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Microbial response over time to hydrologic and fertilization treatments in a simulated wet prairie

Jessica L Mentzer[1], Robert M Goodman[2,3,4] & Teri C Balser[1,2,5]
[1]*Department of Soil Science, University of Wisconsin-Madison, 1525 Observatory Dr., Madison, WI, 53706, USA.* [2]*Gaylord Nelson Institute for Environmental Studies, University of Wisconsin-Madison, 550 N. Park St., Madison, WI, 53706, USA.* [3]*Department of Plant Pathology, University of Wisconsin-Madison, 1630 Linden Dr., Madison, WI, 53706, USA.* [4]*Rutgers Cook College, 88 Lipman Dr. Suite 104, New Brunswick, NJ, 08901, USA.* [5]*Corresponding author**

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Abstract

Naturally occurring wetlands are often threatened by runoff from urban areas, while constructed wetlands are increasingly used to filter sediments and nutrients from urban runoff. To analyze the potential effects of urban runoff on wetland soil microbial activity and composition, we combined phospholipid fatty acid analysis (PLFA) with assays of enzyme activities and nitrification potential (indicators of microbial function) in constructed wetland mesocosms. Mesocosm treatments included nutrient addition treatments (high, low, and none) and hydologic regimes (constant flood, early season flood, and intermittent flood) in a full factorial design. We found that changes in hydrologic regime affected both structural (membrane lipid) and functional (enzyme activities and nitrification potentials) aspects of the microbial community to a greater degree than did nutrient additions. For example, the arbuscular mycorrhizal fungal (AMF) lipid indicator appeared to be highly sensitive to constant flooding. Although hydologic treatments were the dominant factor affecting microbial community structure and function, nutrient additions did appear to alter fungal lipid biomarkers (both mycorrhizal and saprotrophic) and nitrification potentials. In addition, samples taken between June and September indicated a seasonal/temporal progression; for example the effect of the early season flood treatment subsided after flooding ended. Finally, correlation between structure and function data indicate that the study of microbial community structure, in addition to function, may be important for understanding wetland ecosystem function.

Introduction

Wetland ecosystems are both threatened by, and also important to, urban areas (Kercher and Zedler, 2004). Runoff from urban or agricultural areas carries excess water, nutrients, and sediments that potentially alter the physical and chemical environment of wetlands by increasing available phosphorus and nitrogen and increasing water inflow (Watson et al., 1981). Thus, while excess nutrient and water inflow may negatively affect native wetlands (Ewing, 1996; Watson et al., 1981), paradoxically, constructed wetlands are widely used for the filtration of wastewater, storm water, and urban runoff (Gilliam, 1994).

Wetland ecosystems are characterized by hydric soils that support hydrophilic plant communities and have an inflow of water and nutrients

* FAX No: + 1-608-265-2595.
E-mail: tcbalser@wisc.edu

from both surface and subsurface waters with restricted drainage; hydrology also varies seasonally in most wetlands (Mausbach and Parker, 2001). Because of the unusually dynamic physical conditions of wetlands, it is possible that soil microbial community (here defined as the total assemblage of soil microorganisms including potentially interacting and non-interacting portions) function and diversity may also be unique. For instance, the close coupling between aerobic and anaerobic processes in wetland soils (heterotrophic respiration and nitrification are active at a shallow depth, depleting available oxygen and allowing anaerobic, autotrophic processes such as dentrification to dominate below, yet close to, the aerobic zone (Davidsson et al., 1997; Stepanauskas et al., 1996)) may support a widely diverse microbial community. In addition, microbial community structure may vary between permanent and ephemeral wetlands (Boon et al., 1996) or across other wetland types (Borga et al., 1994; Sundh et al., 1997). While prokaryotic organisms may dominate the wetland soil microbial community (Boon et al., 1996), mycorrhizal fungi adapted to wetland conditions are also present (Anupam, 2003) and may vary in species composition with plant species (Ingam and Wilson, 1999) and wetland type (Anupam, 2003).

There is evidence that urban run-off, specifically changes in hydrology and nutrient levels, impacts microbial wetland community function. Increasing water levels may decrease hydrolytic enzyme activities (Freeman et al., 1996; Freeman et al., 1998; Kang et al., 1998; Kang and Freeman, 1999; Pulford and Tabatabai, 1988; Yavitt et al., 2004). Further, increasing dry/wet cycles may alter nitrogen cycling (stimulating denitrification in wet cycles and increasing nitrification in dry cycles) (Qui and McComb, 1996; Tanner et al., 1999; Eaton, 2001; Venterink et al., 2002) or increase enzyme activities (Burns and Ryder, 2001; Corstanje and Reddy, 2004). Nutrient addition may also affect wetland microbial function by increasing processes such as nitrification (Casey et al., 2001; Davidsson and Leonardson, 1998; Davidsson et al., 2002; White and Reddy, 1999) denitrification (Davidsson and Stahl, 2000; Groffman and Crawford, 2003) methanogenesis (Segers, 1998; Weider and Yavitt, 1991) and some enzyme activities (Gusewell and Freeman, 2003; Wright and Reddy, 2001).

Hydrology and nutrient addition may also affect specific microorganisms, such as mycorrhizal fungi (Cornwell et al., 2001; Stevens et al., 2002; Turner and Friese, 1998; Wetzel and VanderValk, 1996) or general microbial community structure (Ravit et al., 2003). However, there is less known about how hydrology and nutrient addition will affect microbial community structure and function together as most studies examine structure or function alone (Mentzer et al., 2006). In particular, microbial structure and function in bulk soil and the rhizosphere (soil adjacent to plant roots) may differ in important ways because of the distinct nutrient dynamics of rhizosphere soil. The rhizosphere receives labile resources from plant roots (Kennedy, 2005) and may also have increased oxygen levels relative to surrounding wetland soil (Weibner et al., 2002; Colmer, 2003). Wetland microbial community structure has also been shown to vary among plant species rhizospheres (Halbritter and Mogyorossy, 2002) and with plant community composition (Borga et al., 1994; Ingam and Wilson, 1999; Sundh et al., 1997). Because rhizosphere microorganisms also impact plant nutrient uptake (Sylvia et al. 2005), the rhizosphere may be important for wetland nutrient cycling.

Temporal variability is another aspect of wetland microbial community response to urban activities. The impacts of anthropogenic disturbance may change over time as the microbial community acclimates and adjusts (Balser and Firestone, 2005), and wetland microbial communities may also shift seasonally (Groffman et al., 1996; Kang and Freeman, 1999). However, while temporal heterogeneities unique to wetland microbial communities may be key to their role in nutrient cycling, most studies have relatively few samples over time.

We investigated the effects of simulated urban runoff (varied hydrology and nutrient addition) on wetland rhizosphere microbial communities in a simulated wet prairie, and quantified changes in microbial community structure and activity over the growing season from May through September. Specifically we asked: Do the structure and function of a soil microbial community change in composition under varied hydrologic or regimes or nutrient levels? Do treatment effects change over the growing season in a constructed wetland? Is

there a relationship between microbial community composition and activity?

Materials and methods

Experimental design

This study was part of a larger project designed to assess the effects of urban runoff and urban activity on a Wisconsin wet prairie plant community (Kercher and Zedler, 2004). We used black stock tanks (0.74 m^3; 0.92 m width, 1.25 m length, 0.65 m tall) as mesocosms, each filled with 15 cm of a fine mason sand (Yahara Materials, Madison, WI, USA) and covered with 30 cm of a pulverized silty loam topsoil (Hammersley, Verona, WI, USA). Mesocosms were planted with native Wisconsin wet prairie species at the University of Wisconsin-Madison Arboretum and grown for two years (2000–2002) before treatments began in June of 2002. The larger experiment included a total of 150 mesocosms arranged into 5 blocks and included treatments designed to test the affects of hydrologic regime, nutrient addition, sediment addition, and simulated grazing on plant species composition (characteristics of urban runoff and urban activity) (Kercher and Zedler, 2004). This study used a subset (focusing only on hydrologic and nutrient treatments) of 50 mesocosms from the larger experiment. The treatments consisted of 3 hydrologic regimes and 3 nutrient additions in a full factorial design with one replication per block in each of 5 blocks. There was also one control (not subjected to treatments) in each block that was watered as needed to sustain plant growth.

Hydrologic treatments were intermittent flood (IF), early season flood (EF), and constant flood (CF). Flooded mesocosms were filled with water 10cm above the soil line and flooded following two-week cycles either during the first two days (IF) or during the entire two weeks (EF and CF) (Figure 1). The EF continued for the first two cycles only (June 17 through July 17) while the CF was flooded June 17 through September 23 (Figure 1). IF and EF mesocosms when not flooded were watered like the control as needed to sustain plant growth. Nutrient loading treatments consisted of high, low, and no nutrients additions using Forever Green Lawn Builder Turf Food® (Eau Claire Crop Oil Company, Eau Claire, Wisconsin, USA) containing 27% nitrogen (1.2% ammonium N; 25.8% urea N), 3% available phosphate (P_2O_5), 4% soluble potash (K_2O), 1% sulfur, and 1% iron (all percents are by weight).

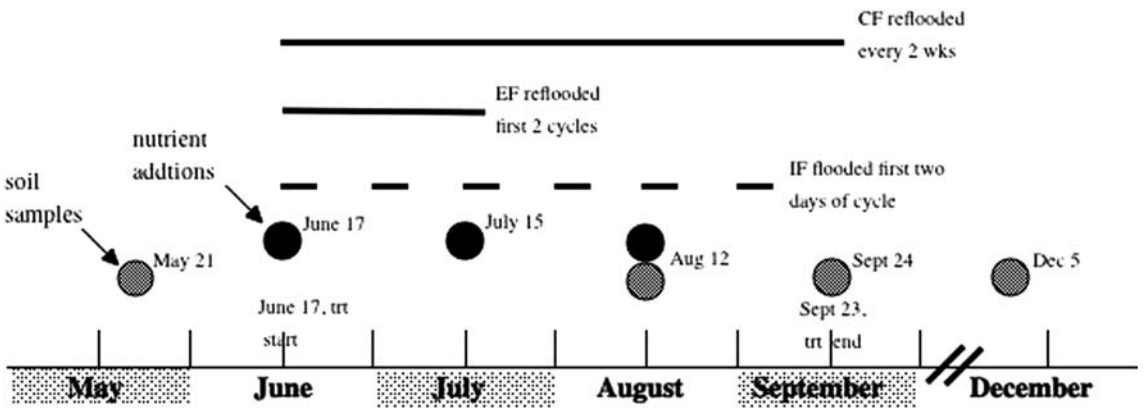

Figure 1. Experimental timeline. Hatch-marks along the line denote the two-week intervals of mesocosm water drawdown and re-flooding. The intermittent flood (IF) treatment was flooded the first two days of each two-week cycle from June 17 through September 23. The early season flood (EF) treatment was flooded for the full two-week cycle after which the water was drained (drawn down) until it was no longer above the soil line, and then the mesocosms were re-flooded. This treatment only continued for the first two cycles only (June 17 through July 17). The CF treatment was flooded for the two-week cycle, drawn down then immediately re-flooded continuously throughout the experiment. Soil sampling dates are indicated with shaded circles: May 21 samples were before the start of treatments, August 12 and September 24 samples were during treatments and correlate with two peaks in plant growth, and December 5 samples were taken after treatments ended and aboveground plant biomass was dead. Black circles indicate nutrient addition dates: June 15th, July 17th, and August 12th.

The high nutrient treatment followed the rate recommended by the fertilizer manufacturer (1 lb/275 sq ft, applied 3–4 times during the growing season) while the low nutrient treatment was 1/4 the recommended rate. The high nutrient treatment was chosen as an endpoint representing the concentration of nutrients that might be potentially carried in urban runoff, and also approximates the lowest level of nutrients that significantly increased the invasibility and biomass of *Typha*×*glauca* biomass in wetland field plots (Woo and Zedler, 2002). The total amount of nutrients added to mesocosms over the 3 applications (Figure 1) (g m^{-2} $year^{-1}$) was 14.21 g N, 1.58 g P, and 2.10 g K in the high treatment and 3.55 g N, 0.395 g P, and 0.526 g K in the low treatment. Our nutrient addition rates are higher than predicted rates (Pitt et al., 1996; WI Department of Natural Resources,) but may be characteristic of constructed wetlands processing flow from large developments.

Soil characteristics

To assess soil chemical and physical characteristics we measured soil texture, soil water content, total carbon and nitrogen (LECO combustion analysis; LECO corporation, St. Joseph, MI) and pH on control mesocosm samples in-house. Texture, nutrient, and mineral analysis, less easily performed in-house, were carried out at the University of Wisconsin-Madison Soil and Plant Analysis Laboratory (SPAL) on samples from control mesocosms (Bray and Kurtz, 1945; Bremmer, 1965; Day, 1965; American Society for Testing and Materials, 1985; Shulte and Hopkins, 1996). Mesocosm soil had a silty loam texture with an average pH of 7.02. Mesocosm soil nutrient status included 2.13% organic matter, 1.14 mg g^{-1} nitrogen, 38 ppm available phosphorus, 435 ppm total phosphorus and 1165 ppm total potassium.

Sampling

Soil samples were taken from each mesocosm on May 21, August 12, September 24, and December 5, 2002 (Figure 1). May and December samples were taken before and after treatment endpoints, respectively. August samples were taken from each mesocosm at the end of a two-week cycle after draw-down of flooded mesocosms; this date also corresponds with three weeks after the end of the early season flood (EF) treatment. September samples were taken from each mesocosm at the end of the last two-week cycle of treatments, after draw-down of flooded mesocosms; this date corresponds with nine weeks after the end of the EF treatment. For each mesocosm sample, four 2-cm by 10-cm cores were taken based on a 9 point randomized grid design. The four cores were combined, thoroughly mixed (the cores were broken up and spread on a tray), and subsampled for the various analyses. Roots were separated from the soil and loosely shaken to remove all but adherent (defined as 'rhizosphere') soil. Separated roots with adherent rhizosphere soil were then frozen until use for lipid analysis and enzyme activities. Fresh bulk soil was used for nitrification assays.

Microbial functional analysis

Enzyme activities were determined based on the method by Waldrop (2004) for several enzymes chosen to represent a variety of soil processes (Table 1). We added rhizosphere samples (2 g) to 100 ml of 5 m*M* bicarbonate buffer at pH 8.0. The soil/buffer slurry was mixed before inoculation into 96-well microtiter plates containing enzyme substrate solutions at 100 μM (with the exception of β-galacatosidase and cellobiohydrolase at 50 μM) methylumberiferal (MUB)-linked substrates in one column per substrate (8 replicates). Remaining wells were used to replicate five quenching standards at concentrations of 0.5, 1.5, 2.0, 2.5, and 3.5 μM MUB (4 replications). We measured fluorescence after 0, 2, and 4 h of incubation at 25 °C. Enzyme activity was then calculated and reported in two ways, the total activity or the 'specific' activity. Total enzyme activity is

Table 1. Enzymes assayed and their respective general function

Enzyme	Enzyme function
β-glucosidase	Cellulose degradation
Cellobiohydrolase	Cellulose degradation
β-xylosidase	Hemicellulose degradation
β-N-glucosaminidase	Chitin degradation
α-glucosidase	Starch degradation
Phosphatase	Polyphosphate degradation

simply the calculated enzyme activity and is expressed as nmol of substrate hydrolyzed h^{-1} g $soil^{-1}$. Specific enzyme activity is the activity for each enzyme divided by the total microbial biomass in the same sample, expressed as nmol of substrate hydrolyzed h^{-1} g $soil^{-1}$ μmol $lipid^{-1}$, and was determined as it may be more related to microbial community structure than total activity (Waldrop et al., 2000). Oven dry equivilent (ODE) soil weight was used in calculations. Although fresh samples are usually used for enzyme analysis, we performed preliminary experiments and determined that frozen soil would yield the same relative results, with slightly higher absolute number, than fresh soil for this study (unpublished data).

Nitrification potential assays were performed on 10 g fresh soil as described by Hart et al. (1994). Nitrate concentration for each sample was determined by colorimetric flow injection analysis (Ruzicka, 1983; LACHAT Instruments, Milwaukee, WI 2001). The rate of change was calculated using the slope function as discussed for enzyme activity rates. Nitrification potential is reported as mg of nitrate h^{-1}g $soil^{-1}$ (ODE).

Lipid analysis

'Signature' lipid biomarkers from the cell membrane of microorganisms (White and Ringelberg, 1998) were used to characterize both microbial biomass (defined as total lipids extracted from soil samples; White et al., 1979) as well as the relative abundance of indicator lipids to determine microbial community structure. Membrane lipids were extracted, purified and identified using steps from a modified Bligh and Dyer (1959) technique for lipid extraction, combined with fatty acid methyl ester analysis (FAME) as described by Microbial ID Inc. (Hayward, CA) (Smithwith et al., 2005). Lipids were extracted from 4 g of homogenized, freeze-dried soil using a chloroform-methanol extraction with a phosphate buffer. We then follow the procedure for FAME as given by Microbial ID Inc.; fatty acids are saponified, followed by strong acid methanolysis. A 2 μl injection of the methyl-esterfied fatty acids was analyzed using a Hewlett-Packard 6890 Gas Chromatograph equipped with a flame ionization detector and an Ultra 2 capillary column (Agilent). Lipid peaks are identified using bacterial fatty acid standards and MIDI peak identification software ("Sherlock microbial identification system", MIDI Inc, Newark, DE). Peak areas were converted to μmol g^{-1} soil using internal standards (9:0 nonanoic methyl ester and 19:0 nonadecanoic methyl ester).

The total μmol lipid/g soil was used as an index of microbial biomass (Balser et al., 2005; Hill et al., 1993; White et al., 1979; Zelles et al., 1992). The relative contribution of various lipid groups was determined by calculating the mol% (moles of a given lipid/total moles lipid per sample). The mol% of chemically similar fatty acids were further combined into groups representing different portions of the microbial community, or 'guilds' (Table 2 shows guilds used for our analysis). Lipids composing less than 0.5 mol% were disregarded. Because an average mol % is reported for each guild, and because not all lipids are used in the guild construction, the total mol% does not equal 100%.

Statistical analysis

Repeated measures analysis, least squares means, and differences of least squares means were conducted using SAS software (SAS Institute, 1999) with nutrient level, hydrologic regime, sample date (day number from 1 to 365) and 'all

Table 2. Fatty acid 'guild' composition

Guild	Generally indicates	Fatty acids included
Branched	Gram-positive bacteria[a]	11:0 anteiso, 14:0 iso, 15:0 iso and anteiso, 16:0 iso, 17:0 iso and anteiso
Monounsaturated	Gram-negative bacteria[b]	14:1 ω5c, 17:1 ω5c, 17:1 ω9c, 18:1ω5c, and 18:1 ω7c
Cyclopropyl	Anaerobic bacteria[b]	17:0 cyclo and 19:0 cyclo ω8c
	Mycorrhizal fungi (AMF)[d]	16:1 ω5c
	Saprotrophic fungi (SF)[d]	18:2 ω6,9c

Referenced from: a. Zelles et al. (1992); b. Wilkinson 1988 d. Balser et al. (2005); Stahl and Klug 1996. Additional references, Federle 1986; Vestal and White 1989.

possible interactions' as variables. Repeated measures analysis did not include baseline data from May, because the analysis was performed to determine treatment differences that were started after the May sample date. Seasonal differences in lipid profiles, enzyme activities, and nitrification potential apart from treatments were determined by repeated measures analysis using data from control mesocosms (see experimental design). In addition to repeated measures analysis, correlation matrices and pair-wise correlations were constructed to determine relationships between lipid guild mol%, enzyme specific activities, and nitrification potentials using JMP software (SAS Institute, 2002).

Results

Specific enzyme activity and nitrification potential

Several enzymes including β-glucosidase, β-xylosidase, cellobiohydrolase, β-N-acetylglucosaminidase, and α-glucosidase, were higher in specific activity in the constant flood (CF) treatment in September (Table 3). The specific activity of alkaline phosphatase showed no significant difference among the treatments (Table 3). There was also a strong interaction between hydrology and nutrient addition within the CF treatment; all enzyme specific activities were higher with high nutrient addition in the CF (with hydrology*nutrient addition as the explanatory variable) (Table 3). Total enzyme activities (not divided by microbial biomass) showed few differences between treatments at any date, with the exception of lower activity of phosphatase in the CF treatment (Table 4). Total enzyme activity was lower in September than August for phosphatase, β-N-acetylglucosaminidase, and β-xylosidase (Table 4).

Nitrification potential data indicated an effect from both nutrient addition and varied hydrologic regime. Nitrification rates from September samples were higher in the CF ($P = 0.0005$) and in the high nutrient addition ($P = 0.0004$) (Table 5).

Microbial community structure

Microbial biomass varied under the hydrologic but not nutrient treatments. There was a decrease in biomass in September in only the

Table 3. Enzyme specific activities in mesocosm treatments[a]

Treatment	β-glucosidase	Cellobiohydrolase	β-xylosidase	β-N-glucosaminidase	α-glucosidase	Phosphatase
August:						
Control	0.084^{ab}	0.008^{c}	0.012^{b}	0.028^{b}	0.018^{bc}	0.256^{a}
IF	0.093^{a}	0.012^{c}	0.014^{b}	0.040^{ab}	0.019^{b}	0.243^{a}
EF	0.178^{a}	0.034^{a}	0.035^{a}	0.069^{a}	0.047^{ab}	0.387^{a}
CF	0.122^{a}	0.016^{b}	0.020^{ab}	0.065^{a}	0.021^{b}	0.182^{ab}
High nutrients						
IF	0.110^{a}	0.009^{c}	0.014^{b}	0.030^{b}	0.027^{b}	0.273^{a}
EF	0.223^{a}	0.015^{bc}	0.024^{a}	0.069^{a}	0.039^{ab}	0.356^{a}
CF	0.201^{a}	0.020^{b}	0.019^{b}	0.088^{a}	0.066^{a}	0.258^{a}
September:						
control	0.049^{b}	0.002^{c}	0.005^{b}	0.018^{b}	0.012^{c}	0.127^{b}
IF	0.049^{b}	0.005^{c}	0.007^{b}	0.017^{b}	0.011^{c}	0.077^{b}
EF	0.040^{b}	0.005^{c}	0.012^{b}	0.017^{b}	0.008^{c}	0.095^{b}
CF	0.132^{a}	0.012^{bc}	0.012^{ab}	0.059^{a}	0.021^{b}	0.136^{ab}
High nutrients						
IF	0.047^{b}	0.007^{c}	0.007^{b}	0.019^{b}	0.014^{bc}	0.077^{b}
EF	0.043^{b}	0.007^{c}	0.008^{b}	0.022^{b}	0.005^{c}	0.117^{b}
CF	0.218^{a}	0.027^{ab}	0.032^{a}	0.091^{a}	0.067^{a}	0.194^{ab}

[a]Values within one column followed by the same letter are not significantly ($P < 0.05$) different ($n = 5$) based on repeated measures analysis. IF = intermittent flood, EF = early season flood, CF = constant flood. Specific enzyme activities are expressed as μmol substrate cleaved h^{-1} g $soil^{-1}$ mol lipid $biomass^{-1}$.

Table 4. Total enzyme activities in mesocosm treatments[a]

Treatment	β-glucosidase	Cellobiohydrolase	β-xylosidase	β-N-glucosaminidase	α-glucosidase	Phosphatase
August:						
Control	23.33[a]	2.40[a]	3.50[ab]	8.18[bc]	5.36[a]	72.03[a]
IF	26.18[a]	3.27[a]	3.85[ab]	11.31[ab]	5.40[a]	63.54[a]
EF	23.48[a]	4.68[a]	5.00[a]	11.05[ab]	7.08[a]	60.07[a]
CF	20.09[a]	2.57[a]	3.77[ab]	10.79[abc]	4.17[a]	27.55[c]
high nutrients						
IF	23.48[a]	1.94[a]	3.12[ab]	7.37[c]	5.77[a]	60.42[a]
EF	22.74[a]	2.67[a]	3.91[ab]	10.61[b]	6.18[a]	60.10[a]
CF	18.30[a]	3.01[a]	3.29[ab]	14.45[a]	9.68[a]	38.96[abc]
September:						
control	16.29[b]	1.02[a]	2.08[b]	6.12[bc]	4.23[a]	43.12[b]
IF	22.53[a]	2.69[a]	3.70[ab]	7.64[bc]	4.45[a]	35.75[bc]
EF	13.06[b]	1.71[a]	2.12[b]	5.84[bc]	2.61[a]	29.92[bc]
CF	18.19[a]	1.59[a]	1.18[b]	9.05[abc]	3.06[a]	19.93[c]
high nutrients						
IF	19.33[a]	2.75[a]	3.43[ab]	8.21[bc]	4.45[a]	32.06[bc]
EF	17.96[a]	1.42[a]	2.19[b]	8.23[bc]	3.17[a]	38.96[b]
CF	26.64[a]	3.38[a]	3.74[ab]	11.50[ab]	5.04[a]	26.77[bc]

[a]Values within one column followed by the same letter are not significantly ($P < 0.05$) different ($n = 5$) based on repeated measures analysis. IF = intermittent flood, EF = early season flood, CF = constant flood. Total enzyme activities are expressed as μmol substrate cleaved h^{-1} g $soil^{-1}$.

Table 5. Nitrification potential in mesocosm treatments[a]

Treatment	Nitrification potential
August:	
control	0.169[st]
IF	0.282[s]
EF	0.367[s]
CF	0.329[s]
By nutrient trt	
No nutrients	0.390[b]
Low nutrients	0.504[b]
High nutrients	0.395[ab]
September:	
Control	0.125[t]
IF	0.431[s]
EF	0.390[s]
CF	0.972[r]
By nutrient trt	
No nutrients	0.350[b]
Low nutrients	0.729[ab]
High nutrients	1.805[a]

[a]Values within the column followed by the same letter are not significantly ($P < 0.05$) different ($n = 5$) based on repeated measures analysis. IF = intermittent flood, EF = early season flood, CF = constant flood. Nitrification potential data are expressed as μg nitrate $hour^{-1}$ g $soil^{-1}$.

constant flood treatment (CF), with a decrease from approximately 350 to 150 μmol lipid/g soil ($P = 0.0009$) (data not shown).

Overall, the various guilds (mycorrhizal fungal indicator, branched fatty acids, cyclopropyl fatty acids, and monounsaturated fatty acids; Table 2) responded differently to hydrologic regime and nutrient treatments, and treatment effects also changed during the course of the experiment. The mycorrhizal fungal (AMF) indicator (16:1 ω5c) relative abundance was approximately 2 fold lower under the CF than the intermittent flood or controls, in both August and September (Figure 2A). The AMF indicator relative abundance was also lower in the early season flood treatment (EF) than the intermittent flood (IF) or controls in August (3 weeks after EF flood cessation). However, by September the AMF indicator relative abundance in the EF composed 35 mol% of the microbial community, similar to the IF and control mesocosms. In contrast branched fatty acids (Gram-positive bacterial indicators) had higher relative abundance in the EF and CF in August and were higher only in the CF in September (Figure 2B). Cyclopropyl fatty acid (Gram-negative and/or anaerobic

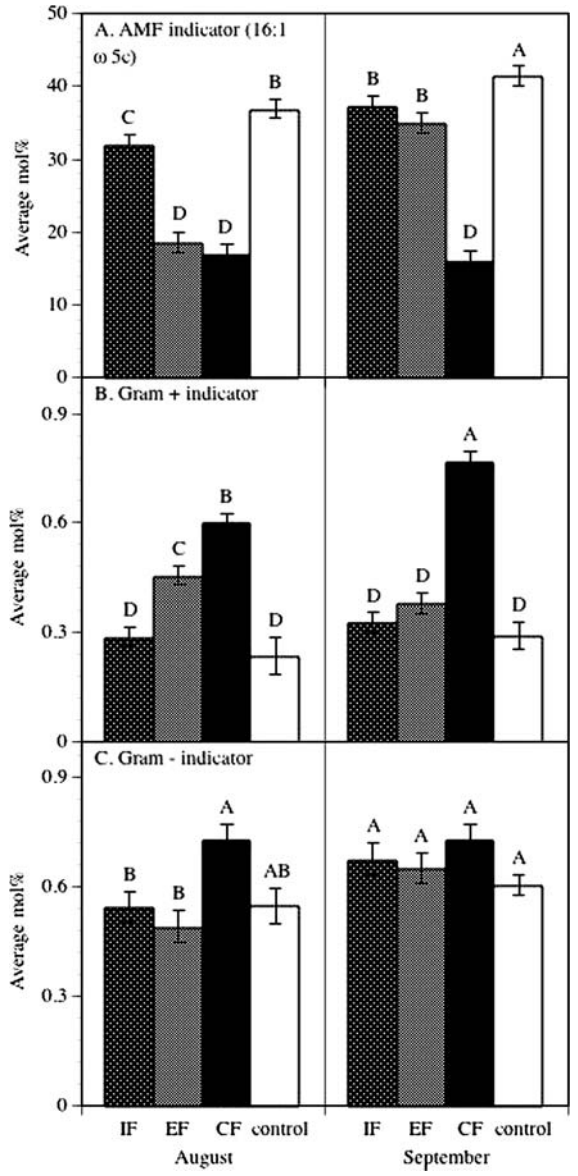

Figure 2. Microbial lipid 'guilds' averaged by flood treatment. The average mol% (average $\text{moles}_{\text{indicator}}$/total moles lipid in each sample) for each hydrologic treatment from August and September sample dates is shown. Note the difference in scale between AMF and the other indicators. Error bars denote one standard error of the mean ($n = 10$). Bars with the same letter within each panel are not significantly different based on repeated measures analysis ($P < 0.05$). IF = intermittent flood treatment, EF = early season flood treatment, CF = constant flood treatment, and control = the experimental control mesocosms. (A) AMF indicator; (B) Gram positive bacterial indicator (branched fatty acids); (C) Gram negative bacterial indicator (monounsaturated fatty acids).

bacterial indicator) relative abundance was similar to that of branched fatty acids, but the trend was not significant. Monounsaturated fatty acid (Gram-negative bacterial indicator) relative abundance was higher under the CF in August, but indicated no treatment effects from hydrologic regime at the September sampling (Figure 2C).

While there were many significant impacts of flood regime on microbial guild relative abundance, there were few significant treatment effects of nutrient addition. The AMF indicator fatty acid was less abundant in nutrient addition treatments (Figure 3A). In contrast the saprotrophic fungal (SF) indicator (18:2 ω6,9c) had a higher relative abundance in the high nutrient addition treatment versus the low nutrient addition treatment (Figure 3B).

Seasonal changes in microbial composition and function

We observed significant changes in microbial composition and function in the control mesocosms during and following the growing season. Microbial biomass (total μmol lipid g soil^{-1}) increased from August to September (repeated measures, significant at $P = 0.0032$) and appeared to decrease from September to December (not statistically significant) (Figure 4). The relative abundance of the AMF indicator increased from May through September (from 30 to 42 mol%) and decreased by December ($P < 0.0001$) (Figure 5, repeated measures analysis). Fungal indicator relative abundance decreased consistently (from 15 to 5 mol%). Of the bacterial indicator guilds only Gram-positive indicators (branched and methyl fatty acids) changed over time, having a higher mol% in May than at other sample dates (data not shown).

There were few differences in functional activity over time. Only phosphatase and β-glucosidase enzyme specific activities displayed a trend; their specific activity decreased through the season with a statistically significant decrease only from August to September (Table 3, repeated measures analysis $P < 0.05$). Nitrification potentials indicated a similar trend, though not significant at $P < 0.05$ (Table 5).

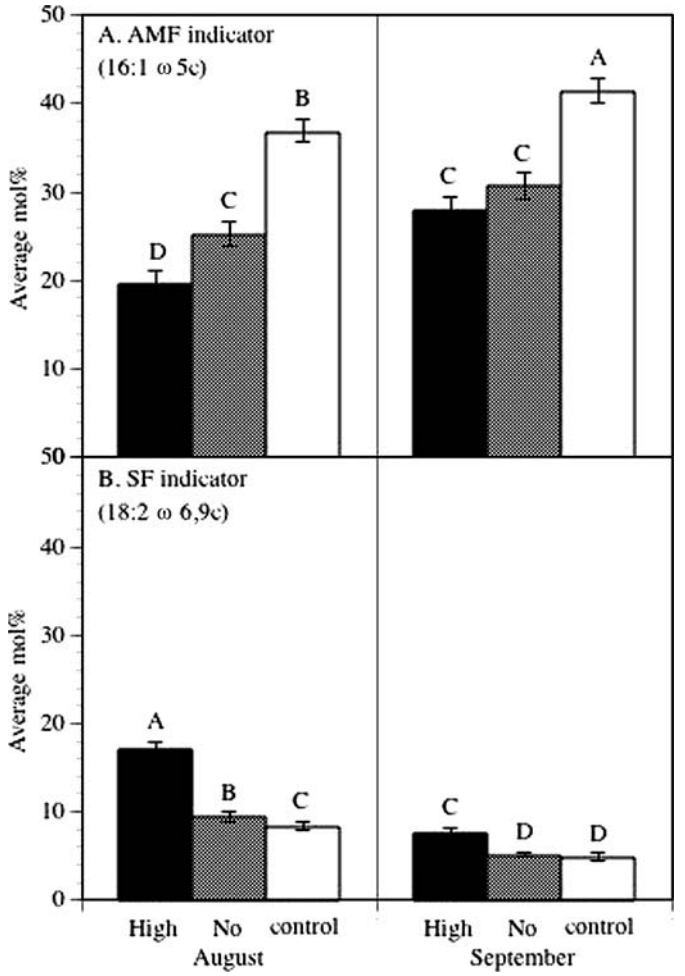

Figure 3. Microbial lipid 'guilds' averaged by nutrient treatment. The average mol% (average $\text{moles}_{\text{indicator}}$/total moles lipid in each sample) for each nutrient treatment from August and September sample dates is shown. Error bars within each panel denote one standard error of the mean ($n = 15$). Bars with the same letter are not significantly different based on repeated measures analysis ($P < 0.05$). (A) arbuscular mycorrhizal fungal (AMF) indicator (16:1 ω5c); (B) saprotrophic fungal (SF) indicator (18:2 ω6,9c).

Correlations between lipid relative abundance and enzyme specific activities

Gram-negative anaerobic indicators (cyclopropyl fatty acids) were negatively correlated with phosphatase and nitrification potential. In contrast Gram-positive indicators (branched fatty acids) were positively correlated with *β*-glucosaminidase,*β*-*N*-acetylglucosaminidase, and *β*-xylosidaseand nitrification potential. With the exception of phosphatase, the AMF indicator fatty acid (16:1ω5c) was significantly negatively correlated with all the enzymes studied (Table 6). The SF fungal indicator was significantly positively correlated only with phosphatase. Gram-negative indicators (monounsaturated fatty acids) were not significantly correlated with any microbial functional assays (Table 6).

Overall effects of flooding and nutrients

In general, after examining relationships among hydrologic regime, nutrient addition, and wetland microbial community structure and activity, we found that prolonged flooding had a larger effect than nutrient loading, altering both compositional and functional aspects of the microbial community. We also found that hydrologic and nutrient treatments appeared to affect portions of the microbial community in distinct ways. In

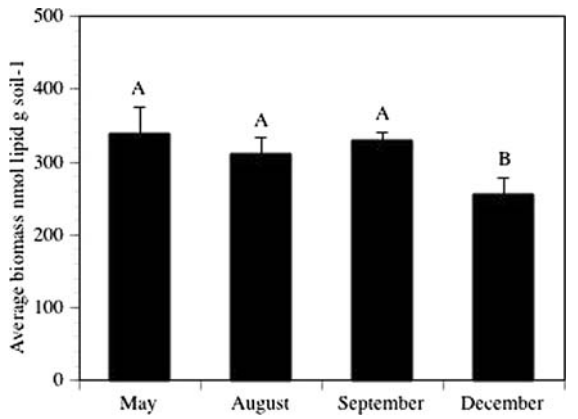

Figure 4. Total microbial biomass at each sample date. The total microbial biomass from controls was averaged for a given sampling date and reported as μmoles lipid/g ODE soil. Error bars denote one standard error ($n = 5$). Bars with the same letter are not significantly different based on repeated measures analysis ($P < 0.05$).

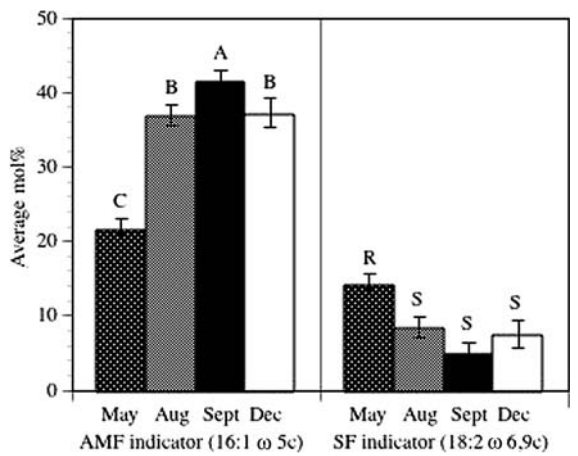

Figure 5. AMF and SF fungal indicators at each sample date. Microbial indicator mol% (average moles indicator/total moles lipid in each sample) from control mesocosms was averaged by sample date, reported as the average mol%. Error bars denote one standard error ($n = 5$). Bars with the same letter are not significantly different based on repeated measures analysis ($P < 0.05$) (a,b,c = AMF, r,s,t = SF) AMF = arbuscular mycorrhizal indicator fatty acid (16:1ω5c); SF = saprophytic fungal indicator fatty acid (18:2 ω6,9c).

particular, the relative abundance of AMF appeared to be greatly reduced in response to excessive flooding while that of Gram-negative anaerobic and Gram-positive bacteria increased. Enzyme specific activities and nitrification potentials also increased under constant flooding. Lastly, we found that nutrient additions and seasonal variations were significant only for AMF, saprotrophic fungi, and soil nitrification potential.

Discussion

Microbial community functional response

Our findings differ from those previously reported in several important ways. Higher enzyme specific activity under increased water was contrary to other studies focused on water manipulation. Enzyme specific activities differed by hydrologic treatment while total enzyme activities showed little difference between treatments; there was higher specific activity under the constant flood (CF) treatment, which could be either due to increased microbial activity or to increased mobility of extant soil enzymes. This increase in specific activity under increased water is in contrast to other studies focused on water manipulation in peatlands. Other studies have found either an increase in enzyme activity under water table draw-down (Freeman et al., 1996), a decrease under increased water (Pulford and Tabatabai, 1988), or little change in enzyme activities under increased water (Freeman et al., 1998). Results are explained by activation of enzymes extant in the peatland, and a lack of increase microbial activity measures (respiration, electron transport activity) that indicate increased production of enzymes. Another explanation for decreased hydrolase activity under flooded condition is the accumulation of phenolic compounds from phenol oxidase inhibition (phenol oxidase requires oxygen to function) (Fremman et al. 2001). Our results may differ because we report specific enzyme activity per unit microbial biomass. As seen by other work, this yields different results than total enzyme activity (Waldrop et al., 2000). Total enzyme activities in our study showed overall little difference between treatments, with the exception of lower phosphatase activity in the CF treatment. It is possible that enzyme activities in our mesocosm soils (which were mineral soils) differ due to the higher pH and lower organic matter content than peat soils. The overall lack of hydrolase

Table 6. Significant correlations between lipid ecological guilds and functional analyses

	Anaerobe	Gm−	Gm+	SF	AMF
β-glucosidase			+*		−*
α-glucosidase					−*
β-xylosidase			+*		−*
Cellobiohydrolase					−*
β-N-acetylglucosaminidase			+***		−***
Phosphatase	−*			+*	
Nitrification potential	+*		+*		

*Value is significant at $P < 0.05$, **value is significant at $P < 0.01$, ***value is significant at $P < 0.001$. Correlations were performed between lipid indicator mol%, enzyme specific activity rates, and nitrification potential rates. Column headings: anaeobe = anaerobic bacterial indicator mol% (cyclopropyl fatty acids); Gm− = Gram negative bacterial indicator mol% (monounsaturated fatty acids); Gm+ = Gram positive bacterial indicator mol% (branched fatty acids); SF = saprotrophic fungal indicator mol% (18:2 ω6,9c); AMF = arbuscular mycorrhizal fungal indicator mol% (16:1 ω5c).

Table 7. Mesocosm plant species

Dominant plant sp.[a]	AM mycorrhizal	Reference	CF recovery	EF recovery
Agrostis stolonifera L.	−	−	−	−
Andropogon gerardii Vitman	+	Shultz et al. (2001)	−	+
Desmodium canadense L.[b]	+ (based on Desmodium sp.)	Rothwell and Vogel (1982); Boddington; Dodd (1998)	−	−
Symphyotrichum novae-angliae L.	+	Rothwell and Vogel (1982)	−	−
Silphium perfoliatum L.	+	Rothwell and Vogel (1982)	−	−
Glyceria striata Lam.	+	Read et al. (1976)	−	−
Verbena hastate L.	+ (based on Verbena sp.)	Rothwell and Vogel (1982)	−	−
Asclepias incarnata L.	−	−	+	+
Spartina pectinata Link.	−	−	+	+
Phalaris arundinacea L.[c]	+	Read et al. (1976); Rothwell and Vogel (1982); Baur et al. (2003)	+	+

Plant biomass data as per Kercher and Zedler (2004). [a]The following are a list of dominant species, for a more complete list see Kercher and Zedler (2004). [b]*D. canadense* is a nitrogen fixing species. [c]P. arundinacea is an invasive species studied in the larger experiment. CF reaction = response of plant species biomass to the constant flood treatment. EF recovery = response of plant species biomass to early season flood treatment after flooding ended.

inhibition in our study could also suggest that there is less accumulation of phenolic compounds.

The interaction between hydrology and nutrient addition within the CF treatment may confound the effects of the CF treatment on enzyme specific activity. It is difficult to discern whether constant flooded conditions directly affected specific enzyme activities or whether there was an indirect effect of hydrologic regime on nutrient availability that caused the increase in enzyme specific activities. Further work is needed to determine the contribution of nutrient additions versus hydrologic regime on specific enzyme activities. While there is evidence that enzyme activities are correlated with nutrient flow in peatlands (Freeman et al., 1997), work could also be done to determine the effect of nutrient additions on enzyme activities, and how nutrient additions and hydrologic regime interact to influence microbial activity.

Relationships between treatments and microbial community structure

In addition to changes in enzyme activities, we observed treatment-related and seasonal shifts in microbial community structure. Fungal lipid indicators (AMF and SF) dominated the rhizosphere microbial community and displayed the most

significant response to treatments in this study (Figures 2 and 3). Arbuscular mycorrhizae in particular were present in high abundance initially and appeared to be sensitive to prolonged flooding and nutrient addition. Rickerl et al. (1994) and Cornwell et al. (2001) also report high levels of mycorrhizal colonization in natural fens and semi-permanent wetlands in South Dakota. In this study, it is possible we observed high AMF abundance because we specifically extracted root/rhizosphere soil lipids. Other studies commonly sample bulk soil, which likely has a lower proportion of AMF. Our sampling technique arguably may allow a more realistic understanding of treatment effects on AMF.

We observed decreased abundance of AMF in response to both nutrient additions and prolonged flooding (Figure 2A). This is consistent with work by Rickerl et al. (1994) and Miller and Bever (1999), demonstrating that AMF species distribution and colonization rates are sensitive to soil water content and sensitive to past water content (i.e., AMF not accustomed to excessive flooding may be especially sensitive to extended periods of flooding). Prior to treatment application our mesocosms were watered but never subjected to flooded conditions (during two years); accordingly, they may have developed AMF communities poorly adapted to excessive moisture. However, despite sensitivity to flooding, there was evidence for short-term resilience in the AMF populations in our study. Three weeks after flooding stopped in the early season flood (EF) treatment AMF indicators were still at half the relative abundance of control levels. By September (9 weeks after flooding had stopped in the EF, Figure 2A) the relative abundance of mycorrhizal indicators in the EF was similar to the controls, suggesting that these AMF have the ability to recover from short-term disturbances.

A decrease in AMF biomass in flooded treatments can also be potentially explained by a decrease in host plant biomass. The pre-treatment mesocosm plant assemblage was comprised of 14–21 plant species to represent a native Wisconsin wet prairie (Kercher and Zedler, 2004). Constant flooding caused abrupt biomass decline in several dominant species while increasing the biomass of other species (Table 7) We did not directly assess which species had mycorrhizal associations, but based on the literature most of the dominant species may support mycorrhizal associations (Table 7). The only dominant species from the early flood treatment, where 9 weeks after flooding ended the AMF levels were similar to control levels, which have reported mycorrhizal associations are *Phalaris arundinacea* and *Andropogon gerardii*.

Mycorrhizal fungi appeared to be sensitive to nutrient treatments in addition to flooding. In high nutrient addition treatments the biomass and relative abundance of the AMF indicator was significantly lower (Figure 3A). These results are supported by several studies (Balser, 2001; Balser et al., 2005; Stevens et al., 2002; Treseder and Allen, 2000; White and Charvat, 1999) that indicate lower AMF abundance in higher fertility soils. It is believed that plant investment in AMF declines under elevated soil nutrient conditions.

While the AMF indicator was negatively affected by constant flooding, Gram-positive and anaerobic microbial indicators were higher in flooded conditions. The physiology of Gram-positive and Gram-negative microorganisms may allow explanation (Firestone and Davidson, 1989); the greater stress tolerance to water content fluctuation of Gram-positive microorganisms could explain why Gram-positive indicators, but not Gram-negative indicators (with the exception of cyclopropyl lipids, an anaerobic bacterial indicator), were more abundant in the constant flood (CF) treatment compared to the same indicators in the controls and other treatments (Fierer and Schimel, 2002; Kieft et al., 1994; Kieft et al., 1987). The CF may represent a stressful environment because this treatment was a drastic change from the first two years of mesocom establishment, which involved no prolonged flooding.

Relationship between microbial community function and structure

Perhaps not surprisingly, we found significant correlations between guilds (as defined by fatty acid analysis) and our functional measurements (Table 6). Gram-positive indicators were positively correlated with nitrification potential and enzyme specific activities for β-glucosidase, β-xylosidase, and β-N-acetylglucosaminidase.

Generally stress-tolerant, and metabolically flexible, Gram-positive bacteria may be more reliant than Gram-negative bacteria on exoenzymes in order to degrade complex molecules into small polymers that can be taken up readily for energy and nutrient uptake (Sylvia et al., 2005). In contrast, Gram-negative anaerobes (indicated by cyclopropyl fatty acids) did not show a significant correlation with enzyme activity (Table 6). Gram-negative microorganisms are more adapted to directly using small molecular nutrients (often from rhizodeposition) as opposed to taking up nutrients from degraded macromolecules (Sylvia et al., 2005). Accordingly, Gram-negative bacteria may show less relationship with enzyme activities than Gram-positive bacteria.

Also of interest were the positive correlation of Gram-positive indicators (branched fatty acid guild) and negative correlation of AMF with β-N-acetylglucosaminidase (a chitinase). It is known that fungal cell walls contain chitin and that bacteria produce chitinases in order to degrade chitin into monomers, which can be taken up as nutrients (Swift et al., 1979). It therefore follows that Gram-positive indicators might have a positive correlation with chitinase while AMF indicators have a negative correlation. It is also interesting that the general fungal, but not mycorrhizal, indicator was correlated with phosphatase activity since mycorrhizae are believed to be involved in plant phosphorus uptake (Sylvia et al., 2005). It may be that AMF play a different role in wetland systems, or that general fungi in this system are more reliant on phosphatase enzymes.

Urban runoff and wetland microbial communities

Microbial community structure and function were affected by both flood and nutrient treatments, known factors of urban runoff. Our results are in agreement with other studies showing changes in microbial communities from hydrologic (Freeman et al., 1998; Turner and Friese, 1998) or nutrient treatments (Cornwell et al., 2001; Ravit et al., 2003), supporting the idea that urban runoff substantially impacts wetland microbial community structure and function. In our study arbuscular mycorrhizal fungi were especially sensitive to flooding, which may impact plant nutrient uptake from excessive moisture caused by urban runoff. We were also able to show changes in treatment effect over time from urban runoff treatments. Arbuscular mycorrhizal fungi (AMF) appeared to recover from short-term flooding, which may indicate that AMF and their associated impact on plant nutrition may only be affected by urban runoff during or directly after times of flooding. AMF also decreased with nutrient addition, suggesting that recovery of AMF from excess flooding may depend on persistence or level of nutrient addition from urban runoff. Bacterial relative abundance and function were also affected by flooding treatments, and while relative abundance appeared to recover from the early season flood, nitrification did not, suggesting that changes in enzyme activity per microbial biomass and nitrification may be persistent or have a longer recovery time after a wetland is returned to pre-runoff conditions. Thus, nutrient cycling mediated by microorganisms may also be altered by urban runoff.

Conclusions

This study represents one of few assessments to date of both microbial community composition and function in wetland soils. Because we focused on the rhizosphere, we may have been better able to determine plant-relevant changes in microbial community components including bacteria, saprotrophic and arbuscular mycorrhizal fungi (AMF). We were also able to assess the sensitivity of AMF to urban runoff factors and found that they may be an indicator of ecosystem health in wetlands. AMF contribute to plant nutrient uptake and can enhance the ability of plants to colonize barren soil (which may be important with plant community establishment in disturbed or reconstructed wetlands). Our results suggest that periodic flooding may increase microbial activity and thereby increase nitrogen cycling and decomposition rates. The variety of treatment effects we found between lipid guilds may indicate that the study of microbial structure, in addition to function, is important for understanding ecosystem function and that timescale differences may be important. These conclusions may be significant for the performance and construction of wetlands used to attenuate runoff and water infiltration.

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