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Title: Bacterial community composition and diversity, and physicochemical properties of soils in mitigation wetlands created in the Virginia piedmont

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### **Abstract**

Soil bacterial communities are essentially involved in biogeochemical cycles in wetlands, yet are rarely examined when assessing ecosystem development of created wetlands. We use amplicon length heterogeneity polymerase chain reaction (LH-PCR) and multi-tag pyrosequencing (MTPS) of 16S ribosomal DNA to characterize the bacterial communities in soil samples collected during two seasons from two created and two natural wetlands in the Piedmont physiographic province of Virginia, USA. Bacterial community profiles and soil physicochemical attributes (percent moisture, C:N, total N, total organic C, pH and soil organic matter) were used to identify community level differences within and between these wetlands specifically in terms of age and hydrologic connectivity to a water body. We found seasonal differences in both physicochemistry and bacterial community dynamics, with moisture content ( $40.2\% \pm 1.8$  and  $29.2\% \pm 2.2$  during the growing and non-growing seasons respectively) as a major factor. Bacterial community diversity was higher during the non-growing season ( $p < 0.01$ ). Soil physicochemical attributes indicated increased soil quality (higher SOM, TOC, TN, percent moisture) with age of wetlands. Bacterial community profiles did not clearly show an age related pattern, with the highest differences in pairwise analysis of similarity (ANOSIM) comparisons between the herbaceous natural wetland and all other wetlands ( $R > 0.9$ ,  $p < 0.05$ ). We did find phyla level negative correlations between the abundance of Acidobacteria and Bacteroides ( $r^2 = 0.51$ ,  $p < 0.01$ ), Firmicutes ( $r^2 = 0.45$ ,  $p < 0.01$ ) and Actinobacteria ( $r^2 = 0.45$ ,  $p < 0.01$ ), indicating pH as a factor. The sensitivity of bacterial communities to soil quality attributes suggests their role as indicators of wetland soil development and can further inform wetland creation and monitoring.