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Nature versus nurture: Functional assessment of restoration effects on wetland services using Nuclear Magnetic Resonance Spectroscopy

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[1] Land-use change has altered the ability of wetlands to provide vital services such as nutrient retention. While compensatory practices attempt to restore degraded wetlands and their functions, it is difficult to evaluate the recovery of soil biogeochemical functions that are critical for restoration of ecosystem services. Using solution ^{31}P Nuclear Magnetic Resonance Spectroscopy, we examined the chemical forms of phosphorus (P) in soils from wetlands located across a land-use gradient. We report that soil P diversity, a functional attribute, was lowest in farmland, and greatest in native wetlands. Soil P diversity increased with age of restoration, indicating restoration of biogeochemical function. The trend in soil P diversity was similar to documented trends in soil bacterial taxonomic composition but opposite that of soil bacterial diversity at our study sites. These findings provide insights into links between ecosystem structure and function and provide a tool for evaluating the success of ecosystem restoration efforts.
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1. Introduction

[2] Wetlands provide a variety of ecosystem services, including nutrient retention [*Mitsch et al.*, 1995]. However, land-use changes have resulted in marked reductions in their services [*Gleason et al.*, 2008], prompting policies and actions for compensatory mitigation through restoration [*U.S. Army Corps of Engineers*, 2008]. While the goal of ecological restoration is to restore impoverished ecosystems and reestablish functions that prevailed prior to their degradation, evaluating the success of restoration efforts has been challenging [*Zedler and Callaway*, 1999]. For example, from the standpoint of ecosystem restoration, recovery of soil biogeochemical functions such as nutrient cycling are among the least understood, but are critical for the successful return of ecosystem services such as nutrient retention.

[3] Ecosystem restoration has become an increasingly popular concept as society seeks to mitigate the impacts of wetland loss and degradation. To understand how ecosystems respond to natural or induced change it is vital to develop indicators of ecosystem function. One such index is nutrient status. However, simply measuring extractable nutrients is clearly inadequate, given the complexity of nutrient cycling and forms that can be utilized by a diverse biota. In undisturbed ecosystems, nitrogen (N) and carbon (C) levels are adjusted through inputs and outputs to the atmosphere; in contrast, P is retained in the biotic and abiotic pools, with relatively small losses [*Brye et al.*, 2002; *Toor et al.*, 2005; *Richardson*, 1999]. This attribute and the tight coupling of P to C [*Tate and Salcedo*, 1988; *Gressel et al.*, 1996; *Sundareshwar et al.*, 2003] and N cycles [*Sundareshwar et al.*, 2003], as well as the sensitivity of chemical speciation of P to land-use changes [*Rubæk et al.*, 1999; *Sundareshwar et al.*, 2001; *Solomon et al.*, 2002; *Turner et al.*, 2003a; *Cade-Menun*, 2005; *Cade-Menun et al.*, 2006], means that the chemical speciation of P across a restoration gradient can provide insights into the restoration of biogeochemical functions in an ecosystem. Here we show that soil P diversity may serve as a time-integrated index of restored biogeochemical functions and associated ecosystem services in forested and prairie wetlands. Specifically, we found that soil P diversity declined upon conversion of these wetlands to agriculture, but increased with restoration age toward those observed in native ecosystems.

2. Materials and Methods

2.1. Study Sites

[4] We compared the diversity of P forms in wetland soils using solution ^{31}P NMR spectroscopy along a gradient of cultivated, restored and undisturbed native (reference) wetlands in the North Carolina coastal plain and the Northern Plains of South Dakota. Within each region we selected wetlands that had the same soil type but differed in their land-use. The North Carolina sites were located in a 975-hectare Carolina Bay complex on Croatan muck and Ponzer muck soils (Terric Haplosaprists) in Cumberland and Beaufort Counties. Much of the site was cleared and ditched in the 1960s for conversion to agriculture, and farmed intensively during the 1970s and 80s. In 2002, soils were collected from 5 wetlands, of which one was an active farmland, 2 previously drained and farmed wetlands that were restored 5 and 8 years prior to sampling, 2 native forested wetlands with no history of cultivation, however one native wetland was logged 50 years prior to sampling.

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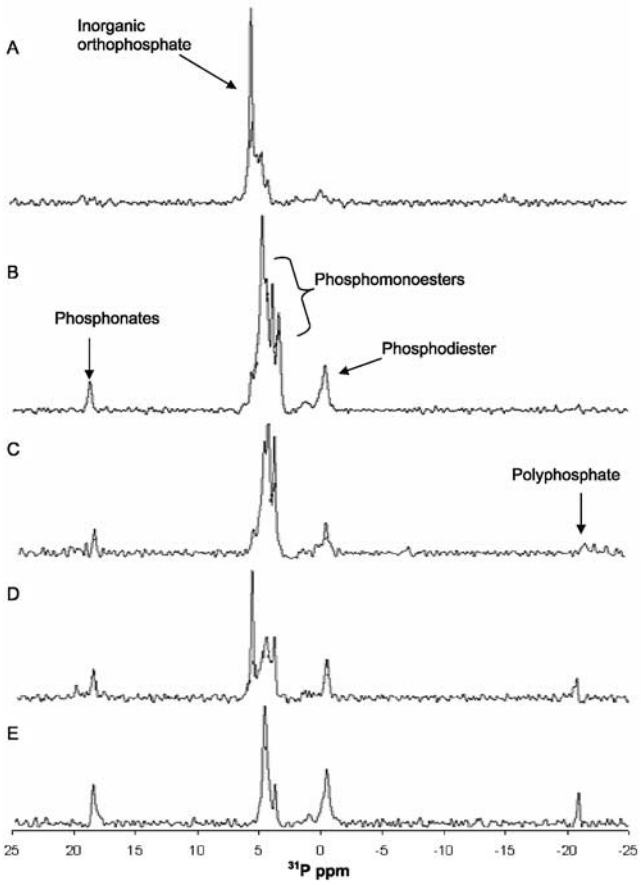

Figure 1. The ^{31}P NMR spectra from NaOH-EDTA extracts of soil samples from wetlands across a network of paired reference and impacted sites in Coastal North Carolina along a land-use gradient. The ^{31}P NMR spectra reveal a link between land-use and chemical diversity. (a) Conventionally tilled agriculture land, (b) and (c) agricultural land under wetland restoration programs for 5 and 8 years, respectively, and (d) and (e) unimpacted Carolina Bay 'reference' wetlands. Restored wetlands show recovery of soil P diversity increasing with age of restoration. The integral values of the peaks contain an estimated 5% uncertainty due to the signal to noise ratio in the samples, but nevertheless demonstrate detectable presence of various P-forms across land use. All spectra are scaled to same vertical magnification.

The three wetlands that were sampled in South Dakota in 2007 were palustrine emergent seasonally-flooded wetlands on Parnell soils (Typic Argiaquolls) under cropland (non-drained), in restoration, and a native prairie. The restored site was a previously-farmed, non-drained wetland that was restored to native grasses in 1991 under the U.S. Department of Agriculture's Conservation Reserve Program (CRP).

2.2. The ^{31}P NMR and Elemental Analyses of Soil Samples

[5] Surface soil from the A horizon were sampled in triplicate from each wetland in North Carolina and in duplicate from each wetland in South Dakota. The replicate soil cores from each wetland were pooled and extracted overnight at room temperature. Extractions were carried out on non-dried samples using a soil:solution ratio that corresponded to approximately 10 g dry sediment:100 ml 0.5 M NaOH + 0.1 M Tetrasodium ethylenediaminetetraacetic acid [EDTA] [*Cade-Menun and Preston*, 1996]. Extraction efficiencies were calculated as a difference in total P between the extracts and sediments samples, and in all cases were greater than 50%. Comparable extraction efficiencies suggest that the relative differences in distribution and abundance of P species at and among sites were not due to extraction artifacts. Data acquisition was performed on un-concentrated extracts after adjusting the pH >12. Solution ^{31}P NMR analyses methods and peak assignments are described by *Sundareshwar et al.* [2001] and *Turner et al.* [2003b]. Total soil P, total C and N were measured in the composite samples used for ^{31}P NMR analyses as described by *Vaithyanathan and Richardson* [1998]. Soil pH were measured in 1:1 soil: water slurries.

3. Results

[6] NMR analyses of soils from North Carolina wetlands revealed that agricultural wetland had the lowest diversity of chemical forms of P (Figure 1a), followed in increasing order by restored (Figures 1b–1c), and native wetlands (Figures 1d–1e). Phosphorus speciation in the agricultural wetland was dominated by inorganic orthophosphate, whereas native wetlands had inorganic orthophosphate, monoester-P, diester-P, polyphosphates and phosphonates. Phosphorus diversity of restored sites was intermediate of that found in native wetlands and farmland. For example, we found phosphonates and diesters in the 5-year-old restored wetland, and polyphosphates in the 8-year-old restored wetland. Soil P diversity appeared to increase with age of restoration and asymptotic profiles approached those observed from native forested wetland (Figures 1b–1e). The trend in restoration of P-forms observed in forested wetlands in North Carolina was also observed in prairie wetlands in South Dakota (Figure S1 and Figure 2).[1]

[7] Besides changes in P forms, restoration also increased the total C, N, and P content of soils. The total soil P for the Carolina Bay sites ranged from 133 mg P/Kg in actively farmed site to 339 mg P/Kg in native wetland, while the 5-yr restored site supported the highest concentration at 800 mg P/Kg. On a volumetric basis, however, the agricultural soils had the highest total P, and the native wetlands had the lowest [*Bruland et al.*, 2003]. Sites also differed in their C and N content with soil carbon increasing linearly from the actively farmed sites to native wetland. Soil C:N ratios progressively declined with decreasing land management and were 27, 24.5, 20, 22.1 and 21.3 for the agriculture, 5-year and 8-year restored sites, and the two native sites, respectively. For the prairie wetlands studied the % C, N and P in soils were 3, 0.2, 0.04; 7, 0.5, 0.07; and 6, 0.5 and 0.06; in active farmland, restored wetland and the native prairie, respectively. Soil pH in Carolina Bay wetlands was 5.97 in active farmland, 4.62 and 4.82 in 5- and 8- year old restored wetlands, and 3.52 and 3.78 in the two reference wetlands, respectively [*Hartman et al.*, 2003]. For the prairie

[1]Auxiliary materials are available in the HTML. doi:10.1029/2008GL036385.

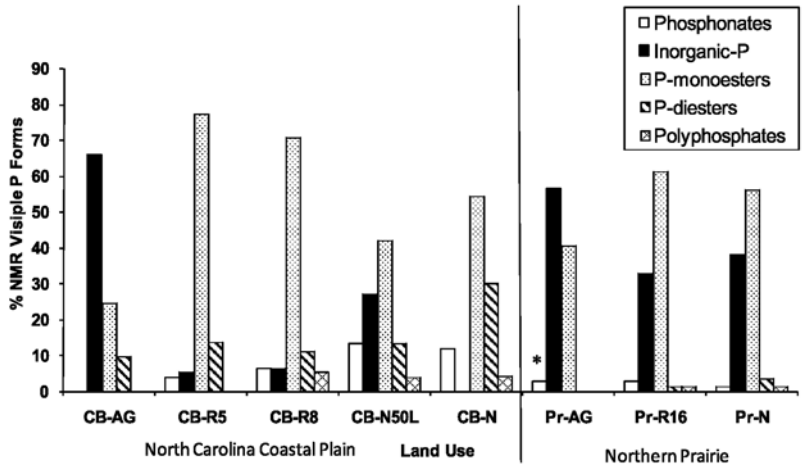

Figure 2. Relative distribution of NMR visible P-forms across land use gradient in wetland complexes from North Carolina coastal plain and the northern plains of South Dakota. Restoration not only decreased the relative contribution of readily available P-forms such as inorganic orthophosphate but also increased the diversity in P-forms in both study sites. However, the magnitude of these changes differed among the wetland complexes. Land use categories include: CB-AG = Carolina Bay-Farmland; CB-R5 = Restored 5 yrs; CB-R8 = Restored 8 yrs; CB-N50L = Native but logged approximately 50 yrs ago; CB-N = Native; Pr-AG = Prairie-Farmland; Pr-R16 = Restored 16 yrs under Conservation CRP; Pr-N = Native. * = Indicates the presence of phosphonate other than the forms present in the restored and native prairie samples. The chemical shift of phosphonates in the Pr-Ag was 18.5 ppm and 20.5 in restored and native prairies (see Figure S1).

wetlands the soil pH were 5.38 in farmland, 5.81 in restored wetlands and 6.77 in native grassland.

4. Discussion

[8] Chemical speciation of P is a function of biogeochemical processes which are affected by land-use. We demonstrated that restoration of biotic and abiotic conditions in wetlands results in a decrease in inorganic orthophosphate and an increase in the abundance of diester and other forms of P (Figure 2). Specifically, the proportion of diester-P in samples from agricultural lands was 65–100% below those observed in reference wetlands, while the restored wetlands showed a 50% recovery in the proportion of diester-P, as has been observed in non-wetland systems [*Rubæk et al.*, 1999; *Solomon et al.*, 2002; *Solomon and Lehman*, 2000]. Whereas P forms such as inorganic orthophosphate or pyrophosphate are readily bioavailable [*Condron et al.*, 1985], other forms such as phosphonates and diesters may be of limited bioavailability as their turnover is controlled by the activity of specific microbial communities [*Kononova and Nesmeyanova*, 2002; *Dyhrman et al.*, 2006], and prevailing environmental conditions such as temperature, precipitation and pH [*Sumann et al.*, 1998; *Hartman et al.*, 2008]. Thus greater P diversity in native ecosystems likely contributes to the measured increase in nutrient retention in undisturbed ecosystems compared to cultivated lands [*Bruland et al.*, 2003]. Our findings thus provide a tool to evaluate the restoration of such ecosystem services.

[9] It is plausible that differences in environmental conditions and microbial communities among cultivated, restored and native wetlands contribute to the observed differences in P forms. For instance, while organic phosphorus compounds (e.g., phosphonates) occur naturally [*Rosenberg*, 1964; *Koukol et al.*, 2008; *Benitez-Nelson et al.*, 2004] and are found in commonly used weed killers, phosphonates were mainly present only in our restored and native wetlands, likely due to biological production outpacing their turnover. Interestingly, for the Carolina Bay wetlands, the trend in soil P diversity reported in this study parallels the trend in soil bacterial taxonomic composition from these sites [*Hartman et al.*, 2008]. The native and restored wetlands shared a more similar soil bacterial community composition and differed from that of the farmland [*Hartman et al.*, 2008] as observed elsewhere [*Potthoff et al.*, 2005]. Importantly, the soil bacterial diversity was greatest in the farmland and lowest in the native sites [*Hartman et al.*, 2008]. This inverse trend in P-forms and microbial diversity implies that soil P diversity is not a function of greater bacterial diversity per se, instead is a result of restoration of key microbial communities leading to recovery of specific biogeochemical functions. While our index of restoration of soil biogeochemical function relates well to trends in soil bacterial taxonomic community composition, it is unclear how it relates to plant diversity and canopy complexity which have been suggested as important attributes for restoring various ecosystem functions [*Zedler et al.*, 2001]. Since soil microbes can be important regulators of plant productivity and diversity [*van der Heijden et al.*, 2008], it could be postulated that P-species diversity likely corresponds to trends in plant community composition as well.

[10] Recent amendments to environmental regulations in the US [*U.S. Army Corps of Engineers*, 2008, 2002] require mitigation to restore ecosystem function. However, there are no reliable ways to account for functional tradeoffs. Our comparative evaluation of changes in P forms in reference and impacted wetlands provides a novel approach to evaluate restoration of soil biogeochemical functions. It complements earlier efforts to develop robust functional assessment tools [*Brinson*, 1993; *Brinson et al.*, 1995, 1998; *Rheinhardt*

et al., 1997; *Smith et al.*, 1995] and utilizes the accepted application of reference conditions for evaluating the efficacy of restoration efforts [*Smith et al.*, 1995; *Brinson and Rheinhardt*, 1996; *Rheinhardt et al.*, 1999]. Besides the recovery of soil P diversity upon restoration, other indices such as soil C:N ratio also indicate restoration of soil biogeochemical functions. Soil C:N ratios in our study declined with decreasing intensity of management, consistent with other observations [*Craft*, 2001; *Elliot*, 1986]. Our study suggests that restoring soil biogeochemical functions in wetlands may require more than 15 years, a timeline remarkably similar to those derived using other indices [*Maul et al.*, 1999; *Craft*, 2001]. While temporal and spatial variability in conventional ecosystem indices hamper our ability to assess the restoration of ecosystem function and services [*Zedler and Callaway*, 1999], our results suggest that changes in soil P diversity may provide a time-integrated assessment of restoration of soil biogeochemical functions in wetlands.

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