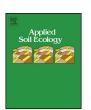
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Multistate assessment of wetland restoration on CO₂ and N₂O emissions and soil bacterial communities



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ABSTRACT

Over the last 200 years, wetlands have been converted to other land uses leading to the loss of approximately 53% of wetlands in the continental United States. In the late 1980's, policies were instated to mitigate further wetland loss through wetland creation and restoration. Restored wetlands provide important ecosystem services, such as filtration of nutrients and wildlife habitat. However, these benefits could be offset by increased greenhouse gas production. We assessed the impact of wetland conversion to agriculture and restoration on CO₂ and N₂O emissions and microbial communities in three land use types: wetlands with native vegetation (natural); wetlands converted to agricultural management (converted); and restored wetlands (restored). Soil properties varied among land use types. Most notably, soils from restored and converted sites had the lowest C and N, and higher pH. Multivariate analysis of soil properties showed the pocosin wetlands in North Carolina separating from all other locations, regardless of land use. Soil bacterial communities showed a similar trend with communities from North Carolina soils separating from the others with no significant effect of land use or season. Furthermore, land use did not have a significant effect on CO₂ or N₂O emissions, although there was significant temporal variation in CO₂ emissions. These findings indicate that while wetland conversion and restoration may alter some soil properties, these alterations do not appear to be great enough to override the underlying geographic and edaphic influences on soil bacterial communities. Furthermore, wetland restoration did not lead to increased N2O emission at the dates sampled.

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1. Introduction

Wetlands across the United States have been drained to create arable land suitable for agriculture and timber production (Brinson and Eckles, 2011; DeSteven and Lowrance, 2011). Urban and rural development have exacerbated this phenomenon, replacing agriculture as the main driver of wetland loss (DeSteven and Lowrance, 2011). In the late 1980's, concern over the dramatic loss of wetlands (Dahl, 1990) and increased recognition of ecosystem services provided by wetlands led to a "no net loss" policy (Bendor, 2009; Mitsch, 1992). Since this time, restoration of drained wetlands has increased, and in 2003 the United States Department of Agriculture (USDA) Conservation Effects Assessment Project (CEAP) began examining the benefits of wetland conservation practices across the U.S. (Brinson and Eckles, 2011).

Wetland restoration aims to restore ecosystem services including the filtration and retention of nutrients, water storage, and

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wildlife habitat (Brinson and Eckles, 2011; DeSteven and Lowrance, 2011; Mitsch and Gosselink, 1993). However, the success of hydraulic restoration can vary (Zedler, 2003). In part, success depends on the intent behind the restoration; wetlands that provide the best habitat and biodiversity may not be as effective at nutrient retention (Hansson et al., 2005; Zedler, 2000). While some studies have shown that restored wetlands can reduce nutrient runoff (Ardón et al., 2010; Jordan et al., 2003; Tanner et al., 2005), others have found that hydraulic restoration can result in the release of nutrients (Steinman and Ogdahl, 2011). Furthermore, like natural wetlands, anoxic conditions in restored wetlands have the potential to produce significant amounts of N_2O when N availability is high, such as in agriculturally impacted areas (Verhoeven et al., 2006).

As mentioned, a common concern with wetland restoration is the potential for increased greenhouse gas emissions, (Brinson and Eckles, 2011; Verhoeven et al., 2006). Increased CO₂ emissions is generally not a concern with wetland restoration (Whiting and Chanton, 2001) as the typically high water content and low O₂ reduces aerobic respiration and thus the production of CO₂. However, ephemeral wetlands that are not permanently saturated may not have conditions leading to a reduction in CO₂ emissions, as

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Morse et al. (2012a) found in examining North Carolina pocosin wetlands. However, these conditions create the potential for increased N2O emissions through two microbially mediated Ncycling processes. The first is nitrification, which results in the oxidation of ammonia (NH₄⁺) to nitrite (NO₂⁻). The second is denitrification, the process of converting nitrate (NO₃⁻) to gaseous nitrogen (N₂). When denitrification is not carried to completion, N₂O can result as a byproduct, particularly when soils are not completely anoxic (Myrold, 1999). Due to its high reactivity and long residency time, a relatively small increase in N2O flux can result in significant greenhouse effect (Schlesinger, 1991). While denitrification in soil is dependent on many factors (Hunter and Faulkner, 2001; Morse et al., 2012b), Peralta and colleagues (2010) reported that restored wetlands had lower denitrification activity than natural wetlands. This finding suggests that wetland restoration may not result in increased N₂O emissions from denitrification. However, a recent study using ¹⁵N tracers demonstrated that in soils with high organic matter and moisture content, nitrification is responsible for a significant portion of N2O emissions (Morse and Bernhardt, 2013). These findings highlight the complexities in predicting N₂O emissions and the need for direct in situ monitoring of greenhouse gases when evaluating the environmental impact of wetland

Removal of nitrogen (N) is biologically driven; therefore, it is important to monitor and evaluate microbial communities when N removal is a restoration goal. Previous findings have shown that soil bacterial communities in wetlands were correlated with pH and land use (Hartman et al., 2008). More recently, Peralta et al. (2010) found that bacterial communities correlated with a number of soil properties, including soil moisture, organic matter, and pH. Due in part to their agricultural legacies, restored wetlands usually have lower carbon (C) content and higher pH compared to natural wetlands (Bruland et al., 2003; Hogan et al., 2004; Morse and Bernhardt, 2013). Because both C and pH can influence microbial communities (Lauber et al., 2009; Lauber et al., 2008), it is not surprising that although hydraulic regimes can be restored almost immediately, biogeochemical functions of restored wetlands can take longer to re-establish (Bendor, 2009; Bruland et al., 2003).

The wetland component of the United States Department of Agriculture (USDA) Conservation Effects Assessment Project (CEAP-Wetland) is being carried out across the United States. In the Mid-Atlantic Region (the area between New England and North Carolina), the USDA CEAP-Wetland study is designed to investigate the effects and effectiveness of wetland conservation practices, with a focus on the provision of ecosystem services. As part of this interdisciplinary effort, our study was designed to test the hypotheses that (1) the hydraulic restoration of converted wetlands will alter soil chemical and physical properties, such that these restored wetlands will begin to approximate their natural counterparts and (2) the effects of hydraulic restoration on soil properties will likewise result in altered structure and function of soil bacterial communities. We achieved the objectives of this study by examining soil properties in natural, converted, and restored wetlands. Additionally, deep 16S sequencing was used to examine the structure of bacterial communities and photoacoustic gas analysis to measure CO₂ and N₂O emissions.

2. Materials and methods

2.1. Site description and study design

As part of the Mid-Atlantic Region CEAP-Wetland project, this study examined three land use types throughout the Mid-Atlantic region (Delaware, Maryland, Virginia, and North Carolina).

- Natural-forested wetlands with native vegetation and no history of drainage.
- Converted-wetlands that were drained and converted to farmland.
- Restored-hydraulically restored wetlands that had been previously drained and converted to farmland.

Restored sites were selected from a list provided by the Natural Resources Conservation Service (NRCS) consisting of farmland restored under Conservation Practice Standard 657 (wetland restoration) or Conservation Practice Standard 646 (shallow water development and management). Detailed information on these conservation practice standards can be found on the NRCS Field Office Technical Guide website (http://www.nrcs.usda.gov/wps/portal/nrcs/main/national/technical/fotg/). After Restored sites were identified, aerial photographs, digital elevation models, and NRCS personnel were used to select corresponding natural and converted sites within 1 to 4km from the restored sites. This study examined twelve MIAR-CEAP sites, including one of each land use type (i.e., natural, restored and converted) in each of four states.

Natural sites in Delaware, Maryland, and Virginia were classified as depressional wetlands (confined to basins or hollows) and were managed by the state or the Nature Conservancy. In North Carolina, pocosin wetlands (acidic, peat soils) within Pocosin Lakes National Wildlife Refuge were used as natural sites. Dominant vegetation in the natural sites included mixed oak, loblolly pine, and sweet gum. Soil series names and soil taxonomy for all sites are presented in Table 1. In all states, Converted sites were drained, filled or leveled prior to 1985 and were typically managed under a corn-wheat-soybean rotation. Hydraulic restoration of the restored sites occurred in 2004 (DE and MD), 2008 (VA), and 2005 (NC). The VA and MD restored sites were replanted, while DE and NC were naturally re-vegetated. Visual inspection prior to site selection insured that restored sites had the appeared similar to the corresponding natural wetlands. The natural and restored wetlands used in this study were seasonally, but not permanently saturated.

2.2. Field sampling

Samples were collected at three time points: spring 2010, fall 2010, and spring 2011. For each site, a digital elevation model in ArcGIS (ESRI, Redlands, CA) and on-site visits were used to identify the lowest wetland topographic position. An approximately 9 m^2 area was randomly selected within the lowest topographic position of each site. At each sampling date, four locations were randomly sampled within this area. One location was used to measure soil surface gas emissions as described below, and the top 10 cm of mineral soil was collected from the three additional locations to create a composite sample for analysis. If present, the organic horizon was removed prior to collecting soil samples. Soils for microbial community analysis were stored at $-80\,^{\circ}\text{C}$, and soils used for pH, C, and N analysis were stored at $4\,^{\circ}\text{C}$ and processed within one week of sampling.

2.3. Soil properties and gas emissions

Soil surface efflux rates were determined using an Innova 1412 photoacoustic gas analyzer, fitted with a 987 optical filter (PAGA; LumaSense Technologies, Santa Clara CA). A static chamber was constructed from a metal beaker with three holes drilled into the base. Two holes were used for inlet and outlet tubes and a rubber stopper was inserted into the third. The chamber was wrapped in an insulating material and covered with aluminium foil to minimize temperature variations and the lip of the beaker was beveled to ease insertion into the soil. For each sampling, the rubber stopper was

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