Geoderma 268 (2016) 119–127

Contents lists available at ScienceDirect

Geoderma

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

Microbial genetic and enzymatic responses to an anthropogenic phosphorus gradient within a subtropical peatland

Elise Morrison [a], S. Newman [b], H.S. Bae [a], Z. He [c], J. Zhou [c], K.R. Reddy [a], A. Ogram [a,*]

[a] Soil and Water Science Department, University of Florida, Gainesville, FL 32611, USA
[b] Everglades Systems Assessment Section, South Florida Water Management District, West Palm Beach, FL 33406, USA
[c] Institute for Environmental Genomics, University of Oklahoma, Norman, OK 73019, USA

ARTICLE INFO

Article history:
Received 1 April 2015
Received in revised form 4 January 2016
Accepted 8 January 2016
Available online 5 February 2016

Keywords:
Everglades
Peat
Nutrient limitation
Phosphatase genes
GeoChip
Enzyme assays

ABSTRACT

Many of the world's peatlands are subject to agricultural runoff, which may impact fundamental biogeochemical processes by altering the concentrations of limiting nutrients. In this study, the distribution of specific microbial genes associated with phosphorus (P) and nitrogen (N) metabolism was compared with potential enzyme activities related to P and N acquisition at five stations along a nutrient gradient in soils of Water Conservation Area 2A (WCA-2 A) of the Florida Everglades, USA. Quantitative PCR was used to compare the relative concentrations of genes encoding alkaline phosphatases (*phoX* and *phoD*) with those encoding dinitrogenase reductase (*nifH*). Combined phosphatase and phosphodiesterase (E_P) activities were compared with leucine aminopeptidase activities (E_N), yielding a measure of potential microbial investment in P acquisition relative to N acquisition. The significant inverse relationship observed between bicarbonate extractable organic P concentrations and ratios of gene copy numbers of *phoX*:*nifH* ($p = 0.049$, $R^2 = 0.77$), and *phoD*:*nifH* ($p = 0.043$, $R^2 = 0.79$), combined with the significant inverse relationship between total P and E_P/E_N ($p = 0.021$, $R^2 = 0.87$), suggest that there is a greater community selection towards P acquisition relative to N acquisition as bicarbonate extractable organic P and total P decrease, suggesting a shift from P limitation to N limitation along the transect. The total number of unique genes detected by the functional microarray GeoChip 3.0 was greatest in an intermediate site, suggesting that the alleviation of nutrient limitation yielded increased functional diversity. The general agreement between genetic and enzymatic data suggests that assessing microbial nutrient demands with molecular techniques is feasible, although future work is needed to apply genetic information as indicators of nutrient enrichment.

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1. Introduction

Wetlands provide numerous important ecosystem services, such as regulation of water quality and quantity, provision of critical habitats for diverse species (including endangered species), and recreation opportunities. Many wetlands, including the Florida Everglades, are subject to nutrient enrichment that impacts many of these services. The majority of the Florida Everglades is underlain by limestone, such that ecosystem productivity was historically limited by phosphorus (P) availability. Much of the original water flow into the Everglades was diverted to the Gulf of Mexico and the Atlantic Ocean to control flooding, and approximately 280,000 ha south of Lake Okeechobee were drained for development of the Everglades Agricultural Area (EAA) (McCray et al., 2012).

Fertilizer runoff from the EAA into what is now the Water Conservation Areas (WCAs; Fig. S1) contained significant levels of nutrients, notably phosphate and sulfate (Castro et al., 2002). The input of phosphate into the phosphate-limited marsh resulted in broad ecosystem level changes, including a change in the dominant vegetation from ridges dominated by low-P adapted sawgrass (*Cladium jamaicense* Crantz) to densely distributed cattail (*Typha* spp.) (Hagerthey et al., 2008; Belanger et al., 1989). The increased primary productivity resulted in increased peat accumulation and associated microbially mediated processes, such as microbial respiration, and methane production (Holmes et al., 2014), in addition to the loss of habitat for numerous species (McCormick et al., 2009).

WCA-2A is characterized by a gradient in water and soil concentrations of P, with the highest concentrations of 53 μg per kg soil in peat nearest to the EAA declining to 5 μg per kg in the interior of the WCA (Turner and Newman, 2005). As expected, the activities and structures of the resident microbial communities are characteristic of the nutrient status along the gradient, and exhibit characteristics of a shift from P-limitation within the center of the marsh to N-limitation in the northern, P-impacted soils (Corstanje et al., 2007). Of note is the existence of a transition zone between the P-limited and N-limited zones.

* Corresponding author at: 2181 McCarty Hall, Soil and Water Science Department, University of Florida, Gainesville, FL 32611-0290, USA.
E-mail address: aogram@ufl.edu (A. Ogram).

http://dx.doi.org/10.1016/j.geoderma.2016.01.008
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The nutrient limitation gradient in WCA-2A has provided an outstanding natural laboratory in which to study the impacts of nutrient enrichment on the structures and activities of microbial communities and microbially mediated biogeochemical cycles. Much of the work previously conducted in WCA-2A has involved identification of indicators of nutrient enrichment, including potential activities of microbial enzymes involved in phosphorus acquisition. The relationships between the activities of enzymes involved in phosphorus (e.g., phosphatase) and nitrogen (e.g., amino peptidase) acquisition have been shown to be generally good indicators of the shift from P- to N-limitation in the soils of WCA-2A (Corstanje et al., 2007; Penton and Newman, 2008). Indicators of nutrient enrichment can be used in developing management strategies as well as providing basic information on how microbial communities react to shifting nutrient limitations. Although extensive work has been conducted on microbial nutrient dynamics, particularly with regard to global cycling (DeLong and Karl, 2006; Arrigo, 2005), extracellular enzymes (Gusewell and Freeman, 2005; Kang and Freeman, 1999) and carbon dynamics (Limpens et al., 2008; Freeman et al., 2001a, 2001b), little work has been conducted to evaluate genetic indicators of nutrient availability within peatland soils.

One of the fundamental ways that microbial communities react to shifting nutrient limitations, such as that observed in WCA-2A, is through changes in community structure. Changes in community structure are reflected in the distribution of specific taxonomic and functional groups of microorganisms, which can be characterized at the genetic level (Zhou et al., 2015; Handelsman, 2004; Torsvik and Ovreas, 2002). Shifts in the relative concentrations of specific functional genes characteristic of methanogens and sulfate reducing prokaryotes along the gradient have been well documented (Bae et al., 2015, 2014; Castro et al., 2002, 2005), as have the distribution of specific phylotypes of primary and secondary fermenters via 16S rRNA gene sequence analysis (Uz et al., 2007, Chauhan et al., 2004).

A significant gap in our understanding of the fundamental mechanisms through which WCA-2A microbial communities respond and adapt to P-enrichment is how the community changes with respect to P acquisition at the genetic level. Increasing phosphatase activities with decreasing P availability in WCA-2A is well known, but nothing is known of potential differences in the genes that encode those enzymes, or of the microorganisms that harbor those genes. Previous work has been conducted on the distribution of genes encoding relatively high and low efficiency phosphatases in marine systems (Sebastian and Ammerman, 2009); however, these studies have only been extended to a few terrestrial studies (Zaheer et al., 2009; Tan et al., 2013; Fraser et al., 2015). In addition, little is known of the potential transformation of reduced organophosphorus compounds such as phosphonates and phytic acid to serve as alternative sources of P in P-limited soils.

To address these gaps in knowledge, we assessed the relative abundances of genes related to P and N cycling along the P gradient in WCA-2A. Abundances of *phoX* and *phoD* (genes encoding high affinity phosphatases), *nifH* (gene encoding dinitrogenase reductase), and the abundances of an array of functional genes related to P-metabolism, including those encoding phytase (*phy*) and polyphosphate kinase (*ppk*) were compared with enzyme assays, including phosphatase (indicator of P-limitation) and leucine aminopeptidase (indicator of N- metabolism) and soil P-fractions at five stations along the nutrient gradient of WCA-2A. This suite of techniques was used to address our overarching hypothesis: that microbial communities will exhibit a response to changes in P-limitation at the genetic level. We found relationships between available P forms, functional genes, and related enzyme activities, suggesting that P enrichment alters the functional diversity of the Everglades' resident microbial communities with respect to P-cycling. Additionally, the phylogenetic diversity of phosphatase (*phoX*) genes, and the functional diversity shown with GeoChip analysis, indicates that nutrient shifts along the WCA-2A gradient impact the diversity of P-cycling genes.

2. Materials and methods

2.1. Sites, sampling, and isolation of environmental DNA for 2013

WCA-2A is located in the southern part of Florida, USA (Fig. S1), and receives half of its water from rainfall, and half from canals and other structures (Turner and Newman, 2005). Agricultural runoff into WCA-2A has led to elevated P and calcium levels, resulting in an anthropogenic P gradient that runs north to south (Turner and Newman, 2005). Five sites were selected along this P gradient and ranged from nutrient impacted (F1 and F3), intermediate (F4), and unimpacted (U3 and E5) (Fig. S1). Three replicate soil cores from 0 to 2 cm depth were taken in August of 2013 using a 10 cm thin-walled, stainless steel sharpened edge corer from each of these five sites. Cores were visually inspected to determine the extent of the unconsolidated flocculent layer. The flocculent (if present) was removed and stored for another study. After removal of the flocculent, the top 0 to 2 cm was measured, and sectioned. Each 0 to 2 cm section was homogenized and subsampled. Subsamples (0 to 2 cm) were immediately frozen on dry ice in the field, and transferred to −80 °C upon returning to the lab (less than four hours). Frozen subsamples were homogenized and ground using liquid nitrogen. Environmental DNA was isolated from the homogenized, ground soils using the PowerSoil DNA isolation kit (MoBio Laboratories, Inc., Carlsbad, CA, USA). The remaining soil samples were homogenized, and all three replicates were composited and stored at 4 °C for further analysis of enzyme activities and P fractionation. Samples were stored at 4 °C for 2 to 3 days prior to conducting assays for phosphatase, β-glucosidase, and leucine aminopeptidase, and 6 days prior to phenol oxidase and peroxidase assays.

2.2. Sites, sampling, and isolation of environmental DNA for 2009

Soils from three of the sites described above were collected for GeoChip analysis in 2009. Sites F1, F4, and U3 were sampled for flocculent and soil from 0 to 2 cm depth. Three replicate soil cores from 0 to 2 cm depth were taken using a 10 cm thin-walled, stainless steel sharpened edge corer from each of these three sites. Cores were visually inspected to determine the extent of the unconsolidated flocculent layer. The flocculent (if present) was removed and the top 0 to 2 cm were measured and sectioned. Each 0 to 2 cm section was homogenized and frozen in the field on dry ice. Upon returning to the lab, they were transferred to a − 80 °C freezer. Triplicate cores were composited, and DNA was isolated from the composited sample and used for further GeoChip analysis (described in Section 2.7).

2.3. Phosphorus fractionation

Soils from the composited cores were analyzed for P fractionation at the University of Florida's Wetland Biogeochemistry Lab. Phosphorus extractions were conducted using the method described by Ivanoff et al. (1998), which quantifies different operationally defined pools of P via chemical fractionation. This fractionation scheme identifies the following organic P (P_o) pools: bicarbonate extractable P_o, microbial biomass P, hydrochloric (HCl) acid extractable P_o, fulvic acid-associated P_o, humic acid-associated P_o, residual P_o, and total P_o. The fractionation scheme identifies the following inorganic P (P_i) pools: bicarbonate extractable P_i, HCl-extractable P_i, and total P_i. Bicarbonate extractable P_o, bicarbonate extractable P_i, and microbial biomass P are generally thought to be the most readily available pools of P; HCl extractable P_o, HCl extractable P_i, and fulvic acid-associated P_o are considered moderately labile; while humic acid-associated P_o and residual P_o are held to be the least available pools. Total P is calculated as the sum of all P_o and P_i pools.

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