TAXONOMIC COMPOSITION AND FUNCTIONAL POTENTIAL OF SEDIMENT MICROBIAL COMMUNITIES VARY BETWEEN FLOODPLAIN WETLANDS WITH DIFFERING MANAGEMENT HISTORIES

by

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ABSTRACT

Wetland ecosystems disproportionately contribute to global biogeochemical cycles, and thus provide valuable ecosystem services. However, land use-change has significantly decreased the global extent of wetlands and subsequently impaired the services they provide to society. Despite considerable restoration and management efforts in recent decades, the functional capacity of wetlands often does not recover, emphasizing the need to understand the factors controlling wetland biogeochemical processes. Microbial communities in wetland sediments mediate these processes; thus understanding how microbial community composition and metabolism differ between wetlands under various restoration and management regimes is necessary to evaluate and inform restoration efforts. Using shotgun metagenomic sequencing, I compared the taxonomic composition and functional potential of sediment microbial communities in three adjacent floodplain wetland units with different hydrological management (i.e., inundation frequency) histories at the Shiawassee National Wildlife Refuge in Saginaw, MI, USA. The wetlands assessed included 1) a newly flooded (formerly drained and farmed) degraded wetland unit, 2) a restored diked and managed deep-water pool unit with intermittent riverine connections and intermediate inundation frequency and 3) a frequently flooded natural backwater wetland with an uninterrupted river connection.

Total microbial community composition and functional potential were significantly different between each wetland unit. Bacterial sequences dominated all metagenomes (~92%), followed by Archaeal sequences (~4%). The relative abundances of aerobic taxa (e.g., *Actinobacteria* and *Thaumarchaeota*) and the genetic potentials of aerobic functions (e.g., cytochrome C oxidases) decreased across the gradient of low-to-

high historic inundation frequencies (degraded wetland to restored wetland to natural wetland), whereas anaerobic taxa (e.g., *Clostridia* and *Methanomicrobia*) and the genetic potentials of multiple anaerobic functions (e.g., anaerobic respiratory reductases, sulfate reduction and methanogenesis) decreased. The functional potential for denitrification was highest in the restored wetland, which may have important implications for the removal of nutrient pollution in this system. Taken together, these results suggest that hydrological management has resulted in microbial communities with differing abilities to mediate biogeochemical cycling, add to the growing body of evidence that restored and natural wetlands often harbor distinct microbial communities and highlight the potential for a microbial framework to inform and evaluate management practices at the Shiawassee National Wildlife Refuge.

Keywords: wetland, microbial communities, functional potential, restoration

INTRODUCTION

Wetland ecosystems contribute significantly to global biogeochemical cycles despite occupying only 5-7% of the global land area (Lehner and Döll, 2004; Schlesinger and Bernhardt, 2013). For example, it is estimated that ~30% of the Earth's soil carbon (C) is stored in wetland soils and sediments (Gorham, 1991; Bridgham et al., 2006), and that wetlands sequester ~830 Tg C y^{-1} (Mitsch et al., 2013). Additionally, wetlands are the largest natural source of atmospheric emissions of methane (CH_4) (~164 Tg CH_4 y^{-1}) (Bridgham et al., 2013), which is a potent greenhouse gas (Donner and Ramanathan, 1980). The ability of wetlands to improve water quality through gaseous nitrogen (N) loss (i.e., denitrification) and sequestration of N and phosphorus (P) in sediments is also well recognized (Zedler, 2003), and wetland-derived dissolved organic C is important to the productivity of connected aquatic ecosystems (Schlesinger and Bernhardt, 2013). However, anthropogenic stresses (e.g., conversion to agricultural land use) have resulted in widespread destruction of wetlands, interrupting the key biogeochemical processes they mediate and significantly impairing their ecological and economic benefits (Zedler and Kercher, 2005). For instance, over half of the original wetland area in the contiguous United States has been lost, subsequently reducing annual C sequestration in U.S. wetlands by an estimated 9.4 Tg C y^{-1} (Bridgham et al., 2006). Despite substantial efforts to restore degraded wetlands and associated ecosystem services (Zedler, 2000; Palmer, 2009), a growing body of evidence suggests full recovery of biogeochemical processes is often unattained due to either slow recovery or equilibration in alternate stable states (Zedler, 2000; Peralta et al., 2010; Moreno-Mateos et al., 2012). Thus, recovery of

biogeochemical functions in restored and managed wetlands requires a more thorough understanding of the factors by which they are controlled.

Microorganisms are the primary mediators of biogeochemical transformations (Falkowski et al., 2008) due to their enormous abundance (Whitman et al., 1998) and ability to utilize numerous C sources, electron donors and terminal electron acceptors (TEAs) (Konhauser, 2009). Taxonomically and metabolically diverse bacterial and archaeal assemblages interact in sediments and soils to drive the biogeochemical processes characteristic of wetland ecosystems (Gutknecht et al., 2006). While anaerobic microbial processes such as denitrification, methanogensis and C sequestration receive considerable attention for their biogeochemical relevance (Peralta et al., 2010; Moreno-Mateos et al., 2012; Bridgham et al., 2013), no single wetland processes can be considered in isolation because each is dependent upon the metabolic byproducts of another (Drake et al., 2009). For instance, methanogenic *Archaea* oxidize a limited set of compounds, all of which are produced by fermentative and acetogenic *Bacteria* (Liu and Whitman, 2008). Thus, any change in the abundance or activity of a specific microbial population could have cascading effects upon the biogeochemical function of an entire community.

Wetland restoration and management typically alter hydrological processes, which in turn influence edaphic and biotic conditions (Zedler, 2000) that drive the composition and activity of microbial communities (Gutknecht et al., 2006). For example, Peralta et al., (2010) demonstrated that soil moisture and nutrient status drove differences in total bacterial community composition and denitrifying functional gene assemblages between restored and natural floodplain wetlands. Similarly, differences in

microbial community composition and methanogenic functional gene abundances between restored and natural freshwater tidal wetlands were related to soil pH, nutrient concentrations and restoration method (Prasse et al., 2015). However, ways in which more comprehensive sets of microbial metabolisms (Laanbroek and Veldkamp, 1982; Drake et al., 2009) differ between restored and natural wetlands remain underexplored, and represent important questions in microbial ecology, restoration ecology and ecological management.

Metagenomics, the random direct sequencing of genomic DNA, is a molecular tool that provides the ability to survey many microbial functions simultaneously (Thomas et al., 2012). Gene-centric metagenomics can be used to quantify genes encoding enzymes involved in biogeochemical transformations, and thus provide a measure of the potential for a microbial community to perform a process (i.e., genetic potential, or functional potential) (Tringe et al., 2005). In this study, I utilized high-throughput metagenomic DNA sequencing to characterize microbial communities in three riverine floodplain wetlands. Community composition and functional potential were determined through the annotation of unassembled sequencing reads. Functional pathways involved in aerobic respiration, polysaccharide degradation, fermentation, acetogenesis, anaerobic respiration (e.g., denitrification, iron reduction, sulfate reduction and methanogenesis), methanotrophy and nutrient utilization were selected to represent a range of microbial metabolisms typical of sediment environments (Laanbroek and Veldkamp, 1982; Drake et al., 2009; Lipson et al., 2013; Schlesinger and Bernhardt, 2013; Kirchman et al., 2014; He et al., 2015). The degraded wetland, restored wetland and natural wetland in this study system have differed substantially in hydrological management for over 60 years,

experiencing the lowest, intermediate and highest inundation frequencies, respectively (Buchanan et al., 2013). Thus, I hypothesized that for a specific function, the genetic potential would be lowest in the degraded wetland, intermediate in the restored wetland and highest in the natural wetland if the function was anaerobic, and the opposite would be observed for an aerobic function. I also expected that functionally relevant taxonomic groups would differ in abundance between wetlands based on their known metabolic capabilities. Variation in taxonomic composition and functional abundances between communities in the wetlands assessed here would reflect the influence of wetland management on the microbial potential to mediate important biogeochemical cycling.

MATERIALS AND METHODS

Site description

The Shiawassee National Wildlife Refuge (SNWR) is located in an erosional depression on the plain of glacial Lake Saginaw in Saginaw County, MI, USA, directly west of the Port Huron Moraine (Arbogast et al., 1997) (**Figure 1a**). Five major rivers (Cass, Flint, Shiawassee, Bad and Tittabawassee) converge at the SNWR to form the Saginaw River, which drains to Saginaw Bay ~35 km downstream of the refuge. Low channel slopes, poorly drained soils and backwater effects associated with conveyance constraints and lake level fluctuations are responsible for the historical prevalence of wetland cover in Saginaw County (Iaquinta, 1994; Buchanan et al., 2013). However, drainage and the disconnection of river floodplains for agricultural and urban development have resulted in a 95% reduction in wetland cover since 1830 (Buchanan et al., 2013). Wetland restoration is of particular interest due to its potential to improve water quality in Saginaw Bay of Lake Huron, which is affected by significant nonpoint source pollution loading (He et al. 2013).

Since the establishment of the SNWR in 1953, a variety of wetland types have been restored or managed on historical floodplains by the United States Fish and Wildlife Service (USFWS), including agricultural land, restored pool and moist soil wetland units with managed hydrology and remnant natural riverine wetlands. The agricultural land and restored wetlands are disconnected from the adjacent rivers by a series of levees and gates to allow for water level control, while the natural wetlands maintain direct hydrological connectivity (Buchanan et al., 2013). Three separately managed wetland units in the Shiawassee River floodplain in the SNWR (**Figure 1b**) were selected as study

sites to compare microbial communities in wetlands subjected to differing hydrological management regimes. The degraded wetland unit (unit D; i.e., SNWR Farm Unit 1) was farmed as row crop agricultural land prior to flooding in spring of 2013. The 'restored' wetland unit (unit R; i.e., SNWR Pool 1A) has been managed with a series of levees and gate structures as deep-water pool wetland. Both unit D and unit R are typically disconnected from the Shiawassee River, except during extreme flooding events. The natural wetland unit (unit N) has maintained direct hydrological connectivity with the Shiawassee River since before settlement. The adjacent location of these wetland units in the Shiawassee River floodplain system ensures a long, shared physiographic history. Yet, for at least the last 60 years, management has caused units D, R and N to have low, intermediate and high inundation frequencies, respectively. Thus, this system provides a useful opportunity to assess management influences on the microbial communities in these three wetland units.

Experimental design and sample collection

At the time of sampling, all wetland units were inundated for at least 4 months due to a significant flood in April 2013. Three replicate plots were haphazardly located in each wetland unit (**Table S1**). Four sediment cores (2.5 cm diameter; 10 cm depth) were collected at equidistant points in a 1 m radius around the center of each plot, and cores were immediately stored on ice. The 4 sediment cores were homogenized in the laboratory to produce a single representative sample for each plot, and gravel and woody debris were removed by hand. Two subsamples (~20 g each) designated for molecular analyses were placed in separate sterile 50 mL centrifuge tubes and stored at -80°C. Remaining sediment was stored at -20°C. All equipment was thoroughly sterilized with

70% ethanol between samples, and all samples were processed and frozen within 12 hours of collection.

DNA extraction, purification and shotgun metagenomic sequencing

Genomic DNA was extracted from ~1.75 g (wet mass; 7 preparation x ~0.25 g) of sediment per sample using the PowerLyzer PowerSoil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA, USA) according to the manufacturer's protocol with modifications. Cell lysis was performed at 3000 RPM for 45 seconds with a PowerLyzer 24 Bench Top Bead-Based Homogenizer (MoBio Laboratories, Carlsbad, CA, USA), and preparations were subsequently centrifuged for 3 minutes to fully pellet suspended clays. An additional wash step with 100% ethanol was performed. Each wash step included a 5 minute incubation at room temperature prior to centrifugation. DNA was eluted with 100 uL of molecular-grade water. The 7 replicates were pooled after verification of quality. Extracted DNA was purified using the PowerClean DNA Clean-Up Kit (MoBio Laboratories, Carlsbad, CA, USA). Triplicate preparations were performed per sample (3 x 150 uL extracted DNA) according to the manufacturer's protocol before being combined onto a single spin filter to concentrate the DNA. Wash steps and elution were performed as above. DNA quality was verified with gel electrophoresis and a NanoDrop 8000 Spectrophotometer (Thermo Scientific, Waltham, MA, USA), and DNA concentration was determined using the Quant-iT PicoGreen dsDNA Assay Kit (Life Technologies, Carlsbad, CA, USA) with a BioTek Synergy HT Multi-Detection Microplate Reader (BioTek Instruments, Winooski, VT, USA). DNA was stored at -80°C prior to sequencing. Library preparation and sequencing of the 9 purified DNA samples were performed by the University of Michigan DNA Sequencing Core

(http://seqcore.brcf.med.umich.edu/). Samples were multiplexed onto one lane of an Illumina HiSeq 2500 sequencer in Rapid mode to generate 9 metagenomic libraries of 150 bp single-end sequencing reads.

Data analysis

Sequence quality was assessed with FastQC (Andrews, 2010). Cutadapt (Martin, 2011) was used to remove adapter-contaminated sequences and to trim the first 3 low-quality base calls from the 5' end of each sequence. Metagenomes were randomly subsampled without replacement to an equivalent depth with seqtk (https://github.com/lh3/seqtk). The unassembled metagenomes were then uploaded to the Metagenomics Rapid Annotation using Subsystem Technology server (MG-RAST) (Meyer et al., 2008) for annotation. Preprocessing pipeline conditions for dereplication, a minimum phred score of 20 and a maximum of 5 low-quality bases per sequence were selected.

Taxonomic annotations were determined in MG-RAST by the Best Hit method against the SEED database, and functional annotations were determined against the SEED Subsystems database in MG-RAST (Meyer et al., 2008; Overbeek et al., 2014). Only annotations with a maximum E-value of $1x10^{-5}$, a minimum identity of 60% and a minimum alignment length of 25 amino acids for proteins and 25 bp for rRNA were retained. Taxonomic annotations were grouped by domain, phylum and class, whereas functional annotations were analyzed at the SEED Subsystems Level 3. The subsystems approach is a functional annotation approach that is well-suited to broadly assessing the functional potential of a community, and a function within the Subsystems Level 3 hierarchy is analogous to a metabolic pathway (Overbeek et al., 2005). Taxonomic and

functional abundances were normalized to the number of sequences that passed quality control (QC) (Fierer et al., 2012) in MG-RAST. Thus, the relative abundance of a function or a taxonomic group refers to the number of sequences associated with that function or group per quality sequence. As a result, abundances are relativized to metagenome size and are therefore comparable between metagenomes. As an example, the statement '*Denitrification* is greater in unit R than in units D and N,' can be read as 'the relative number of sequences attributed to the function *denitrification* is greater in unit R than in units D and N.' Names of Subsystems Level 3 functions are italicized for clarity. All statistical tests were performed on relative abundances. Taxonomic abundances are presented as a percentage of annotated taxonomic sequences for ease of discussion, and functions are presented as reads per million reads as to be readily understandable. Species richness was estimated in MG-RAST. Data are stored on MG-RAST under the identification numbers 4622914.3 - 4622922.3.

Ordinations were developed from principle coordinates analysis (PCoA) of pairwise distances between metagenomes (i.e., plots), calculated for phylum, class, all functions and selected functions. Statistical significance of pairwise distances between units was determined by PerMANOVA (Anderson, 2001). Calculation of pairwise distances (determined by Bray-Curtis similarity), PCoA and PerMANOVA were performed in PRIMER and PERMANOVA+ (version 6; Clarke and Gorley 2006). The significance of differences in taxonomic and functional abundances was determined by one-way ANOVA. ANOVA *P*-values were corrected for a false discovery rate of 0.05 using the Benjamini-Hochberg procedure (Benjamini and Hochberg, 1995) to avoid type I error due to multiple comparisons. Significance of pairwise differences in abundance

was determined by Tukey's HSD test for tests with significant main effects (i.e., Benjamini-Hochberg adjusted $P < 0.05$). ANOVA and Tukey's HSD tests were performed with the 'manova' and 'TukeyHSD' functions in R (version 3.1.2; R Core Team 2014) and RStudio (version 0.98.1103; RStudio Team 2015). Z-scores were calculated by metagenome for each of the selected functions.

RESULTS

Metagenomic sequencing

Illumina high-throughput sequencing generated 9 metagenomic libraries (one library per plot) totaling 172,481,129 sequences (15,202,749 to 26,846,396 sequences per metagenome; **Table 1**). After initial quality control (QC) and subsampling, 14,620,507 sequences per metagenome were uploaded to MG-RAST, and an average of 14,144,680 ± 45,353 sequences passed the MG-RAST QC pipeline (**Table 1**). From the post-QC sequences, an average of 12,780,744 ± 116,702 protein features were predicted per metagenome, of which 4,057,245 ± 103,112 were identified (**Table 1**).

Taxonomic community composition

The taxonomic composition of microbial communities in the degraded (unit D), restored (unit R) and natural (unit N) wetland units clearly separated in ordination space and differed significantly at both the phylum and class levels (PerMANOVA, $P < 0.001$; all pairwise unit comparisons were highly significant at $P < 0.001$; **Figure 2a and 2b** for phylum and class ordinations, respectively; full PerMANOVA results are included in **Table S5**). Additionally, species richness differed significantly between units, and was highest in unit N, intermediate in unit R and lowest in unit D ($P < 0.001$, $P < 0.001$ and $P = 0.0064$ for N-D, R-D and R-N comparisons, respectively **Table 2**; full ANOVA results are included in **Table S6**).

The domain *Bacteria* dominated the metagenomes of all wetland units (**Table 2 and Figure 3a**). However, *Bacteria* were less abundant in unit N (89.72% ± 0.21%) than unit R (92.00% ± 0.32%; $P < 0.001$) and D (93.43% ± 0.09%; $P < 0.001$), but did not differ between units D and R ($P = 0.1148$). In contrast, *Archaea* were more abundant in

unit N (5.19% ± 0.30%) than unit R (3.53% ± 0.32%; $P = 0.0010$) and unit D (2.63% ± 0.04%; $P < 0.001$), and more abundant in unit R than in unit D ($P = 0.0068$; **Table 2 and Figure 3b**). The relative abundance of *Eukaryota* was less than 0.5% in each unit, and did not differ between units ($P = 0.0849$; **Table 2**; full ANOVA results at the domain level are included in **Table S6**).

Proteobacteria, *Actinobacteria* and *Firmicutes* together dominated communities in all wetland units, accounting for between 66% (unit N) and 74% (unit D) of annotated sequences (**Table 2 and Figure 3a**). *Proteobacteria* were more abundant in units R and N than in unit D ($P = 0.0011$, $P = 0.0107$ for R-D and N-D comparisons, respectively; **Table 2 and Figure 3a**), but did not differ between units R and N ($P = 0.1010$).Within the *Proteobacteria*, the abundance of both *Betaproteobacteria* (8.67% ± 0.14%, 11.62% ± 0.91% and 12.98% ± 0.40% for units D, R and N, respectively; $P < 0.001$, $P = 0.0013$ and $P = 0.6377$ for N-D, R-D and R-N comparisons, respectively; **Figure 4a**) and *Gammaproteobacteria* (7.03% ± 0.04%, 8.79% ± 0.84% and 10.26% ± 0.3% for units D, R and N, respectively; $P = 0.0014$, $P = 0.0084$, $P = 0.2081$ for N-D, R-D and R-N comparisons, respectively; **Figure 4a**) was greater in units N and R than unit D, but did not differ between units R and N. *Deltaproteobacteria* were more abundant in unit N than units R and D, and more abundant in unit R than unit D (10.27% ± 0.29%, 15.48% ± 1.36% and 19.59% ± 0.52% for D, R and N, respectively; $P < 0.001$, $P < 0.001$ and $P = 0.0096$ for N-D, R-D and R-N comparisons, respectively; **Figure 4a**), while *Alphaproteobacteria* were more abundant in unit D than units R and N, and more abundant in unit R than unit N (18.37% ± 0.56%, 12.80% ± 1.19% and 7.98% ± 0.15% for D, R and N, respectively; $P < 0.001$ for all pairwise comparisons; **Figure 4a**). The

abundance of *Actinobacteria* declined substantially from unit D (23.94% ± 0.78%) to unit R (15.05% ± 2.75%), and from unit R to unit N (5.57% ± 0.14%; $P = 0.0020$, $P < 0.001$ and $P = 0.0012$ for R-D, N-D and R-N comparisons, respectively; **Table 2 and Figure 3a**). The relative abundance of *Firmicutes* was greatest in unit N and lowest in unit D, with unit R intermediate ($P < 0.001$ for all pairwise comparisons; **Table 2 and Figure 3a**). *Bacilli* and *Clostridia* were the dominant classes belonging to the *Firmicutes* (**Figure 4c**). *Bacilli* were more abundant in units R and N than in unit D, but did not differ between units R and N (1.73% ± 0.03%, 1.94% ± 0.02 and 2.15% ± 0.03% for units D, R and N, respectively; $P < 0.001$, $P < 0.001$ and $P = 0.1260$ for R-D, N-D and R-N comparisons, respectively; **Figure 4c**). The relative abundance of *Clostridia* was lowest in unit D and highest in unit N, with unit R intermediate (3.01% ± 0.06%, 4.55% ± 0.31% and 6.40% ± 0.12% for unit D, unit R and unit N, respectively; $P < 0.001$ for all pairwise comparisons; **Figure 4c**; full ANOVA results for bacterial classes are included in **Table S7**; see **Table S4** for relative abundances). Significant differences in relative abundance between at least two units were detected for several less abundant bacterial phyla, including *Acidobacteria*, *Bacteroidetes*, *Chlorobi*, *Chloroflexi*, *Cyanobacteria*, and *Planctomycetes* (**Table 2 and Figure 3a**; full ANOVA results for bacterial phyla are included in **Table S6**).

Euryarchaeota were the dominant archaeal phylum in all units, and were most abundant in unit N, least abundant in unit D, and intermediate in unit R ($P < 0.001$, $P = 0.0012$ and $P = 0.0030$ for R-D, N-D and R-N comparisons, respectively; **Table 2 and Figure 3b**). Within the phlyum *Euryarchaeota*, the *Archaeoglobi* (0.078% ± 0.002%, 0.142% ± 0.012% and 0.263% ± 0.021% for units D, R and N, respectively),

Methanobacteria (0.086% ± 0.002%, 0.191% ± 0.023% and 0.254% ± 0.010% for units D, R and N, respectively), *Methanococci* (0.0967% ± 0.001%, 0.147% ± 0.017% and 0.295% ± 0.013% for units D, R and N, respectively), *Methanomicrobia* (0.782% ± 0.044%, 1.792% ± 0.313% and 2.580% ± 0.209% for units D, R and N, respectively), *Methanopyri* (0.032% ± 0.002%, 0.048% ± 0.005% and 0.093% ± 0.004% for units D, R and N, respectively) and *Thermoplasmata* (0.058% ± 0.001%, 0.068% ± 0.003% and 0.111% ± 0.004% for units D, R and N, respectively) were each more abundant in unit N, least abundant in unit D and of intermediate abundance in unit R ($P < 0.05$ for each pairwise unit comparison; **Figure 4b**; full ANOVA results for archaeal classes are included in **Table S7**, and relative abundances in **Table S3**). In contrast to the *Euryarchaeota*, the relative abundance of *Thaumarchaeota* was greatest in unit D, lowest in unit N and intermediate in unit R ($P < 0.001$, $P < 0.001$ and $P = 0.0406$ for R-D, N-D and R-N comparisons, respectively; **Table 2 and Figure 3b**). *Crenarchaeota* were more abundant in unit N than units D or R, but did not differ between units D and R ($P < 0.001$, $P = 0.5748$ and $P < 0.001$ for R-D, N-D and R-N comparisons, respectively; **Table 2 and Figure 3b**; full ANOVA results for archaeal phyla are included in **Table S6**).

Community functional potential

Community functional potential clearly separated in ordination space, and differed significantly between each unit for all (**Figure 5a**) and selected (**Figure 5b**) SEED Subsystems Level 3 functions (PerMANOVA, $P < 0.001$; all pairwise unit comparisons were highly significant at $P < 0.001$; full PerMANOVA results are included in **Table S5**). Significant differences in abundance between at least two units (one-way ANOVA, $P < 0.05$) were detected for all selected functions except *fermentations: mixed*

acid. The functional potential of multiple functions was highest in unit D, intermediate in unit R and lowest in unit N, including *CO dehydrogenase, terminal cytochrome C oxidases, fermentations: lactate, nitrate and nitrite ammonification* and *alkylphosphonate utilization* (**Table 3 and Figure 6**). In contrast, the potentials of multiple functions were highest in unit D, intermediate in unit R and lowest in unit N, including *pyruvate:ferredoxin oxidoreductase, anaerobic respiratory reductases, methanogenesis, methanogenesis from methylated compounds, ribulose monophosphate pathway, particulate methane monooxygenase (pMMO), Nitrogen fixation, cellulosome, xyloglucan utilization* and *sulfate reduction-associated complexes* (**Table 3 and Figure 6**). The genetic potentials of *Fe(III) respiration – Shewanella type, methanogenesis strays, methanopterin biosynthesis, dissimilatory nitrite reductase* and *phosphate transporter and PHO regulon* were greater in units R and N than in unit D, but not significantly different between units R and N (**Table 3 and Figure 6**). Potentials of *acetogenesis from pyruvate, butanol biosynthesis* and *serine-glyoxylate cycle* were greatest in unit N, but did not differ between units D and R, whereas those of *soluble methane monooxygenase (sMMO)* and *chitin and N-acetylglucosamine utilization* were greatest in unit D but did not differ between units R and N (**Table 3 and Figure 6**). The genetic potentials of *acetone butanol ethanol synthesis* and *denitrification* were greatest in unit R but did not differ between units D and N (**Table 3 and Figure 6**). The potential of *acetoin, butanediol metabolism* was greatest in unit R, intermediate in unit N and lowest in unit D, whereas that of *acetyl-CoA fermentation to butyrate* was higher in unit R than unit N (**Table 3 and Figure 6**; full ANOVA results for selected functions are included in **Table S9**).

DISCUSSION

Broad microbial community differences between wetland units

Restoration and management alter conditions in wetland soils and sediments such as redox potential (E_h), pH, N and P concentrations and OM content (Zedler, 2000; Bossio et al., 2006; Peralta et al., 2010), that are important in structuring microbial communities (Gutknecht et al., 2006; Lauber et al., 2009; Peralta et al., 2014). As such, restoration has been shown to affect microbial community composition, functional gene assemblages and activity (Peralta et al., 2010; Prasse et al., 2015). I observed clear separations in ordination space between three units with different management histories for both taxonomic composition (**Figure 2**) and functional potential (**Figure 5**), which were supported by highly significant PerMANOVA results. Thus, each wetland in this study supports a taxonomically and functionally distinct microbial community. Differences in microbial community characteristics can have important implications for ecosystem processes (Nemergut et al., 2014) and the highly significant differences at all taxonomic levels and functional subsets examined indicate that the potential for biogeochemical mediation varies substantially between communities in the three wetland units. Moreover, our results add to the growing body of evidence that numerous characteristics of microbial communities differ between restored and natural wetlands (Bossio et al., 2006; Peralta et al., 2010; Prasse et al., 2015). Below, I discuss differences between the wetlands in relevant microbial functions and taxonomic groups, and suggest implications and potential drivers of these differences.

Polysaccharide degradation and the generation of key intermediate metabolites

In the anaerobic degradation of OM, fermentative bacteria hydrolyze polysaccharides into oligo- and monosaccharides (Vymazal, 2005; Schlesinger and Bernhardt, 2013). The genetic potential of *cellulosome* and *xyloglucan utilization* functions increased between units D, R and N, while the potential of the *Chitin and N-acetylglucosamine ultilization* function was higher in unit D. Cellulose and xyloglucan (a type of hemicellulose) are structural constituents of plant tissue (Scheller and Ulvskov, 2010), whereas chitin (a polymer consisting of N-acetylglucosamine monomers) is a primary component of fungal cell walls (Bartnicki-Garcia, 1968). My results suggest the greatest importance of plant-derived C in the natural wetland, and the least importance in the degraded wetland, possibly due to seasonal removal of crop residues (Yang et al., 2013). However, cellulosomes belong exclusively to anaerobic bacteria (Schwarz, 2001). It is therefore possible that the observed abundance pattern of the *cellulosome* function is instead due to parallel shifts in populations of known cellulolytic anaerobes (e.g., *Clostridia*; **Figure 4c**) (Schwarz, 2001), resulting from hydrology-driven anoxia rather than differences in the biochemistry of C inputs. This latter hypothesis is supported by the high relative abundance of *Actinobacteria* in unit D (and to a lesser extent unit R), as many species within this largely aerobic phylum are cellulolytic (Anderson et al., 2012). Moreover, although some cellulosome-containing *Bacteria* have been isolated from soils, composts, mud and sewage, most are ruminal *Bacteria* (Schwarz, 2001). Therefore, the genetic potential of this function likely does not provide an accurate representation of cellulolytic bacterial assemblages in freshwater sediments. The examination of a wider range of genes encoding cellulose and hemicellulose degrading enzymes would be more

informative. In contrast to the cellulose and hemicellulose degradation functions assessed here, the functional potential for chitin utilization is highest in the degraded wetland, possibly due to greater fungal biomass (and thus greater contributions of fungal necromass to OM inputs) under the aerobic conditions maintained by prolonged agricultural management (Brady and Weil, 2008).

Aside from a general underrepresentation in unit N, I observed no consistent differences in fermentative and acetogenic pathways between the three wetland units (**Figure 6**). For example, the genetic potential of *fermentations: lactate* function was highest in unit D, intermediate in unit R and least lowest in unit N, while no variation in the functional potential of *fermentations: mixed acid* was observed (**Figure 6**). Both fermentative and acetogenic metabolisms are relatively understudied in wetlands (Drake et al., 2008, 2009), and taken together, my results suggest that the genetic potentials of functions within these metabolisms vary differentially due to the SNWR wetland management strategy. This may be because fermentative pathways are found in both facultative and obligate anaerobes, the population dynamics of which can be differentially influenced following recent soil inundation (i.e., as occurred in unit D, and to a lesser extent unit R) (Degelmann et al., 2009). Perhaps most importantly, these results suggest that the prevalence of specific fermentation products may differ between units, which could impact the downstream metabolisms by which they are utilized. Fermentation and acetogenesis are key 'intermediary ecosystem metabolisms' that link the hydrolysis of polysaccharides to terminal respiratory pathways (Drake et al., 2009). More specifically, fermentative bacteria convert hydrolytic products into organic acids, alcohols, CO_2 and hydrogen gas (H_2), which acetogens may then utilize to produce

acetate (Drake et al., 2008). In turn, microorganisms that obtain energy through methanogenesis, sulfate reduction, metal reduction and denitrification, catabolize fermentative and acetogenic metabolites as electron donors (Konhauser, 2009), often in a highly substrate-specific manner (Liu and Whitman, 2008; Pester et al., 2012). As a result, the observed differences in acetogenic and fermentative functional potentials in the SNWR wetland units may indirectly affect key terminal ecosystem processes (e.g., methanogenesis). Elucidating relationships between these microbial functions and the factors driving acetogenic and fermentative genetic potentials may be necessary for the development of successful management strategies at the SNWR.

Respiratory pathways

The ultimate control over microbially-mediated wetland processes is hydrology, as the slow diffusion of O_2 through water limits its availability in sediments and shifts the dominance of respiratory metabolisms from aerobic to anaerobic (Schlesinger and Bernhardt, 2013). Thus, I hypothesized that the abundance of aerobic respiratory functions and organisms would be greatest in unit D, followed by unit R and lastly unit N (lowest, intermediate and highest inundation frequencies, respectively), while anaerobic respiratory pathways and obligate anaerobes would exhibit the opposite trend. As predicted, the potential of the *cytochrome c oxidases* function, an indicator of aerobic respiration (Lipson et al., 2013), and the abundances of predominantly aerobic taxa such as *Actinobacteria* (Anderson et al., 2012) and *Thaumarchaeota* (Offre et al., 2013) were highest in unit D, followed by unit R and unit N (**Figure 3 and Figure 6**). All known members of the *Thaumarchaeota* are involved in the aerobic oxidation of ammonia, or nitrification (Pester et al., 2011; Offre et al., 2013), and it is therefore likely that

nitrification is more important to N cycling in unit D than in unit R, and more important in unit R than unit N. In contrast, the potentials of *anaerobic respiratory reductases* and the abundances of obligate anaerobes such as *Clostridia* (Schwarz, 2001) and *Methanomicrobia* (Liu and Whitman, 2008) were highest in unit N, followed by unit R and finally unit D.

The competitive advantage attained through the reduction of a specific TEA, and thus the abundance of both the pathway and organism by which it is utilized, largely depends upon E_h and the availability of the TEA in a particular habitat (Canfield and Thamdrup, 2009; Peralta et al., 2014). Denitrification in wetlands has received considerable attention due to its ability to remove N pollution (Vymazal, 2007; Peralta et al., 2010), and the potential for *denitrification* was highest in unit R, whereas units D and N did not differ (**Figure 6**). This is likely because hydrological manipulation has resulted in an E_h low enough in unit R that full reduction of NO_3^- to N_2 is the most thermodynamically favorable respiratory metabolism, but not so low that NO_3^- is reduced faster than made available (presumably the case in unit N). Additionally, the functional potential of *nitrate and nitrite ammonification*, representing dissimilatory nitrate reduction to ammonium (DNRA) (Morrissey et al., 2013), was greatest in unit D, suggesting a shift in the relative contribution of N removal pathways. It is important to note that edaphic characteristics such as C availability have been found to structure both DNRA (Morrissey et al., 2013) and denitrifier (Wallenstein et al., 2006) assemblages, and could therefore also influence the functional potential of N removal pathways in this system. In contrast to denitrification, the genetic potentials of functions related to iron (III) and SO_4^{2-} reduction generally differed as expected (increased from unit D to unit R

to unit N), although that of *Fe(III) reduction – Shewanella type* did not differ between units R and N (**Figure 6**). The importance of SO_4^{2-} reduction to C mineralization in freshwater systems has recently been recognized (Pester et al., 2012), and the detection of sulfate reduction complexes in our metagenomes indicates these metabolisms may serve nontrivial roles in C cycling at the SNWR.

Methane emissions are a primary consideration when restoring, creating or managing wetlands (Mitsch et al., 2013), and the net flux of methane from sediments is controlled by the relative rates of methane production from the reduction of CO_2 as a TEA (methanogenesis) and methane oxidation (methanotrophy) (Walter and Heimann, 2000). The potentials of the *methanogenesis* and *methanogenesis from methylated compounds* functions were highest in unit N, intermediate in unit R and lowest in unit D (**Figure 6**) lending support to my hypotheses regarding anaerobic metabolisms. All five classes of methanogenic *Archaea* (*Methanobacteria, Methanococci, Methanomicrobia, Methanopyri* and *Thermoplasmata*) (Liu and Whitman, 2008) were detected in each metagenome, and the relative abundances of each class paralleled that of the *methanogenesis* function (**Figure 4b and Figure 6**). Aceticlastic and hydrogenotrophic methanogenesis typically dominate freshwater environments (Liu and Whitman, 2008), but the presence of the *methanogenesis from methylated compounds* function and methylotrophic methanogenic *Thermoplasmata* (Poulsen et al., 2013) suggest a role for methylotrophic methanogenesis in these units. Aerobic methane oxidation is carried out by type I and type II methanotrophs. Type I methanotrophs belong to the *Alphaproteobacteria* and utilize the ribulose monophosphate pathway for formaldehyde assimilation, while type II methanotrophs belong to the *Gammaproteobacteria* and

assimilate formaldehyde through the serine cycle (McDonald et al., 2008). The *ribulose monophosphate pathway* function was more abundant in unit N, of intermediate abundance in unit R and least abundant in unit D, while the genetic potential of the *serine-glyoxylate cycle* function was lower in unit N than in either unit R or unit D (**Figure 6**). Interestingly, the trends in both pathways did not match those of the *Alpha-* or *Gammaproteobacteria*, respectively (**Figure 4a**). Particulate methane monooxygenase (pMMO) and soluble methane monooxygenase (sMMO), key methanotrophic enzymes varying in taxonomic associations (McDonald et al., 2008), exhibit opposite trends in genetic potential (**Figure 6**). While it is clear that methanogenic functional potential is greatest in the natural wetland and lowest in the degraded wetland, resolving the relative abundance and taxonomic affiliation of different methanogenic and methylotrophic pathways would require analyzing these metagenomes at finer taxonomic and functional resolutions. The use of custom gene databases (Orellana et al., 2014) and phylogenetic sequence placement approaches (Filipski et al., 2015) would likely yield such resolution, but are beyond the scope of this work.

Nutrient utilization

The potential for the sequestration and removal of N and P motivates many wetland restoration and creation projects (Zedler, 2003; Vymazal, 2007). The fates of N and P in wetlands are diverse. These nutrients can be sequestered or removed through sedimentation, adsorption to soil particles, immobilization in incompletely decomposed OM, by gaseous loss (in the case of N), or mobilized by various biotic and abiotic mechanisms (e.g., OM mineralization) (Vymazal, 2007). Microbial uptake can be an important, albeit small, P sink (Vymazal, 2007), and I therefore assessed two functions

related to microbial P utilization. The Pho regulon is involved in bacterial responses to P starvation (Santos-Beneit, 2015) and the abundance of a phosphatase-encoding gene (*PhoD*) within this regulon was found to be negatively correlated with soil P concentrations in a greenhouse experiment (Fraser et al., 2015). Thus, lower observed functional potential of the Pho regulon in unit D may indicate a less P-limited habitat (**Figure 6**). The genetic potentials of the *alkylphosphonate utilization* function suggest that organic P is a more important source of P in unit D than in unit R, and in unit R relative to unit N (Cook et al., 1978). The functional potential for *nitrogen fixation* exhibits the opposite trend of *alkylphosphonate utilization*, indicating that N may also less available in the natural wetland unit. These results may suggest differences in sediment nutrient concentrations between wetland units, but these hypotheses must be verified by chemical analyses. It is unclear as to how the results of P cycling functions may affect P removal or mobilization in SNWR wetlands, although enhanced potential for bacterial P uptake may suggest a lower likelihood for release of P from sediments (Vymazal, 2007). Since SNWR wetlands may serve as a source of P loading to the Saginaw River (Scott, 2014), these mechanisms warrant further investigation.

Ecological and management considerations

Presumably, communities in the three wetland units assessed here were similar in taxonomic composition and functional potential prior to anthropogenic manipulation. I observed significant differences between the microbial communities in each wetland that suggest management practices have substantially altered their capacity to mediate ecologically and economically valuable biogeochemical processes. From an ecological perspective, communities in the degraded and restored wetlands have been subjected to

press disturbances (Bender et al., 1984), where environmental conditions by which microbial communities are affected (namely hydrology) have been altered for a long period of time. Specifically, the degraded wetland was disconnected from the Shiawassee River floodplain and drained, creating long-term oxic conditions in a habitat that was formerly wetter and more anoxic. Similarly, the restored wetland unit has been largely disconnected from natural riverine hydrology and subjected to muted water level fluctuations to create alternate years of pooled and moist soil conditions as part of the USFWS management strategy. Microbial communities under press disturbances may shift to alternative stable states, in which stabilized community composition and functional potential differ from the pre-disturbance state (Shade et al., 2012). Thus, it may be unreasonable to expect natural and restored microbial communities in this system to return to equivalency if artificial hydrological conditions are maintained. However, assessing the recovery trajectory of these communities through time, as well as their relationships to ecological process rates, will be necessary to determine whether slow recovery or the attainment of an alternative stable state has resulted in the differences I observed and how these differences influence ecosystem function. The degraded wetland unit we assessed is scheduled to be restored by the USFWS (Buchanan et al., 2013) and will provide an excellent opportunity for an extended temporal study of microbial responses to restoration.

Ultimately, the management of wetland ecosystems to achieve desired functionality requires a predictive understanding of the drivers of wetland microbial community composition and functional potential and the relationship of these characteristics to ecosystem processes. Such knowledge will allow for 1) the use of

microbial indicators to assess the status of a restored or managed wetland and 2) the ability to manipulate environmental conditions to attain certain microbial functions. It is important to note that this study did not include replication at the level of wetland units. Thus, I cannot generalize my results to predict the responses of microbial communities in other SNWR wetland units to the management strategies employed at the three units assessed here. Additionally, I have quantified the genetic capacity of microbial functions to be carried out rather than actual process rates. The relationship between community characteristics and ecosystem processes is not necessarily straightforward (Nemergut et al., 2014), but combining community analyses with the measurement of process rates would improve our understanding of such linkages in this study system (Gutknecht et al., 2006) and determine whether observed changes in genetic potential are realized as differences in biogeochemical functioning. Despite these limitations, my results are relevant to the restoration and management of the three wetland units assessed here. For example, maximizing denitrification to remove eutrophying N pollution and minimizing the emissions of methane are two primary goals when restoring or creating wetlands (Peralta et al., 2010; Mitsch et al., 2013). I observed that the functional potential for denitrification was highest in the restored wetland community, whereas this same community had an intermediate methanogenic potential. Moreover, hydrological manipulation appears to be fundamental to structuring microbial communities in the three wetland units assessed in this study, although my results also suggest potential driving roles for variation in both C inputs and sediment nutrient concentrations. Distance-based linear modeling has been successfully used to explain variation in metagenomic data with environmental parameters (Kelly et al., 2014), and could be utilized with this dataset to

elucidate the role of additional driving factors. While hydrological manipulation will remain the principle means of restoration and management in this system (Buchanan et al., 2013), understanding how edaphic characteristics structure microbial communities would provide insights into more targeted management practices to manipulate microbial processes.

Conclusions

The broad approach I have applied here demonstrates that microbial metabolic pathways involved in biogeochemical cycling in these wetlands differ substantially, but not uniformly, across a gradient of floodplain connectivity and hydrology. Furthermore, the results of this study add to the growing body of evidence that numerous characteristics of microbial communities differ between natural wetlands and those that are considered restored and degraded. Elucidating differences in relevant metabolic pathways at finer taxonomic and functional resolutions, as well as their drivers, will be necessary to better explain how taxonomic composition and functional potential of soil and sediment microbial communities are controlled in the SNWR and other wetland systems. Additional information of this kind and its relationship to process rates may someday allow management practices to be tailored toward desired microbially-mediated ecological functions. A primary goal of restoration and management is to recover the valuable biogeochemical processes afforded by natural wetland ecosystems, and this study helps emphasize that understanding the microbial framework underlying wetland functions is necessary to both inform and evaluate our restoration and management practices.

TABLES

Table 1: Summary of sequence quality control (QC) and general annotation data. 'Pre-upload' refers to sequence processing done prior to submission to MG-RAST; all other categories are from the MG-RAST QC and annotation pipelines.

	Value for:								
	Degraded Wetland			Restored Wetland			Natural Wetland		
Category	D1	D2	D3	R1	R2	R3	N1	N2	N3
Pre-upload read count									
Raw	21,614,914	19,992,945	19,599,899	19,027,498	15,202,749	17,611,254	15,996,877	16,588,597	26,846,396
Trimmed	20,762,035	19,214,509	18,842,179	18,288,285	14,620,507	16,932,765	15,361,247	15,952,369	25,788,504
Subsampled	14,620,507	14,620,507	14,620,507	14,620,507	14,620,507	14,620,507	14,620,507	14,620,507	14,620,507
Pre-upload read length, bp									
Raw	151	151	151	151	151	151	151	151	151
Trimmed	148	148	148	148	148	148	148	148	148
Post-QC									
Read count	14,117,564	14,085,025	14,099,642	14,153,824	14,118,436	14,138,893	14,203,004	14,167,554	14,218,179
Mean read length, bp	140 ± 20	139 ± 21	140 ± 20	140 ± 20	140 ± 20	140 ± 20	141 ± 19	141 ± 19	141 ± 19
Mean GC content, %	62 ± 11	62 ± 11	62 ± 10	61 ± 10	62 ± 10	61 ± 10	57 ± 11	57 ± 11	57 ± 11
Post-processing									
Predicted protein features	12,655,920	12,592,633	12,693,867	12,869,910	12,784,501	12,739,869	12,899,119	12,879,889	12,910,985
Identified protein features	4,095,109	4,121,593	4,096,545	4,114,378	4,162,895	4,151,804	3,912,389	3,956,028	3,904,461
Predicted rRNA features	154,634	160,224	155,113	146,101	150,878	146,200	133,278	134,780	136,598
Identified rRNA features	5,685	5,790	5,718	5,694	5,614	5,330	5,353	5,426	5,666
Post-annotation									
Identified functional categories	3,168,144	3,195,520	3,170,954	3,242,389	3,273,982	3,276,788	3,085,812	3,110,256	3,066,974

Table 2: Mean species richness and mean relative abundances (% of annotated sequences) at the domain and phylum level with standard deviation (SD). Unit comparison denotes statistical significance based on Tukey's HSD adjusted *P*-values; $P < 0.05$.

	Value for:						
	Degraded Wetland (D)		Restored Wetland (R)		Natural Wetland (N)		Unit comparison
Classification	Mean	SD	Mean	SD	Mean	SD	(D N R)
Richness (species)	622	8	708	14	748	7	(a b c)
Domain							
Archaea	2.625	0.035	3.530	0.322	5.193	0.302	(a b c)
Bacteria	93.447	0.087	91.969	0.321	89.719	0.213	(a a b)
Eukaryota	0.300	0.005	0.309	0.011	0.352	0.020	(a a a)
Other	3.628	0.065	4.191	0.011	4.737	0.089	-
Archaeal phyla							
Crenarchaeota	0.354	0.011	0.368	0.009	0.644	0.025	(a a b)
Euryarchaeota	1.518	0.042	2.888	0.386	4.352	0.273	(a b c)
Korarchaeota	0.029	0.001	0.056	0.005	0.116	0.003	(a b c)
Nanoarchaeota	0.004	0.000	0.003	0.001	0.008	0.001	(a a b)
Thaumarchaeota	0.719	0.061	0.215	0.070	0.073	0.004	(a b c)
Bacterial phyla							
Acidobacteria	4.485	0.156	3.760	0.414	3.044	0.084	(a b c)
Actinobacteria	23.936	0.776	15.052	2.747	5.565	0.143	(a b c)
Aquificae	0.202	0.007	0.285	0.012	0.380	0.015	(a b c)
Bacteroidetes	3.480	0.183	3.155	0.336	5.118	0.330	(a a b)
Chlamydiae	0.086	0.005	0.103	0.009	0.118	0.002	(a b b)
Chlorobi	0.748	0.029	1.224	0.121	1.678	0.027	(a b c)
Chloroflexi	2.704	0.057	3.937	0.076	4.388	0.133	(a b b)
Cyanobacteria	2.434	0.070	2.827	0.156	2.663	0.023	(a b a)
Deferribacteres	0.056	0.004	0.100	0.009	0.151	0.006	(a b c)
Deinococcus-Thermus	0.897	0.014	1.010	0.031	0.910	0.017	(a b a)
Dictyoglomi	0.118	0.004	0.218	0.020	0.366	0.011	(a b c)
Elusimicrobia	0.030	0.001	0.051	0.007	0.080	0.001	(a b c)
Firmicutes	4.793	0.091	6.551	0.330	8.635	0.147	(a b c)
Fusobacteria	0.075	0.002	0.111	0.014	0.167	0.001	(a b c)
Planctomycetes	2.616	0.036	1.974	0.087	1.932	0.103	(a b c)
Proteobacteria	44.720	0.506	49.228	1.788	51.550	0.141	(a b b)
Spirochaetes	0.175	0.006	0.241	0.029	0.314	0.001	(a b c)
Synergistetes	0.101	0.002	0.183	0.018	0.258	0.001	(a b c)
Tenericutes	0.029	0.001	0.035	0.003	0.049	0.003	(a a b)
Thermotogae	0.304	0.009	0.505	0.043	0.753	0.012	(a b c)
Verrucomicrobia	1.055	0.043	0.968	0.083	1.158	0.062	(a a a)
Unclassified Bacteria	0.401	0.004	0.450	0.018	0.443	0.016	(a b ab*)

*N does not differ significantly from D or R.

Table 3: Relative abundances of selected SEED Subsystem Level 3 functions, presented as reads per million reads.

	Value for:					
	Degraded Wetland (D)		Restored Wetland (R)		Natural Wetland (N)	
Function	Mean	SD	Mean	SD	Mean	SD
Acetogenesis						
CO dehydrogenase	1142.3	48.3	790.2	235.2	221.6	11.7
Acetogenesis from pyruvate	2807.7	31.7	2764.8	74.1	2364.9	36.7
Pyruvate:ferredoxin oxidoreductase	403.4	10.3	654.7	34.6	781.8	3.3
Aerobic respiration						
Terminal cytochrome C oxidases	875.7	11.2	812.1	37.0	597.8	10.7
Anaerobic respiration						
Anaerobic respiratory reductases	1369.0	28.9	2250.9	128.7	2754.9	52.9
Fermentation						
Acetoin, butanediol metabolism	645.7	12.4	766.7	28.0	711.8	11.7
Acetone butanol ethanol synthesis	2069.0	27.9	2174.9	60.3	2027.1	24.4
Acetyl-CoA fermentation to butyrate	2470.2	27.7	2570.5	91.1	2332.6	13.9
Butanol biosynthesis	1953.6	24.3	2047.2	68.1	1815.2	9.1
Fermentations: Lactate	662.7	5.6	583.4	33.9	471.5	8.3
Fermentations: Mixed acid	928.0	35.6	962.0	49.1	1012.1	33.4
Iron reduction						
Fe(III) respiration - Shewanella type	4.4	0.4	6.0	0.8	5.9	0.3
Methanogenesis						
Methanogenesis	140.0	8.6	536.7	95.6	928.3	39.3
Methanogenesis from methylated compounds	15.5	1.3	48.7	7.4	70.0	3.9
Methanogenesis strays	8.1	0.9	52.5	10.5	70.6	8.5
Methanopterin biosynthesis	249.6	5.7	282.0	11.6	292.6	2.7
Methanotrophy						
Ribulose monophosphate pathway	18.2	1.7	28.7	4.6	44.9	0.8
Particulate methane monooxygenase (pMMO)	0.3	0.2	1.5	0.2	3.5	0.6
Serine-glyoxylate cycle	7469.1	49.1	7558.6	121.7	6905.1	17.4
Soluble methane monooxygenase (sMMO)	14.3	0.9	2.0	0.5	0.6	0.1
Nitrogen metabolism						
Denitrification	255.1	11.0	317.1	16.4	274.5	11.3
Dissimilatory nitrite reductase	141.6	3.3	186.5	5.0	179.0	5.6
Nitrate and nitrite ammonification	914.2	36.2	819.5	16.8	633.4	13.5
Nitrogen fixation	153.7	8.7	296.5	34.4	398.3	35.9
Phosphorus metabolism						
Alkylphosphonate utilization	176.4	4.3	128.4	16.9	66.9	4.7
Phosphate transporter and PHO regulon	1235.8	23.9	1421.5	26.8	1454.6	27.3
Polysaccharide degradation						
Cellulosome	44.2	2.9	53.4	1.7	65.5	2.8
Chitin and N-acetylglucosamine utilization	465.2	15.0	407.5	7.5	390.3	18.0
Xyloglucan utilization	77.2	4.5	122.0	17.9	185.2	9.0
Sulfur reduction						
Sulfate reduction-associated complexes	23.5	2.9	224.8	40.5	360.3	7.4

FIGURES

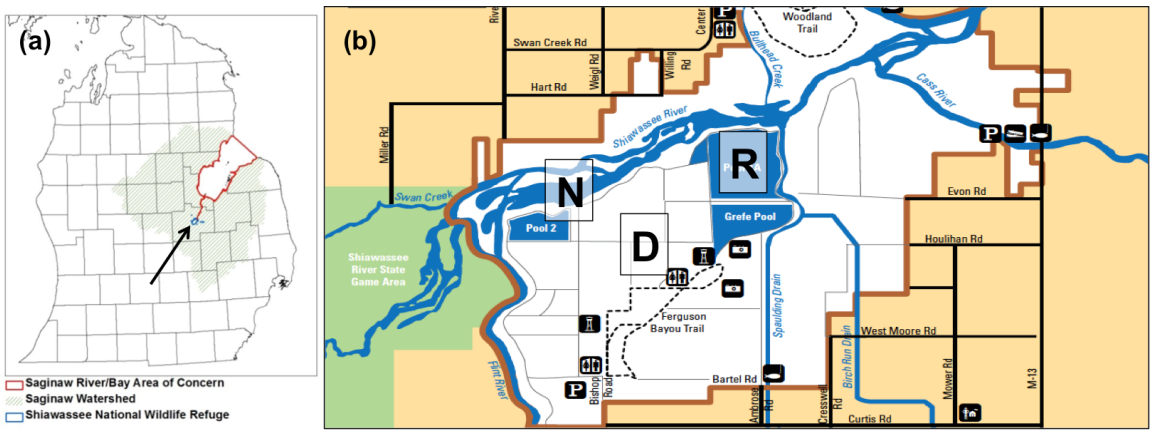

Figure 1: Locations of the Shiawassee National Wildlife Refuge in Saginaw County, MI, USA (**a**) (Buchanan et al., 2013), and the wetland units assessed in this study (**b**) (http://www.fws.gov/refuge/Shiawassee/visit/plan_your_visit/brochures.html); unit D (Farm Unit 1), unit R (Pool 1A) and unit N (Shiawassee River backwaters) (see **Table S1**).

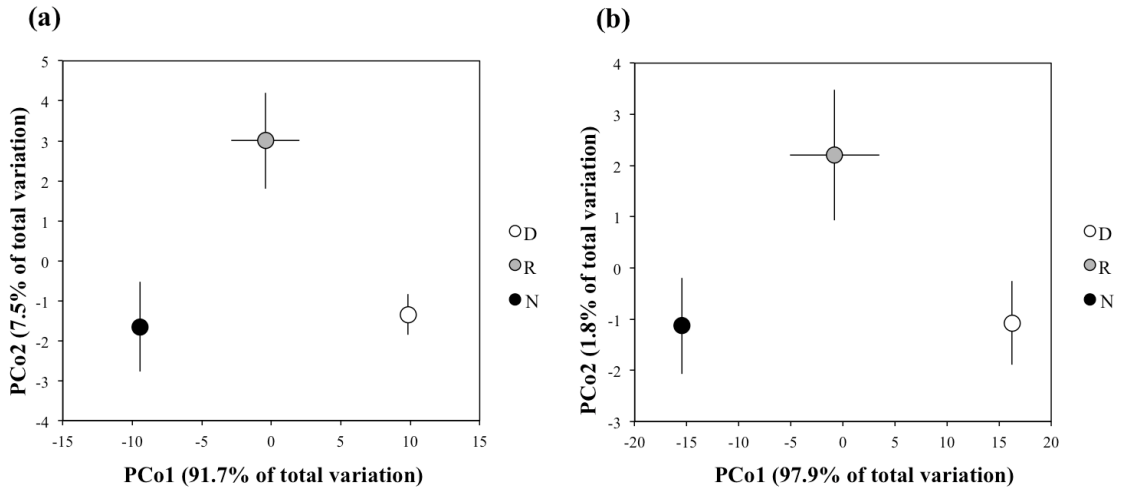

Figure 2: Ordinations developed from principle coordinates analysis (PCoA) of Bray-Curtis similarity at phylum (**a**) and class (**b**) taxonomic levels. Main effects and all pairwise unit comparisons were significant by PerMANOVA ($P < 0.05$; **Table S5**) at both taxonomic levels.

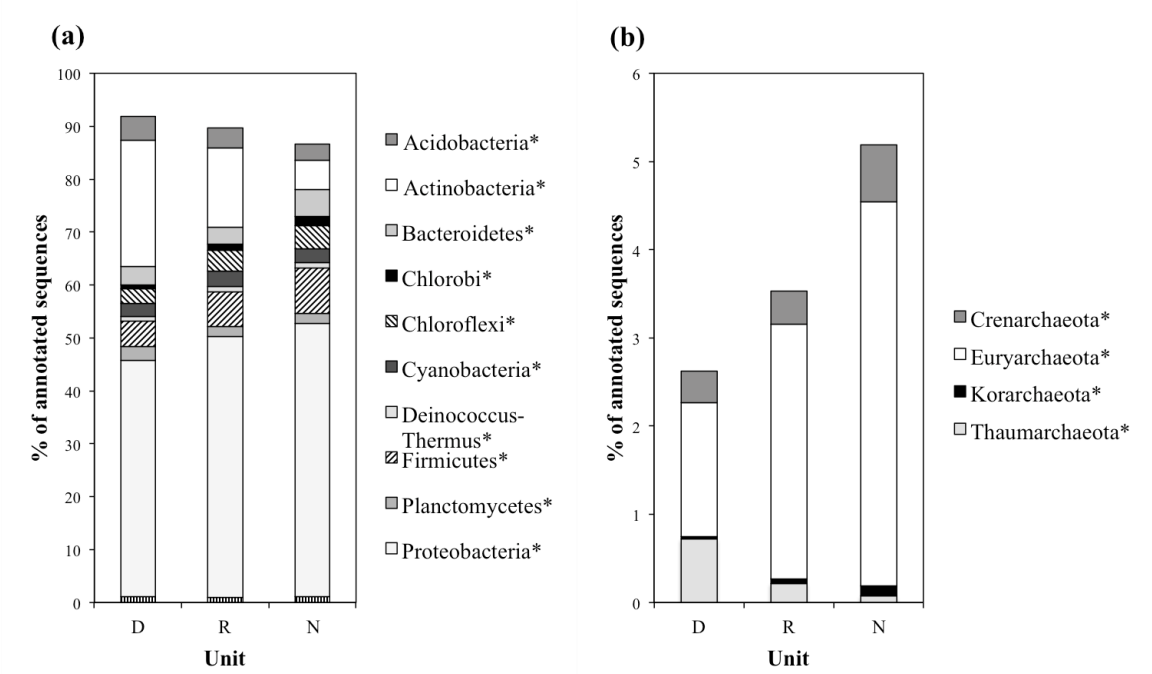

Figure 3: Relative abundance of dominant bacterial (**a**) and archaeal (**b**) phyla, presented as mean percentage of annotated sequences by wetland unit. Abundances for *Eukaryota* and "other" are not shown. *, main test significant difference detected by one-way ANOVA ($P < 0.05$; **Table 2 and Table S6)**.

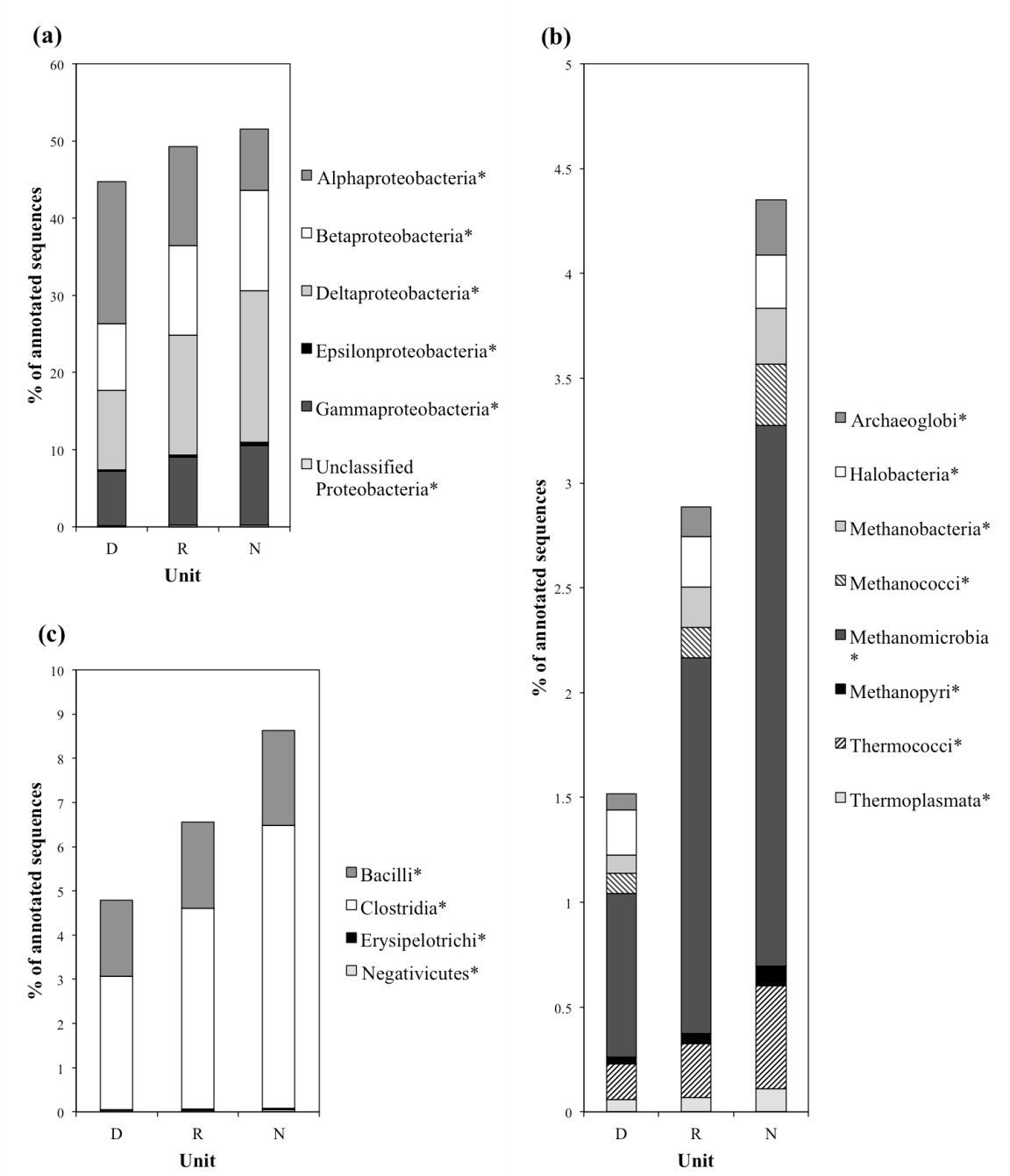

Figure 4: Relative abundances of classes belonging to the *Proteobacteria* (**a**) *Euryarchaeota* (**b**) and *Firmicutes* (**c**), presented as mean percentage of annotated sequences by unit. *, main test significant difference detected by one-way ANOVA ($P < 0.05$; **Table S7 and Table S8**).

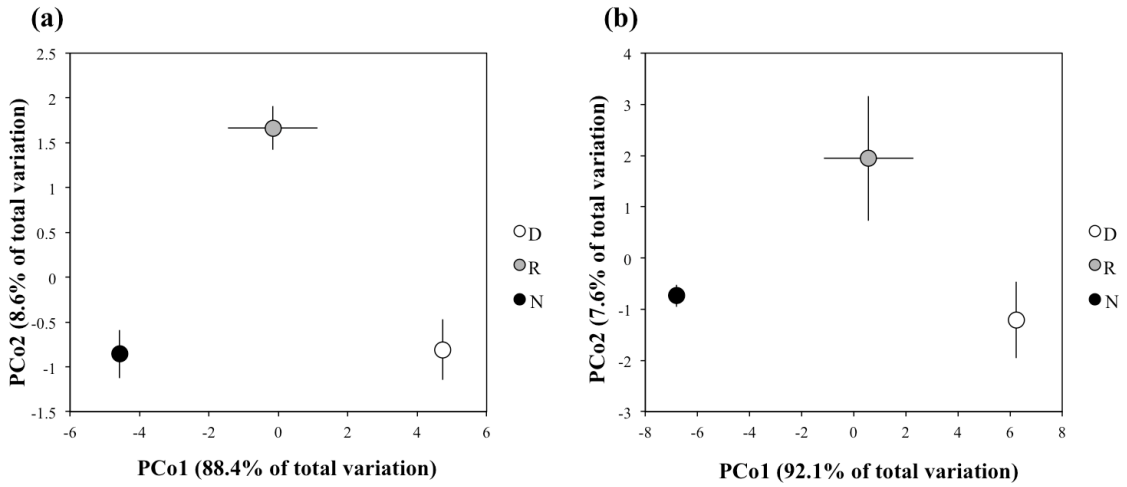

Figure 5: Ordinations developed from principle coordinates analysis (PCoA) of Bray-Curtis similarity for all (**a**) and selected (**b**) SEED Subsystems Level 3 functions. Main effects and all pairwise unit comparisons were significant by PerMANOVA ($P < 0.05$; **Table S5**).

Figure 6: Selected SEED Subsystems Level 3 functions involved in important microbial functions in wetlands, represented as Z-scores calculated from relative function abundances. Warm colors and cool colors correspond to high and low Z-scores, respectively. Unit comparisons indicate differences in mean function abundances (**Table 3**); different letters indicate significant difference between means (one-way ANOVA, Tukey's HSD adjusted $P < 0.05$; **Table S9**).

	Value for:									
	Degraded Wetland			Restored Wetland			Natural Wetland			
Function	D1	D2	D3	R1	R2	R3	N1	N2	N3	Unit comparisons
Acetogenesis										
CO dehydrogenase	1.11	0.88	1.04	-0.47	0.46	0.53	-1.15	-1.19	-1.21	(a b c)
Acetogenesis from pyruvate	0.63	0.91	0.71	0.21	0.90	0.54	-1.38	-1.11	-1.42	(a a b)
Pyruvate:ferredoxin oxidoreductase	-1.24	-1.32	-1.20	0.48	0.09	0.16	1.03	1.00	0.99	(a b c)
Aerobic respiration										
Terminal cytochrome C oxidases	0.92	0.79	0.96	0.06	0.53	0.59	-1.34	-1.33	-1.19	(a b c)
Anaerobic respiration										
Anaerobic respiratory reductases	-1.28	-1.24	-1.19	0.45	0.07	0.10	1.13	1.00	0.96	(a b c)
Fermentation										
Acetoin, butanediol metabolism	-1.24	-1.29	-0.88	0.54	1.11	1.55	0.20	0.18	-0.18	(a b c)
Acetone butanol ethanol synthesis	-0.68	0.06	-0.24	0.21	1.70	1.49	-0.48	-0.95	-1.10	(a b a)
Acetyl-CoA fermentation to butyrate	-0.13	0.36	0.10	0.07	1.54	1.35	-0.96	-1.19	-1.15	(a a b*)
Butanol biosynthesis	-0.09	0.37	0.14	0.37	1.64	1.02	-1.23	-1.06	-1.16	(a a b)
Fermentations: Lactate	1.12	0.99	1.07	-0.33	0.31	0.40	-1.21	-1.08	-1.27	(a b c)
Fermentations: Mixed acid	-1.29	0.02	-1.08	0.78	0.05	-1.15	0.29	1.60	0.77	(a a a)
Iron reduction										
Fe(III) respiration - Shewanella type	-0.74	-1.03	-1.64	1.06	-0.36	1.22	0.14	0.53	0.81	(a b b)
Methanogenesis										
Methanogenesis	-1.16	-1.16	-1.12	0.32	-0.13	-0.17	1.27	1.08	1.07	(a b c)
Methanogenesis from methylated compounds	-1.27	-1.21	-1.16	0.50	0.09	-0.10	0.96	1.23	0.94	(a b c)
Methanogenesis strays	-1.27	-1.24	-1.22	0.73	0.11	0.08	1.28	0.75	0.78	(a b b)
Methanopterin biosynthesis	-1.49	-0.93	-1.26	0.99	-0.12	0.19	0.72	0.94	0.96	(a b b)
Methanotrophy										
Ribulose monophosphate pathway	-1.14	-0.88	-1.11	0.22	-0.56	-0.13	1.25	1.22	1.12	(a b c)
Particulate methane monooxygenase (pMMO)	-0.82	-1.02	-1.17	-0.19	-0.33	-0.09	0.73	1.28	1.61	(a b c)
Serine-glyoxylate cycle	0.34	0.64	0.53	0.36	1.12	0.88	-1.30	-1.23	-1.34	(a a b)
Soluble methane monooxygenase (sMMO)	1.30	1.47	1.20	-0.60	-0.46	-0.60	-0.77	-0.74	-0.79	(a b b)
Nitrogen metabolism										
Denitrification	-1.13	-0.48	-1.13	1.76	0.68	1.08	-0.02	-0.06	-0.70	(a b a)
Dissimilatory nitrite reductase	-1.20	-1.21	-1.47	0.67	0.70	1.10	0.55	0.68	0.18	(a b b)
Nitrate and nitrite ammonification	0.93	1.31	0.75	0.12	0.38	0.23	-1.30	-1.12	-1.30	(a b c)
Nitrogen fixation	-1.20	-1.25	-1.09	0.47	-0.14	0.04	1.42	0.78	0.97	(a b c)
Phosphorus metabolism										
Alkylphosphonate utilization	1.17	0.99	1.09	-0.31	0.31	0.27	-1.07	-1.20	-1.26	(a b c)
Phosphate transporter and PHO regulon	-1.55	-1.19	-1.13	0.78	0.35	0.32	0.84	1.04	0.53	(a b b)
Polysaccharide degradation										
Cellulosome	-1.33	-1.15	-0.73	-0.18	0.11	-0.23	0.89	1.14	1.47	(a b c)
Chitin and N-acetylglucosamine utilization	1.08	1.69	0.90	-0.41	-0.15	-0.56	-0.97	-0.30	-1.28	(a b b)
Xyloglucan utilization	-1.17	-1.02	-0.99	0.27	-0.18	-0.47	0.98	1.24	1.34	(a b c)
Sulfur reduction										
Sulfate reduction-associated complexes	-1.22	-1.22	-1.19	0.45	-0.09	0.08	1.10	1.08	1.01	(a b c)

*N does not differ significantly from D (Tukey's HSD adjusted $P = 0.0523$).

SUPPLEMENTAL MATERIAL

Table S1: Location of study plots in the degraded (D), restored (R) and natural (N) wetland units at the Shiawassee National Wildlife Refuge in Saginaw County, MI, USA.

	Value for:	
Study Plot	Latitude	Longitude
Degraded Wetland (Unit D)		
D1	43.348961°	-84.028456°
D2	43.349067°	-84.02845°
D3	43.34905°	-84.028389°
Restored Wetland (Unit R)		
R1	43.3575°	-84.009972°
R2	43.358528°	-84.008667°
R3	43.357694°	-84.009722°
Natural Wetland (Unit N)		
N1	43.355383°	-84.040067°
N2	43.355567°	-84.040083°
N3	43.3557°	-84.039883°

Table S2: Mean normalized relative abundances (reads per million reads) of taxonomic annotations at domain and phylum levels with standard deviations (SD). Annotations are based on SEED database.

	Value for:					
	Degraded Wetland (D)		Restored Wetland (R)		Natural Wetland (N)	
Classification	Mean	SD	Mean	SD	Mean	SD
Domain						
Archaea	8507.3	160.7	11353.9	876.5	15462.4	866.4
Bacteria	302853.4	1468.0	296030.5	5067.9	267188.6	2877.9
Eukaryota	972.2	14.4	994.5	28.1	1048.2	50.9
Other[a]	11759.4	239.7	13491.1	220.3	14106.7	333.5
Archaeal phyla						
Crenarchaeota	1145.7	34.7	1186.0	31.2	1918.0	66.5
Euryarchaeota	4921.0	126.7	9283.6	1110.0	12958.4	787.5
Korarchaeota	94.9	2.7	180.1	14.3	344.7	9.6
Nanoarchaeota	14.1	0.3	10.5	2.1	23.9	2.4
Thaumarchaeota	2331.6	208.7	693.7	231.7	217.3	9.5
Bacterial phyla						
Acidobacteria	14533.9	429.6	12110.3	1434.0	9064.2	260.6
Actinobacteria	77584.0	2924.9	48527.2	9475.0	16575.7	586.3
Aquificae	656.2	18.4	916.7	32.8	1130.3	38.3
Bacteroidetes	11280.5	629.2	10145.8	956.7	15237.1	897.2
Chlamydiae	278.6	14.0	332.2	25.3	352.4	1.9
Chlorobi	2425.2	82.4	3935.2	334.9	4997.3	33.8
Chloroflexi	8763.5	145.2	12673.4	313.1	13070.8	509.4
Cyanobacteria	7888.1	186.2	9102.4	588.3	7929.7	145.6
Deferribacteres	182.4	10.6	322.5	23.7	448.5	14.8
Deinococcus-Thermus	2907.4	28.3	3251.8	135.0	2711.0	73.6
Dictyoglomi	381.5	10.9	700.2	56.1	1088.5	28.9
Elusimicrobia	95.8	2.3	163.3	19.2	237.0	3.6
Firmicutes	15532.4	219.4	21077.6	768.2	25713.6	454.5
Fusobacteria	242.8	5.7	356.5	39.8	497.1	1.5
Planctomycetes	8478.3	112.9	6350.6	198.7	5755.4	329.6
Proteobacteria	144930.1	956.8	158399.3	3649.0	153518.8	1675.1
Spirochaetes	566.8	17.4	775.6	82.8	934.3	10.1
Synergistetes	327.1	6.7	587.7	48.1	769.7	9.7
Tenericutes	95.2	2.8	113.5	7.1	145.6	10.1
Thermotogae	985.9	25.2	1624.4	115.1	2243.5	40.0
Verrucomicrobia	3417.7	141.3	3114.7	235.8	3448.0	167.7
Unclassified Bacteria	1300.0	19.3	1449.9	73.3	1319.9	59.9

[a]Includes viral, unclassified and unassigned sequences.

Table S3: Mean normalized relative abundances (reads per million reads) of taxonomic annotations for archaeal classes with standard deviations (SD). Annotations are based on SEED database.

Classification		Value for: Degraded Wetland (D)		Restored Wetland (R)		Natural Wetland (N)	
Phylum	Class	Mean	SD	Mean	SD	Mean	SD
Crenarchaeota	Thermoprotei	1145.7	34.7	1186.0	31.2	1918.0	66.5
Euryarchaeota	Archaeoglobi	252.2	6.2	457.2	32.9	782.2	58.7
Euryarchaeota	Halobacteria	698.4	13.3	780.4	35.0	756.0	27.0
Euryarchaeota	Methanobacteria	277.3	6.6	612.6	66.0	793.4	17.6
Euryarchaeota	Methanococci	314.2	4.0	472.9	47.4	876.9	35.3
Euryarchaeota	Methanomicrobia	2534.5	138.5	5757.6	924.2	7681.5	611.2
Euryarchaeota	Methanopyri	103.0	5.1	154.8	14.1	277.1	10.1
Euryarchaeota	Thermococci	554.3	6.5	829.0	56.1	1460.2	22.8
Euryarchaeota	Thermoplasmata	187.1	2.8	219.2	7.0	331.1	10.8
Korarchaeota	Korarchaeota[a]	94.9	2.7	180.1	14.3	344.7	9.6
Nanoarchaeota	Nanoarchaeota[a]	14.1	0.3	10.5	2.1	23.9	2.4
Thaumarchaeota	Thaumarchaeota[a]	2331.6	208.7	693.7	231.7	217.3	9.5

[a]Unclassified sequences belonging to corresponding phylum.

Table S4: Mean normalized relative abundances (reads per million reads) of taxonomic annotations for bacterial classes with standard deviations (SD). Annotations are based on SEED database.

		Value for:					
		Degraded Wetland (D)		Restored Wetland (R)		Natural Wetland (N)	
Classification							
Phylum	Class	Mean	SD	Mean	SD	Mean	SD
Acidobacteria	Solibacteres	10393.8	278.0	8639.6	1093.8	6310.6	175.4
Acidobacteria	Acidobacteria[a]	4140.1	153.1	3470.6	341.1	2753.6	85.6
Actinobacteria	Actinobacteria[b]	77584.0	2924.9	48527.2	9475.0	16575.7	586.3
Aquificae	Aquificae[b]	656.2	18.4	916.7	32.8	1130.3	38.3
Bacteroidetes	Bacteroidia	1628.6	101.9	1899.0	382.4	3757.0	262.1
Bacteroidetes	Cytophagia	3349.0	203.7	2535.3	197.9	3646.5	210.4
Bacteroidetes	Flavobacteriia	2269.5	148.3	2065.9	239.3	3491.9	282.6
Bacteroidetes	Sphingobacteriia	2176.2	184.3	1482.6	136.0	2341.3	158.7
Bacteroidetes	Bacteroidetes[a]	1857.2	49.5	2163.0	34.4	2000.4	13.2
Chlamydiae	Chlamydiia	278.6	14.0	332.2	25.3	352.4	1.9
Chlorobi	Chlorobia	2425.2	82.4	3935.2	334.9	4997.3	33.8
Chloroflexi	Chloroflexi[b]	5499.1	128.2	8707.7	190.2	8479.8	420.1
Chloroflexi	Dehalococcoidetes	486.0	18.2	1209.5	198.9	2443.5	105.3
Chloroflexi	Thermomicrobia[b]	2778.4	12.3	2756.2	302.3	2147.6	87.1
Cyanobacteria	Gloeobacteria	1450.4	33.9	1344.4	108.6	1033.5	21.4
Cyanobacteria	Cyanobacteria[a]	6437.7	154.6	7758.0	479.8	6896.2	126.0
Deferribacteres	Deferribacteres[b]	182.4	10.6	322.5	23.7	448.5	14.8
Deinococcus-Thermus	Deinococci	2907.4	28.3	3251.8	135.0	2711.0	73.6
Dictyoglomi	Dictyoglomia	381.5	10.9	700.2	56.1	1088.5	28.9
Elusimicrobia	Elusimicrobia[b]	95.8	2.3	163.3	19.2	237.0	3.6
Firmicutes	Bacilli	5610.5	80.0	6249.1	25.1	6410.9	120.8
Firmicutes	Clostridia	9760.0	171.0	14625.9	786.0	19068.7	341.9
Firmicutes	Erysipelotrichi	59.9	0.8	77.7	3.1	88.6	1.6
Firmicutes	Negativicutes	102.1	1.9	124.9	1.4	145.4	3.5
Fusobacteria	Fusobacteriia	242.8	5.7	356.5	39.8	497.1	1.5
Planctomycetes	Planctomycetia	8478.3	112.9	6350.6	198.7	5755.4	329.6
Proteobacteria	Alphaproteobacteria	59548.8	1703.6	41238.0	4367.2	23755.1	667.5
Proteobacteria	Betaproteobacteria	28089.0	487.1	37372.9	2568.3	38665.3	1298.7
Proteobacteria	Deltaproteobacteria	33278.5	816.2	49778.5	3671.0	58324.0	1404.2
Proteobacteria	Epsilonproteobacteria	708.2	13.0	987.5	94.7	1404.5	27.0
Proteobacteria	Gammaproteobacteria	22780.3	44.3	28272.1	2291.0	30565.7	1019.1
Proteobacteria	Proteobacteria[a]	525.4	17.1	750.4	38.4	804.3	15.3
Spirochaetes	Spirochaetia	566.8	17.4	775.6	82.8	934.3	10.1
Synergistetes	Synergistia	327.1	6.7	587.7	48.1	769.7	9.7
Tenericutes	Mollicutes	95.2	2.8	113.5	7.1	145.6	10.1
Thermotogae	Thermotogae[b]	985.9	25.2	1624.4	115.1	2243.5	40.0
Verrucomicrobia	Opitutae	2654.4	107.9	2357.9	173.8	2543.1	117.9
Verrucomicrobia	Verrucomicrobiae	322.5	20.3	321.6	38.4	437.5	34.7
Verrucomicrobia	Verrucomicrobia[a]	440.8	20.4	435.1	24.6	467.4	17.7
Unclassified Bacteria	-	1300.0	19.3	1449.9	73.3	1319.9	59.9

[a]Unclassified sequences belonging to the corresponding phylum.
[b]Class name same as phylum.

Table S5: Results of PerMANOVA tests for taxonomic abundances, all and selected SEED Subsystems Level 3 functions.

	Main test		Pairwise tests		
	Statistic		Statistic		
Classification or function	Pseudo-F	P	t	P	Unique permutations
Phylum					
Main	75.7	< 0.001		-	252
D, R		-	5.9	< 0.001	10
D, N		-	24.1	< 0.001	10
R, N		-	5.3	< 0.001	10
Class					
Main	90.6	< 0.001		-	253
D, R		-	6.2	< 0.001	10
D, N		-	28.0	< 0.001	10
R, N		-	5.3	< 0.001	10
All functions					
Main	51.3	< 0.001		-	253
D, R		-	5.0	< 0.001	10
D, N		-	14.1	< 0.001	10
R, N		-	4.7	< 0.001	10
Selected functions					
Main	71.6	< 0.001		-	253
D, R		-	4.9	< 0.001	10
D, N		-	21.7	< 0.001	10
R, N		-	6.0	< 0.001	10

Table S6: Results of one-way ANOVA tests for taxonomic abundances at domain and phylum levels.

	Main test			Pairwise comparisons			
	Statistic			Tukey's Adj. *P*-value			
Classification	F-value	P-value	B-H adj. P-value	N-D	R-D	R-N	(D R N)
Richness	125.5	< 0.001	-	< 0.001	< 0.001	0.0064	(a b c)
Domain							
Archaea	71.2	< 0.001	< 0.001	< 0.001	0.0068	0.0010	(a b c)
Bacteria	89.3	< 0.001	< 0.001	< 0.001	0.1148	< 0.001	(a a b)
Eukaryota	3.8	0.0849	0.0849	-	-	-	(a a a)
Archaeal phyla							
Crenarchaeota	257.7	< 0.001	< 0.001	< 0.001	0.5748	< 0.001	(a a b)
Euryarchaeota	78.0	< 0.001	< 0.001	< 0.001	0.0012	0.0030	(a b c)
Korarchaeota	479.0	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	(a b c)
Nanoarchaeota	40.7	< 0.001	< 0.001	0.0017	0.1275	< 0.001	(a a b)
Thaumarchaeota	113.7	< 0.001	< 0.001	< 0.001	< 0.001	0.0406	(a b c)
Bacterial phyla							
Acidobacteria	29.3	< 0.001	0.0010	< 0.001	0.0341	0.0127	(a b c)
Actinobacteria	84.9	< 0.001	< 0.001	< 0.001	0.0020	0.0012	(a b c)
Aquificae	176.3	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	(a b c)
Bacteroidetes	30.4	< 0.001	< 0.001	0.0029	0.2959	< 0.001	(a a b)
Chlamydiae	15.5	0.0043	0.0048	0.0040	0.0184	0.3662	(a b b)
Chlorobi	125.2	< 0.001	< 0.001	< 0.001	< 0.001	0.0015	(a b c)
Chloroflexi	134.7	< 0.001	< 0.001	< 0.001	< 0.001	0.4121	(a b b)
Cyanobacteria	10.6	0.0106	0.0115	0.9894	0.0156	0.0182	(a b a)
Deferribacteres	178.0	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	(a b c)
Deinococcus-Thermus	27.6	< 0.001	0.0012	0.0827	0.0082	< 0.001	(a b a)
Dictyoglomi	275.1	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	(a b c)
Elusimicrobia	116.4	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	(a b c)
Firmicutes	276.8	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	(a b c)
Fusobacteria	90.2	< 0.001	< 0.001	< 0.001	0.0024	< 0.001	(a b c)
Planctomycetes	114.7	< 0.001	< 0.001	< 0.001	< 0.001	0.0453	(a b c)

Table S6 continued: Results of one-way ANOVA tests for taxonomic abundances at domain and phylum levels.

	Main test			Pairwise comparisons			
	Statistic			Tukey's Adj. *P*-value			
Classification	F-value	*P*-value	B-H adj. *P*-value	N-D	R-D	R-N	(D R N)
Proteobacteria	24.6	0.0013	0.0015	0.0107	0.0011	0.1010	(a b b)
Spirochaetes	42.2	< 0.001	< 0.001	< 0.001	0.0048	0.0176	(a b c)
Synergistetes	181.4	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	(a b c)
Tenericutes	36.4	< 0.001	< 0.001	< 0.001	0.0507	0.0041	(a a b)
Thermotogae	229.8	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	(a b c)
Verrucomicrobia	2.9	0.1283	0.1283	-	-	-	(a a a)
Unclassified Bacteria	6.4	0.0326	0.0339	0.9022	0.0381	0.0652	(a b ab*)

Table S7: Results of one-way ANOVA tests for archaeal class abundances.

Classification		Main test Statistic			Pairwise comparisons Tukey's Adj. P-value			
Phylum	Class	F-value	P-value	B-H adj. P-value	N-D	R-D	R-N	(D R N)
Crenarchaeota	Thermoprotei	257.7	< 0.001	< 0.001	< 0.001	0.5748	< 0.001	(a a b)
Euryarchaeota	Archaeoglobi	140.7	< 0.001	< 0.001	< 0.001	0.0016	< 0.001	(a b c)
Euryarchaeota	Halobacteria	7.5	0.0234	0.0248	0.0846	0.0218	0.5377	(a b ab*)
Euryarchaeota	Methanobacteria	131.2	< 0.001	< 0.001	< 0.001	< 0.001	0.0034	(a b c)
Euryarchaeota	Methanococci	215.6	< 0.001	< 0.001	< 0.001	0.0031	< 0.001	(a b c)
Euryarchaeota	Methanomicrobia	48.8	< 0.001	< 0.001	< 0.001	0.0021	0.0248	(a b c)
Euryarchaeota	Methanopyri	220.7	< 0.001	< 0.001	< 0.001	0.0022	< 0.001	(a b c)
Euryarchaeota	Thermococci	522.7	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	(a b c)
Euryarchaeota	Thermoplasmata	295.6	< 0.001	< 0.001	< 0.001	0.0051	< 0.001	(a b c)
Korarchaeota	Unclassified Korarchaeota	479.0	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	(a b c)
Nanoarchaeota	Unclassified Nanoarchaeota	40.7	< 0.001	< 0.001	0.0017	0.1275	< 0.001	(a a b)
Thaumarchaeota	Unclassified Thaumarchaeota	113.7	< 0.001	< 0.001	< 0.001	< 0.001	0.0406	(a b c)

*N does not differ significantly from D or R.

Table S8: Results of one-way ANOVA tests for bacterial class abundances.

		Main test			Pairwise comparisons			
Classification		Statistic			Tukey's Adj. P-value			
Phylum	Class	F-value	P-value	B-H adj. P-value	N-D	R-D	R-N	(D R N)
Acidobacteria	Solibacteres	28.9	< 0.001	0.0011	< 0.001	0.0397	0.0117	(a b c)
Acidobacteria	Unclassified Acidobacteria	29.4	< 0.001	0.0011	< 0.001	0.0235	0.0174	(a b c)
Actinobacteria	Actinobacteria	84.9	< 0.001	< 0.001	< 0.001	0.0020	0.0012	(a b c)
Aquificae	Aquificae	176.3	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	(a b c)
Bacteroidetes	Bacteroidia	53.6	< 0.001	< 0.001	< 0.001	0.4910	< 0.001	(a a b)
Bacteroidetes	Cytophagia	23.8	0.0014	0.0017	0.2522	0.0066	0.0013	(a b c)
Bacteroidetes	Flavobacteriia	33.6	< 0.001	< 0.001	0.0015	0.5576	< 0.001	(a a b)
Bacteroidetes	Sphingobacteriia	24.1	0.001361	0.0017	0.4665	0.0045	0.0015	(a b a)
Bacteroidetes	Unclassified Bacteroidetes	55.3	< 0.001	< 0.001	0.0064	< 0.001	0.0034	(a b c)
Chlamydiae	Chlamydiia	15.5	0.0043	0.0049	0.0040	0.0184	0.3662	(a b b)
Chlorobi	Chlorobia	125.2	< 0.001	< 0.001	< 0.001	< 0.001	0.0015	(a b c)
Chloroflexi	Chloroflexi	125.9	< 0.001	< 0.001	< 0.001	< 0.001	0.5979	(a b b)
Chloroflexi	Dehalococcoidetes	173.0	< 0.001	< 0.001	< 0.001	0.0012	< 0.001	(a b c)
Chloroflexi	Thermomicrobia	11.6	0.0086	0.0093	0.0127	0.9877	0.0150	(a a b)
Cyanobacteria	Gloeobacteria	31.5	< 0.001	< 0.001	< 0.001	0.2074	0.0031	(a a b)
Cyanobacteria	Unclassified Cyanobacteria	15.0	0.0046	0.0053	0.2267	0.0040	0.0291	(a b c)
Deferribacteres	Deferribacteres	178.0	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	(a b c)
Deinococcus-Thermus	Deinococci	27.6	< 0.001	0.0012	0.0827	0.0082	< 0.001	(a b a)
Dictyoglomi	Dictyoglomia	275.1	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	(a b c)
Elusimicrobia	Elusimicrobia	116.4	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	(a b c)
Firmicutes	Bacilli	74.5	< 0.001	< 0.001	< 0.001	< 0.001	0.1260	(a b b)
Firmicutes	Clostridia	255.4	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	(a b c)
Firmicutes	Erysipelotrichi	145.5	< 0.001	< 0.001	< 0.001	< 0.001	0.0017	(a b c)
Firmicutes	Negativicutes	236.5	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	(a b c)
Fusobacteria	Fusobacteriia	90.2	< 0.001	< 0.001	< 0.001	0.0024	< 0.001	(a b c)
Planctomycetes	Planctomycetia	114.7	< 0.001	< 0.001	< 0.001	< 0.001	0.0453	(a b c)

Table S8 continued: Results of one-way ANOVA tests for bacterial class abundances.

		Main test			Pairwise comparisons			
Classification		Statistic			Tukey's Adj. P-value			
Phylum	Class	F-value	P-value	B-H adj. P-value	N-D	R-D	R-N	(D R N)
Proteobacteria	Alphaproteobacteria	128.6	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	(a b c)
Proteobacteria	Betaproteobacteria	35.2	< 0.001	< 0.001	< 0.001	0.0013	0.6377	(a b b)
Proteobacteria	Deltaproteobacteria	90.5	< 0.001	< 0.001	< 0.001	< 0.001	0.0096	(a b c)
Proteobacteria	Epsilonproteobacteria	111.9	< 0.001	< 0.001	< 0.001	0.0024	< 0.001	(a b c)
Proteobacteria	Gammaproteobacteria	22.9	0.0016	0.0018	0.0014	0.0084	0.2081	(a b b)
Proteobacteria	Unclassified Proteobacteria	98.5	< 0.001	< 0.001	< 0.001	< 0.001	0.0950	(a b b)
Spirochaetes	Spirochaetia	42.2	< 0.001	< 0.001	< 0.001	0.0048	0.0176	(a b c)
Synergistetes	Synergistia	181.4	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	(a b c)
Tenericutes	Mollicutes	36.4	< 0.001	< 0.001	< 0.001	0.0507	0.0041	(a a b)
Thermotogae	Thermotogae	229.8	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	(a b c)
Verrucomicrobia	Opitutae	3.6	0.0930	0.0948	-	-	-	(a a a)
Verrucomicrobia	Verrucomicrobiae	12.9	0.0067	0.0074	0.0110	0.9994	0.0106	(a a b)
Verrucomicrobia	Unclassified Verrucomicrobia	2.0	0.2148	0.2148	-	-	-	(a a a)
Unclassified Bacteria	Unclassified Bacteria	6.4	0.0326	0.0339	0.9022	0.0381	0.0652	(a b ab*)

*N does not differ significantly from D or R.

Table S9: Results of one-way ANOVA tests for selected SEED Subsystems Level 3 functions.

	Main test			Pairwise comparisons			
	Statistic			Tukey's HSD adj. *P*			
Function	F	*P*	B-H adj. *P*	N-D	R-D	R-N	(D R N)
Acetogenesis							
CO dehydrogenase	33.6	< 0.001	< 0.001	< 0.001	0.0477	0.0058	(a b c)
Acetogenesis from pyruvate	68.4	< 0.001	< 0.001	< 0.001	0.5880	< 0.001	(a a b)
Pyruvate:ferredoxin oxidoreductase	254.1	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	(a b c)
Aerobic respiration							
Terminal cytochrome C oxidases	118.7	< 0.001	< 0.001	< 0.001	0.0348	< 0.001	(a b c)
Anaerobic respiration							
Anaerobic respiratory reductases	219.3	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	(a b c)
Fermentation							
Acetoin, butanediol metabolism	30.8	< 0.001	< 0.001	0.0123	< 0.001	0.0276	(a b c)
Acetone butanol ethanol synthesis	10.4	0.0111	0.0119	0.4670	0.0439	0.0105	(a b a)
Acetyl-CoA fermentation to butyrate	13.9	0.0056	0.0063	0.0523	0.1474	0.0046	(a a b*)
Butanol biosynthesis	23.1	0.0015	0.0019	0.0162	0.0769	0.0013	(a a b)
Fermentations: Lactate	66.5	< 0.001	< 0.001	< 0.001	0.0075	0.0013	(a b c)
Fermentations: Mixed acid	3.4	0.1048	0.1048	0.0925	0.5810	0.3402	(a a a)
Iron reduction							
Fe(III) respiration - Shewanella type	8.1	0.0199	0.0206	0.0371	0.0257	0.9490	(a b b)
Methanogenesis							
Methanogenesis	129.9	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	(a b c)
Methanogenesis from methylated compounds	95.1	< 0.001	< 0.001	< 0.001	< 0.001	0.0042	(a b c)
Methanogenesis strays	51.0	< 0.001	< 0.001	< 0.001	0.0011	0.0661	(a b b)
Methanopterin biosynthesis	25.7	0.0011	0.0015	0.0011	0.0049	0.2802	(a b b)
Methanotrophy							
Ribulose monophosphate pathway	64.7	< 0.001	< 0.001	< 0.001	0.0103	0.0012	(a b c)
Particulate methane monooxygenase (pMMO)	47.0	< 0.001	< 0.001	< 0.001	0.0310	0.0021	(a b c)
Serine-glyoxylate cycle	64.5	< 0.001	< 0.001	< 0.001	0.3829	< 0.001	(a a b)
Soluble methane monooxygenase (sMMO)	449.6	< 0.001	< 0.001	< 0.001	< 0.001	0.0758	(a b b)

*N does not differ significantly from D.

Table S9 continued: Results of one-way ANOVA tests for selected SEED Subsystems Level 3 functions.

	Main test			Pairwise comparisons			
	Statistic			Tukey's HSD adj. *P*			
Function	F	*P*	B-H adj. *P*	N-D	R-D	R-N	(D R N)
Nitrogen metabolism							
Denitrification	17.5	0.0031	0.0036	0.2447	0.0028	0.0172	(a b a)
Dissimilatory nitrite reductase	77.6	< 0.001	< 0.001	< 0.001	< 0.001	0.2055	(a b b)
Nitrate and nitrite ammonification	103.6	< 0.001	< 0.001	< 0.001	0.0074	< 0.001	(a b c)
Nitrogen fixation	53.3	< 0.001	< 0.001	< 0.001	0.0023	0.0123	(a b c)
Phosphorus metabolism							
Alkylphosphonate utilization	83.5	< 0.001	< 0.001	< 0.001	0.0032	< 0.001	(a b c)
Phosphate transporter and PHO regulon	61.6	< 0.001	< 0.001	< 0.001	< 0.001	0.3314	(a b b)
Polysaccharide degradation							
Cellulosome	53.4	< 0.001	< 0.001	< 0.001	0.0100	0.0027	(a b c)
Chitin and N-acetylglucosamine utilization	22.9	0.0015	0.0019	0.0016	0.0060	0.3618	(a b b)
Xyloglucan utilization	62.9	< 0.001	< 0.001	< 0.001	0.0085	0.0015	(a b c)
Sulfur reduction							
Sulfate reduction-associated complexes	152.1	< 0.001	< 0.001	< 0.001	< 0.001	0.0011	(a b c)

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