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Long-term denitrification rates in created riverine wetlands and their relationship with environmental factors

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ABSTRACT

Created wetlands that mimic structure and functions of natural wetlands have been widely applied mainly as a means for water quality improvement. Denitrification is a process that is considered to be important, especially in wetlands with their anaerobic soils, for the removal of nitrogen. In this study, we assessed the temporal and spatial patterns of denitrification from created wetlands over a relatively long time scale of 6 years. We found that denitrification rates were highly variable, ranging from 2.1 to 5288 $\mu g\,m^{-2}\,h^{-1}$ over that 6-year study period. Overall denitrification followed expected seasonal patterns, depending on temperature and nitrate concentrations in the surface water. We also found higher denitrification in open water and deep marsh areas than in shallow and wetland edge areas, likely the result of oxygen depletion in deeper water areas. Denitrification was also regulated by hydrologic regime. The highest mean denitrification rate ($506\,\mu g\,m^{-2}\,h^{-1}$) was observed in 2004 when the wetlands received artificial hydrologic pulses and the lowest rates ($217\,\mu g\,m^{-2}\,h^{-1}$) were observed in 2008, when wetlands were drier than in other years. The presence/absence of vegetation had little influence of spatial and seasonal variations of denitrification. However, annual variations of denitrification tended to be related to vegetation productivity as indicated by aboveground net primary productivity. This result suggests that denitrification rates may have been suppressed by plants creating aerobic condition in their rhizospheres. The high spatio-temporal patterns of denitrification seen in these created wetlands were caused mainly by a complex interaction of hydrology, nitrate-nitrogen concentrations and temperature.

1. Introduction

Nitrogen, the most prominent non-point pollutant, is discharged from agricultural and urban areas and causes eutrophication in aquatic ecosystems, particularly in coastal waters. Given that wetlands are known as significant sinks of nitrogen, particularly nitrate-nitrogen by denitrification and vegetation uptake (Gold et al., 1998; Mitsch and Gosselink, 2007), the creation and restoration of wetlands have been attempted many times to improve water quality (Mitsch et al., 2001, 2005).

Created wetlands are self-designing ecosystems which can maintain functions naturally by using natural energy sources, such as sunlight and nutrient inflow (Mitsch and Jørgensen, 2004). This self-designing nature of created wetlands lessens the cost and efforts for wetland operation and management, but also allows temporal variations of environmental conditions over the longer term as the ecosystems self-design. These changes in environmental conditions of created wetlands over time further lead to high temporal variability in biogeochemical process rates such as denitrification in wetlands. However, long-term biogeochemical process rates and their relationship with environmental changes in created wetlands are poorly observed and understood in most wetland restoration and creation cases.

Denitrification is a microbial process that removes nitrate nitrogen from wetlands by transforming into the gasses dinitrogen and nitrous oxide (Martin et al., 1999; Mitsch and Gosselink, 2007). This process is considered as a main N removal mechanism in wetlands, particularly in created wetlands for wastewater treatment where often over 70% of total nitrogen removal efficiency have been obtained (Kadlec and Knight, 1996). Various factors such as temperature, nitrate concentration, and organic matter content and quality affect the denitrification process, and vegetation

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community, growth rates, or soil texture and chemistry are known as distal regulators (Paul and Clark, 1996). Vegetation could enhance denitrification rates by providing organic carbon and microbial habitat (Brix, 1997). Plant uptake itself, on the other hand, is considered only short-term nitrogen storage since inorganic nitrogen can be released back to the system by decomposition (Philippot and Hallin, 2005; Reddy et al., 1999), although plant uptake accounted for 16 to 75% of total nitrogen removal in wetlands (Reddy and DeBusk, 1987).

Seasonal variability of denitrification rates, with high rates in summer and low rates in winter, are mainly due to temperature dependency of denitrification process (e.g. Bachand and Horne, 2000; Hernandez and Mitsch, 2007; Song et al., 2012). The changes in nitrate concentration at inflows, associated with fertilizer usage and precipitation patterns, also caused temporal variation of denitrification rates (Hernandez and Mitsch, 2007). In the longer term, the changes in organic matter contents as a created wetland develops, or the changes in hydrologic regime modify the denitrification rates. For example, the organic matter and carbon content increased dramatically over 15-year operation of created wetlands (Mitsch et al., 2012) and aboveground live plant and litter masses were associated with the age of restored wetland (Ballantine and Schneider, 2009). These increases of organic carbon and litter materials in wetlands over time can stimulate denitrification rates. Denitrification rates are variable as such in both short-term (seasonal) and longer term (annual) due to complex regulators of denitrification and the environmental heterogeneity of wetlands (Groffman et al., 2009). This high variability of denitrification rates may result in changes in nitrogen removal efficiency in wetlands over time. Despite increases in wetland creation and the importance of denitrification process as a nitrogen removal mechanism, how denitrification rates changes over time, especially during long term operational periods of created wetlands, have been of little focus.

In this study, we aimed to understand long-term, seasonal and annual denitrification rates in created wetlands and their regulating factors. For this, we measured in situ denitrification rates and environmental parameters using same methods and in the same locations over 6 years. Along with temporal pattern in denitrification rates, we also hypothesized that the transverse gradient in wetlands associated with water level and presence and absence of vegetation may differentiate denitrification rates.

2. Materials and methods

2.1. Site description

This study was conducted at the Wilma H. Schiermeier Olentangy River Wetland Research Park (ORWRP) located in Columbus, OH, USA. Two 1-ha wetlands were created in 1993–1994. The nearby third-order Olentangy River water was pumped through these two experimental wetlands continuously, and inflow water flow to each wetland was identical for both basins. The hydroperiods of wetlands were variable depending on river flow and precipitation patterns, resulting in seasonal water level fluctuation with high levels in the wet season (December–June) and low in the dry season (July–November) (Mitsch et al., 2012). The bottom of both wetlands is shaped transversely deep in the center with no vegetation present (refer to as open water area hereafter) and surrounded by shallower parts dominated by emergent plants (marsh areas). Marsh areas again were separated to deeper (deepwater marsh) and shallower (shallow marsh) areas by difference of water depth, in this study.

2.2. Sampling and measurements

Water samples were collected weekly from inflow and outflow areas in both wetlands in 2004, 2005, 2008 and 2009. Nitrate concentrations were analyzed by sulfanilamide colorimetric method (USEPA, 1983) using QuickChem Autoanalyzer (Lachat, Lachat QuickChem Autoanalyzer IV). Aboveground net primary productivity (ANPP) was estimated using aboveground biomass measured in the middle of August each year. Aboveground biomass from 16 quadrats (1 m × 1 m) in wetlands was harvested and peak biomass as ANPP ($g\,m^{-2}\,yr^{-1}$) were estimated (Mitsch et al., 2012). Water temperature and water depth was measured in locations during each time of denitrification measurement.

2.3. Denitrification and denitrifying enzyme activity measurement

Denitrification was measured at four types of communities in the experimental wetlands: open water areas in the center of wetlands, deepwater marshes, shallow marshes and edge areas. The edge includes seasonally flooded areas that are characterized as a boundary between the wetland and upland. Measurements were conducted monthly from all of these communities in 2004 and 2005, while edge areas were excluded in 2008 and deep marsh areas in 2009.

Denitrification was measured using the in situ acetylene blocking method (Tiedje, 1982; Tiedje et al., 1989). To minimize the diurnal temperature effect on in situ measurements, all samples were taken between 11:00 am and 4:00 pm. Duplicated 4-cm diameter and 80-cm high PCV chambers were inserted 10 cm deep into the sediment at each sampling site 24 h prior to the sampling in each location. Acetylene gas was injected into the chambers until it took 10% (v/v) of headspace volume. When the sampling area was inundated as in most of the open water some vegetated areas, acetylene gas was injected into the sediment through 1-cm diameter tube connected to the gas cylinder. Headspace gas samples were then collected 30 min after the acetylene addition every 20 min for 2 h. Nitrous oxide produced by denitrification accumulated in the headspace was analyzed using a gas chromatograph equipped with electron capture detector (Shimazu, GC-14A) and Porapak-Q columns. Dissolved nitrous oxide content in overlying water was estimated based on its solubility (Weiss and Price, 1980). The slope of the changes in nitrous oxide concentration is used to estimate the denitrification rate.

Denitrifying enzyme activity measurements were conducted twice: June 2005 and August 2008. For denitrifying enzyme activity, same acetylene blocking method was used in sampled sediments in the lab after adding sufficient substrates. Ten to 15 g of fresh sediment samples from each sampling location were added into oxygen-free vials and amended with 200 ml of (1) glucose, (2) nitrate-nitrogen and (3) combined substrates (Hernandez and Mitsch, 2007). In order to prevent microbial growth during the analysis, 0.01 g of chloramphenicol was added in each vial. Ten percent of the headspace in the vials was replaced with acetylene gas and the slurries were incubated at room temperature. Headspace samples were collected every 6 h until the linearity of accumulated nitrous oxide contents lasted (ca. for 30 h). Nitrous oxide concentration analysis and the rate calculation were conducted in the same way as denitrification measurements.

2.4. Data analysis

Friedman Repeated Measures ANOVA and separate Bonferroni post hoc analyses were applied to compare denitrification between years and locations. Replication in each sampling location was