous oxide concentrations with time was selected (Song et al., 2010; Ye et al., 2015). At each plot, three sub-plots (each 1 m × 1 m) of top soil samples (0–20 cm) were randomly selected. A total of 54 soil samples for each hydrological phase were kept in an icebox when transporting to the laboratory. Soil samples for molecular analysis were preserved at −80 °C.

2.3. Analysis of basic soil properties

Methods for analyzing soil physicochemical characteristics are described in detail by Ye et al. (2017). In brief, soil moisture and bulk density were determined gravimetrically by weighing fresh soils, oven-drying intact soil cores at 105 °C for 24 h, and reweighing. Soil pH was measured using a soil-to-water ratio of 2:1 (by weight) with Fisher Scientific AR15 (Waltham, MA) pH probe. Soil temperature was measured using a temperature probe within the 0–20 cm layer. Soil organic C, total N, and total C and N concentrations of roots were quantified by a C/N Analyzer (Flash, EA, 1112 Series, Italy). For NH_4^+-N and NO_3^--N analysis, soils were extracted with 2 M KCl (1:10 soil: extraction ratio) for 1 h and the extracts