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Effects of soil chemical characteristics and water regime on denitrification genes (*nirS*, *nirK*, and *nosZ*) abundances in a created riverine wetland complex

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ABSTRACT

The impacts of site-specific characteristics, such as wetland type, water regime, and soil chemical parameters, on the abundance of denitrifying genes encoding nitrite (*nirS* and *nirK*) and nitrous oxide (*nosZ*) reductases, and their proportions in a bacterial community were investigated in a river diversion wetland complex using quantitative real time polymerase chain reaction. The water regimes of the wetlands had the greatest effect on all denitrification gene parameter values, except *nirK* abundance. While the proportion of *nirS* genes dominated other targeted genes, and their abundance and proportion in bacterial community was highest in constantly flooded areas, *nosZ* and *nirK* gene proportions were highest in occasionally flooded areas.

Environmental factors had different effects on the abundances and proportions of *nirS*, *nirK*, and *nosZ* genes. The abundance of the *nirS* gene and ratio of *nirK* and *nirS* genes were affected by soil pH, and *nirK* gene proportions in the bacterial community were related to the NO_3-N concentration in soil; however, the nature of these relationships varied in different wetlands and transitional areas, respectively. This finding suggests that microbes related to denitrification in soils of different wetland types do not respond similarly to the same environmental variables.

1. Introduction

From the time of the industrial revolution, humans have interrupted the natural nitrogen cycle through the intensive use of inorganic fertilizers, the cultivation of N_2-fixing plants on a large scale, and the burning of fossil fuels to satisfy the continuously rising global demands for food and energy (Vitousek et al., 1997; Gruber and Galloway, 2008). From 1960 to 2000 global nitrogen fertilizer consumption increased by 800% (Fixen and West, 2002). One of the negative effects of the intensified use of fertilizers is an increase in coastal eutrophication and, consequently, the formation of temporary or persistent zones of hypoxia (dead zones) that affect more than 245,000 km^2 of marine ecosystems (Diaz and Rosenberg, 2008). To address some of these problems, wetlands have been used worldwide for the removal of excessive nitrogen from polluted surface and subsurface waters (Jordan et al., 2011).

The removal or reduction of nitrogen compounds in water flowing through wetlands has been attributed mainly to denitrification, the process by which nitrogen compounds are converted to nitrogen gas and returned to the atmosphere. Bacterial denitrification is a facultative anaerobic respiratory process that consists of sequential reaction steps in which nitrate and nitrite are reduced to nitric and nitrous oxides, and finally to nitrogen gas (N_2) via four enzymatic complexes (Zumft, 1997). Nitrite reductases (cytochrome cd_1 encoded by the *nirS* gene and a Cu-containing enzyme encoded by the *nirK* gene) reduce nitrite (NO_2) to nitric oxide (NO). Presumably, most denitrifiers possess a complete denitrification pathway that also includes a nitrous oxide reductase encoding gene (*nosZ*), which catalyses the final step, the reduction of N_2O to N_2. Recent phylogenetic analysis of the *nosZ* gene revealed two distinct clades, which mainly differ in the translocation pathway of nitrous oxide reductase across the membrane, and were almost equally represented in free-water treatment wetland and fen soils (Jones et al., 2013). However, in approximately one-third of the denitrifying bacteria, this gene is missing and the process ends with the reduction of NO to N_2O (Jones et al., 2008), a greenhouse gas contributing to climate

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change (IPCC, 2007) and acts as a catalyst in the destruction of the stratospheric ozone layer (Ravishankara et al., 2009).

For many years, the main focus in wetland research has been on relationships between environmental factors and denitrification-related gas emissions (Teiter and Mander, 2005; Hernandez and Mitsch, 2006, 2007) or potential denitrification activity (Kjellin et al., 2007; Gardner and White, 2010; Fellows et al., 2011). Vymazal (2007) and Faulwetter et al. (2009) have shown that denitrification in wetlands is controlled by oxygen and nitrate content; organic matter quantity, quality, and availability; redox potential; temperature; pH; and soil type. However, more recent molecular-based studies have yielded contradicting results, demonstrating the lack of clear understanding which environmental factors are affecting the denitrification genetic potential (Philippot et al., 2009; Bárta et al., 2010; Enwall et al., 2010; Dandie et al., 2011; García-Lledó et al., 2011).

In addition to physicochemical parameters, hydrological factors such as flooding patterns (Hernandez and Mitsch, 2006, 2007), the extent of soil water saturation (Bateman and Baggs, 2005), and water residence time (Kjellin et al., 2007) are other important factors affecting denitrification. The hydrological flow determines the oxygen availability and the exchange of nutrients (organic carbon, nitrate, etc.) between water and sediments affecting the overall rate of denitrification in systems with fluctuating water regime (Baldwin and Mitchell, 2000; Rassam et al., 2006). Although a number of studies have focused on the impact of changes on water regimes (pulsing floods) on denitrification rates (Hernandez and Mitsch, 2006, 2007; Song et al., 2010; Fellows et al., 2011; Batson et al., 2012), the mechanism behind this is still not completely understood.

The United States Midwest is one of the most productive agricultural regions in the world, but excessive nutrient use has caused several environmental problems in the Gulf of Mexico (Mitsch and Day, 2006). In order to reduce the excessive nutrient concentrations in waterbodies, a whole ecosystem wetland experiment has been undertaken at the Wilma H. Schiermeier Olentangy River Wetland Research Park (ORWRP) in OH (USA) to investigate the treatment potential of managed river diversion systems. Of the many studies conducted in the ORWRP (Mitsch et al., 2012), only those of Song et al. (2010, 2012) focused on microbial levels, evaluating the relationship between environmental factors and *nirS* gene abundance and diversity.

The aim of the current study was to evaluate the effects of site-specific characteristics such as soil chemical parameters, water regimes, and wetland type on the bacterial community and its denitrification potential using the abundances of denitrifying genes (*nirS*, *nirK*, and *nosZ*) in the soil and sediments of the ORWRP river diversion wetland complex.

2. Materials and methods

2.1. Study site description

This study was carried out in the ORWRP complex located adjacent to the campus of Ohio State University in Columbus, USA (lat 40.021° N, long 83.017° E). The ORWRP includes three different wetland basins: two 1 ha kidney-shaped freshwater marshes (W1 and W2) and a 3 ha river diversion wetland (oxbow) on the floodplain of the Olentangy River (Fig. 1).

The freshwater marshes consist of deepwater basins surrounded by shallower marsh areas which were either planted (W1) or colonized naturally (W2) (Mitsch et al., 1998). For the last 15 years water from the Olentangy River has been continuously pumped into these wetlands using a formula that relates pumping rates to river stages (on average, 626–1552 m^3/d) (Mitsch et al., 2012). For the first 10 years, the water depth in the deep water basins and shallow transitional marsh areas generally fluctuated between 50–80 cm and 20–40 cm, respectively (Mitsch et al., 2005). Currently, water depths range from 30 to 60 cm in the deep water basins and from 0 to 30 cm in the shallow marsh areas (Batson et al., 2012).

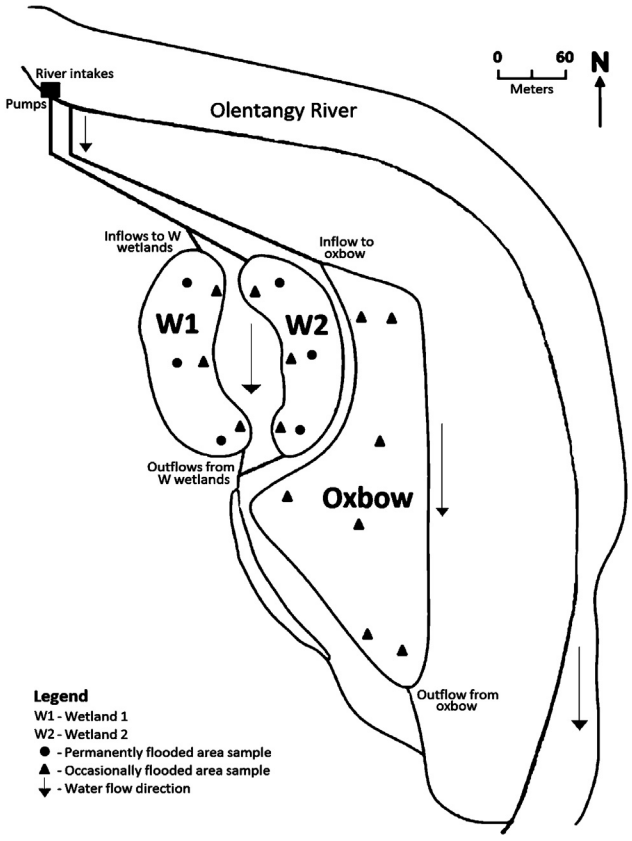

Fig. 1. Sampling sites in the Olentangy River Wetland Research Park study area.

The oxbow wetland, planted with 21 different plant species (Fink and Mitsch, 2007), receives water through a check valve if the water level in the river is higher than in the wetland. Water from the oxbow flows back into the Olentangy River by gravity through an outflow control weir (about 300 m from the inflow). When the river elevation is lower than the wetland, the check valve closes, keeping water from spilling back to the river (Mitsch and Day, 2006). On average, the oxbow wetland receives 7–8 natural flood pulses per year (Fink and Mitsch, 2007).

2.2. Soil sampling

In March 2009, 19 samples (approximately 500 g each) were collected in one replicate from the topsoil layer (0–15 cm) of the W1, W2, and oxbow wetlands (Fig. 1). Samples from two zones of the W1 and W2 wetlands were taken along longitudinal gradients from inflow to outflow: three samples per wetland were taken from the shallow occasionally flooded transition edges and three per wetland from the permanently flooded open water zones. Seven samples were taken from the oxbow, also following the longitudinal gradient from inflow to outflow. Samples were placed in airtight plastic bags and transported to the laboratory on ice (within three days). Subsamples for chemical and molecular analyses were stored at +4 °C and −20 °C, respectively. Chemical analyses of the soil samples were performed within one week after the sampling.