



Assessment of microbial community structure and function within two oligotrophic peatlands

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Background

Soil microbial communities are highly complex networks that are comprised of many diverse microbial taxa. Conserved genes, such as the 16S rRNA gene, can be used as phylogenetic markers to determine the composition of prokaryotic (Bacteria and Archaea) members of soil microbial communities (Klindworth et al., 2013). Putative interactions can be found between these taxa by analyzing highly correlated microbial groups with network analyses (Barberan et al., 2012), which can provide insights into how these communities may influence biogeochemical cycling. Additionally, the quantification of microbial functional genes, such as those encoding for alkaline phosphatase (*phoD* and *phoX*), and dinitrogenase reductase (*nifH*) can provide information about potential microbial activity related to phosphorus (P) acquisition and nitrogen (N) fixation, respectively.

In this study, we characterize the prokaryotic communities along nutrient gradients within two oligotrophic (low-nutrient) peatlands: the Everglades in South Florida, and San Pond Sak in Panama. Both have well characterized phosphorus (P) gradients that have led to documented changes in vegetation communities and biogeochemical cycling. Triplicate cores were collected from sites along each gradient, and DNA was isolated from each replicate. DNA was sequenced for 16S rRNA genes and networks were created to determine potential microbial interactions. Functional genes for alkaline phosphatase (*phoD* and *phoX*) and dinitrogenase reductase (*nifH*), were quantified via quantitative PCR (qPCR) to assess potential microbial investment in P and N acquisition along the P gradients.

Objectives

OBJECTIVE 1

To determine if prokaryotic microbial community composition shifts along each nutrient gradient.

OBJECTIVE 2

To create phylogenetic networks for highly correlated microbial taxa and assess how network connectivity differs between sites along both gradients.

OBJECTIVE 3

To evaluate how functional genes for P and N acquisition respond to P availability, to determine relative microbial investment in P acquisition relative to N acquisition.

Hypotheses

We hypothesize that (1) prokaryotic community composition will shift along the two P gradients, (2) that phylogenetic network density will be enriched at the intermediate and low-P sites, due to increased diversity of microbial interactions, and that (3) the relative microbial investment in P acquisition will increase as P availability decreases along changing nutrient gradients.

Approach

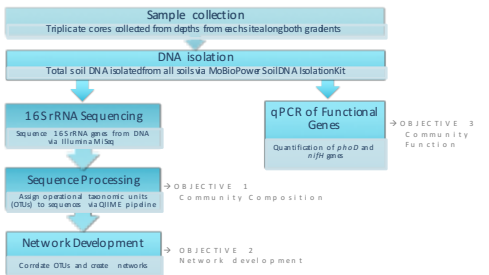


FIGURE 1: Methods used to achieve the objectives of this study

Results

OBJECTIVE 1: Community Composition

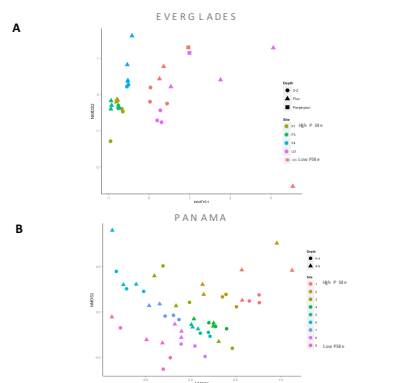


FIGURE 2: NMDS showing differences in community composition along the Everglades (A) and Panama (B) gradients. Community composition was seen to shift along each gradient, likely due to differences in nutrient availability. ADONIS was run for each location, and significant differences (p-value=0.001) were seen in community composition between sites along both gradients.

OBJECTIVE 2: Phylogenetic Networks

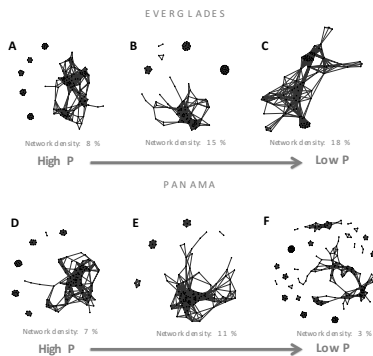


FIGURE 3: Phylogenetic networks along the Everglades gradient for high-P (A), intermediate-P (B), and low-P (C) sites, and along the Panama gradient, for high-P (D), intermediate-P (E), and low-P (F) sites. Nodes are microbial OTUs and edges are putative interactions. Only OTUs with significant ($p < 0.05$) correlations were retained. Network density was assessed to determine how putative microbial interactions may shift along the gradient. Greater density suggests more putative microbial interactions at a site. The Everglades high-P site in had suppressed network density relative to the intermediate and low-P sites, while Panama had greatest network density in the intermediate and low-P sites, suggesting potential differences in microbial interactions along both P gradients.

Results

OBJECTIVE 3: Community Function

Factor 1	Factor 2	P value	R ²
En: En	Total P	0.0214	-0.87
<i>phoX</i> / <i>nifH</i>	Bicarbonate: R	0.049	-0.77
<i>phoD</i> / <i>nifH</i>	Bicarbonate: R	0.043	-0.79

En = Phosphatase + Phosphatase activity
En: En = Leucine Amino acid activity
EnEn = P acquisition: N acquisition
phoX, *phoD* = Alkaline phosphatase
nifH = dinitrogenase reductase
phoX/*nifH* = P acquisition: N acquisition

TABLE 1: Quantification of functional genes for P acquisition (*phoX* and *phoD*) and N fixation (*nifH*) was conducted to assess relative investment in P acquisition to N acquisition along the Everglades transect. Enzyme assays were conducted as well, to support the qPCR data. An inverse relationship was seen for all measures, indicating that the relative abundance of *phoD*, *phoX*, and *nifH* is sensitive to P availability, and the greatest relative microbial investment in P acquisition was seen at the low-P site.

Conclusions & Future Work

OBJECTIVE 1

We found that prokaryotic community composition differed significantly between sites suggesting that changes in site characteristics are driving differences in microbial community structure. Further analyses are being conducted to determine what environmental characteristics (i.e. P availability, C quality, vegetation community) are driving these differences in community structures.

OBJECTIVE 2

Networks we developed for all sites along both gradients. We found that network density tended to be greater in the intermediate and low-P sites, for the Everglades gradient and was greatest at the intermediate site for the Panama gradient. These results suggest that there may be fundamental differences in how microbial communities interact when subjected to different nutrient availabilities, and that there may be differences in the nature of microbial interactions between the Everglades and Panama gradients. Further work is necessary to elucidate the mechanisms that are driving the differences seen between the two sites along both transects. It is possible that greater microbial diversity at the intermediate sites may drive increased microbial interactions. Additional analyses will be conducted to determine the most important microbial groups within the phylogenetic networks to better understand how their interactions may influence nutrient cycling along both transects.

OBJECTIVE 3

The relative abundance of functional genes for P and N acquisition was sensitive to P availability, and was greatest in the low-P sites, suggesting greater microbial investment in P acquisition in low-P environments. Further work is being conducted to assess the abundance of these functional genes at the Panama gradient, to see if similar trends are seen. Additional work is being conducted to characterize the microorganisms that carry these functional genes, to provide insights into the microorganisms that are driving P cycling within oligotrophic peatlands.

Acknowledgements

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References

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Klindworth, A. et al. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Research* 41, doi:10.1093/nar/gks008 (2013).