Microb Ecol (2012) 64:893–908
DOI 10.1007/s00248-012-0090-2

MICROBIOLOGY OF AQUATIC SYSTEMS

Detrital Floc and Surface Soil Microbial Biomarker Responses to Active Management of the Nutrient Impacted Florida Everglades

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Received: 27 March 2012 / Accepted: 23 June 2012 / Published online: 26 July 2012

Abstract Alterations in microbial community composition, biomass, and function in the Florida Everglades impacted by cultural eutrophication reflect a new physicochemical environment associated with monotypic stands of *Typha domingensis*. Phospholipid fatty acid (PLFA) biomarkers were used to quantify microbial responses in detritus and surface soils in an active management experiment in the eutrophic Everglades. Creation of open plots through removal of *Typha* altered the physical and chemical characteristics of the region. Mass of PLFA biomarkers increased in open plots, but magnitude of changes differed among microbial groups. Biomarkers indicative of Gram-negative bacteria and fungi were significantly greater in open plots, reflective of the improved oxic environment. Reduction in the proportion of cyclopropyl lipids and the ratio of Gram-positive to Gram-negative bacteria in open plots further suggested an altered oxygen environment and conditions for the rapid growth of Gram-negative bacteria. Changes in the PLFA composition were greater in floc relative to soils, reflective of rapid inputs of new organic matter and direct interaction with the new physicochemical environment. Created open plot microbial mass and composition were significantly different from the oligotrophic Everglades due to differences in phosphorus availability, plant community structure, and a shift to organic peat from marl-peat soils. PLFA analysis also captured the dynamic inter-annual hydrologic variability, notably in PLFA concentrations, but to a lesser degree content. Recently, use of concentration has been advocated over content in studies of soil biogeochemistry, and our results highlight the differential response of these two quantitative measures to similar pressures.

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Introduction

Wetland biogeochemical cycles are mediated by complex detrital microbial communities structured by a suite of short- and long-term environmental conditions, both abiotic (e.g., oxygen availability and hydroperiod) and biotic (e.g., plant community composition and stoichiometry) [15, 16, 30, 35]. Fungi are important aerobic degraders of higher plant cell walls [34, 52, 53], and biomass can vary with water depth, dissolved oxygen (DO), or nutrient availability [10, 12, 31]. Similarly, Gram-negative bacteria (GNB) abundance tends to be greater in aerobic soils relative to those under persistent anoxia [11]. When stressed (e.g., poor substrate quality and anaerobic conditions), GNB produce cyclopropyl lipids in place of mono-unsaturated fatty acids and reduce growth rates [11, 58]. Biomass and metabolic activity of other microbial groups, including strict and facultative aerobes and anaerobes such as actinobacteria, Gram-positive bacteria (GPB), sulfate-

reducing bacteria (SRB), and methanogens, will also vary with redox potential, plant community composition, or substrate quality and availability [2, 5, 24, 30]. Composition and biomass of microbial groups responding to environmental conditions affect turnover of organic matter (OM) and nutrients [15, 38], and ultimately peat development [27, 28].

The microbial consortium is sensitive to environmental modification. Assessing changes in the microbial community are therefore important to wetland restoration strategies (e.g., [11, 57]). The ability to rapidly assess changes in microbial community composition is useful for tracking the trajectory of environmental manipulations. Phospholipid fatty acids (PLFAs) are an effective microbial monitoring tool since there are several biomarkers to coarsely quantify active functional groups [54], and PLFAs turnover rapidly after cell death ([60] but see [21]). Abundance of PLFA biomarkers has been related to total microbial biomass, environmental condition, and process rates. For example, PLFAs have been correlated with bacterial cell numbers and mass [20, 59] and substrate induced respiration biomass [3, 44], hydrology and substrate quality [10, 11, 31], and enzyme activity [43]. Ratios of specific PLFAs have also been used to infer environmental conditions or physiological state of the microbial community. The fungi to bacteria (F/B) ratio has been positively correlated with organic matter content and dry conditions (i.e., aerobic) and negatively with pH [10, 20, 31]. The ratio of GPB/GNB may increase under flooded conditions, corresponding with decreased oxygen availability [4, 5, 11]. PLFAs have been used in wetlands to quantify microbial compositional changes in response to management and land use changes or comparing degraded and reference conditions (e.g., [6, 10, 12]).

Oligotrophic regions of the Florida Everglades ecosystem have been significantly altered due to excessive nutrient (phosphorus (P)) inputs [26, 41]. The result is replacement of the characteristic ridge-and-slough landscape by dense monotypic cattail (*Typha domingensis* Pers) stands [26]. Microbial responses in P-impacted regions relative to oligotrophic conditions include greater sulfate reduction and methanogenesis [41]. Despite eutrophication increasing decomposition rates [41], increased inputs of recalcitrant emergent macrophyte OM with hypoxic conditions have accelerated peat accretion [15]. In response, active restoration strategies of *Typha*-dominated, eutrophic regions are being pursued (see [46]).

The primary objective of this study was to use PLFA analysis to assess floc and surface soil (0–5 cm) microbial structure responses to the active management of emergent macrophyte removal for the accelerated recovery of P-impacted regions of the Everglades. We hypothesized that removal of *Typha* would alter detrital microbial communities in response to the new abiotic and biotic conditions created by shifting ecosystem structure back to a submersed macrophyte and periphyton community. A secondary objective was to assess the results of active management on detrital PLFA composition relative to that of sloughs in the desired oligotrophic Everglades (i.e., reference conditions). Reference conditions also provide a baseline for interpretation of effects of eutrophication and management on alterations in PLFA biomass, composition, and potential function.

Materials and Methods

Study Location

The study was conducted in Water Conservation Area-2A (WCA-2A), a northern Everglades impoundment. The experimental rational, design, and environmental results of the active management strategy have been detailed elsewhere ([46] http://www.sfwmd.gov). Briefly, two sets of triplicate-paired 6.25-ha plots were established in a region dominated by *T. domingensis* (Pers) and a transitional region containing a 50:50 mixture of *T. domingensis* and *Cladium jamaicense* (Crantz) (Fig. 1). The former region, designated E, is highly enriched, with average surface water TP>30 μg L^{-1}, floc TP>1,200 mg kg^{-1}, and soil (0–5 cm) TP>1,200 mg kg^{-1}, whereas the latter, designated T, is moderately enriched, with average surface water TP> 14 μg L^{-1}, floc TP>1,000 mg kg^{-1}, and soil TP>900 mg kg^{-1}. Floc here is defined as the pourable detrital layer above the consolidated soils. Paired plots consisted of a dense emergent macrophyte control (designated C) and an open treatment plot created by the application of herbicides and a controlled burn (designated O). Three additional 6.25-ha plots were established in the oligotrophic region, with surface water TP< 10 μg L^{-1}, floc TP<400 mg kg^{-1}, and soil TP<300 mg kg^{-1}, to serve as reference conditions (designated UC) (Fig. 1). Numerous differences were documented in the plant structure and physicochemical environments among regions and between treatments (see [46] for a more comprehensive documentation). Briefly, active management resulted in the removal of approximately 80 % of the emergent macrophyte cover. In created opening plots, water column TP concentrations (15–35 %) and TP contents in floc (20–50 %) and soil (2–16 %) were greater relative to controls, but differences were more marked across regions [46]. Dissolved oxygen, percent saturation, and photosynthetic active radiation (PAR) were more than 2-fold greater for the created open than control plots [46]. Whereas PAR was similar among regions, dissolved oxygen and percent saturation were greater for the reference region than for open plots in the transitional and enriched regions. Periphyton biomass at reference sites was generally comparable to emergent macrophyte biomass in control plots [46].

Floc and Soil Sampling

Floc and the top 5 cm of soil were sampled during a 1-week period in 8 January 2007, 16 July 2007, 10 September 2007,

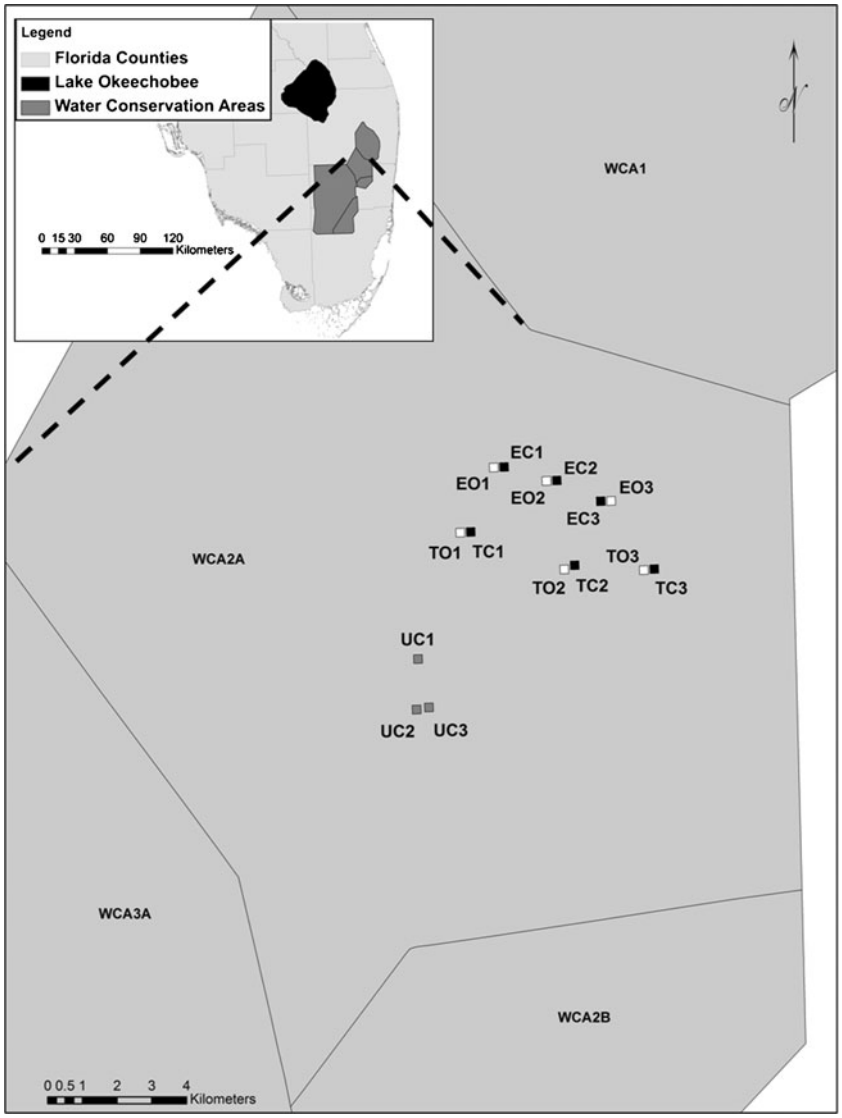

Figure 1 Study location within northern WCA-2A. Plots are numbered from west (*left*) to east (*right*). The first letter for each site refers to plots from: *E* plots in the highly phosphorus-enriched region, *T* in the transitional region, and *U* in the un-impacted, reference region. The second letter *O* refers to open, treatment plots and *C* for control plots

21 January 2008, 30 June 2008, 20 October 2008, 6 July 2009, and 5 October 2009. Samples were collected using a thin-walled 10 cm internal diameter (ID) stainless steel coring tube from three sub-plots within a plot. Floc was separated from consolidated soils by pouring the detrital fraction into a large Ziploc™ bag after removal of large leafy material. The 0- to 5-cm soil layer was extruded in the field and placed in a separate labeled bag. Both fractions were stored on ice for transport to the laboratory where the sub-plot fractions were pooled for analysis.

PLFA Analysis and Functional Group Designations

An approximate 50 g wet weight sub-sample of floc or soil was lyophilized for a minimum of 48 h in a FreeZone 6 Liter Console Freeze Dry System (Labconco, Kansas City, MO). Lyophilized samples were then shipped to Microbial Insights, Inc. (Rockford, TN) for PLFA extraction and quantification. Samples were prepared and extracted using a modified Bligh and Dyer method [8, 59] by adding a methanol(MeOH)/chloroform/0.05M potassium phosphate buffer (2:1:0.8) to a sample and mixing for 4–18 h on an orbital shaker. Chloroform was added to the supernatant to obtain a final MeOH/chloroform ratio of 1:1. The solution was then shaken briefly by hand and centrifuged. The lower organic layer was recovered and the solvent removed with a gentle stream of dry nitrogen. The dried total lipid extract was then re-suspended in chloroform and fractionated into neutral lipids, glycolipids, and phospholipids using different elution solvents (non-polar to polar) through 300–500 mg silicic acid column, depending on sample size [25, 60].

The phospholipid fraction was eluted using MeOH and dried under nitrogen gas. Phospholipids were then trans-esterified to fatty acid methyl esters (FAMEs) using mild alkaline methanolysis [25]. The sample was re-suspended in 1 mL of methanolic 0.2M potassium hydroxide and

1 mL of MeOH, heated for 30 min at 60 °C, cooled to room temperature, diluted with 2 mL of hexane/chloroform (4:1), and neutralized with 200 μL of 1 N glacial acetic acid in nanopure water. An equal volume of nanopure water was added, vortexed, centrifuged, and the organic layer recovered. The aqueous phase was washed twice with hexane/chloroform (4:1) and added to the recovered organic phase. The organic phase was then dried under a stream of nitrogen.

FAMEs were quantified by gas chromatography and/or gas chromatography–mass spectrometry (GC/MS) on a Hewlett Packard (HP) 5890 or 6890 GC with flame ionization detection (FID) equipped with a HP 7673 auto-sampler. Prior to injection of 1 to 3 μL, samples were diluted in hexane containing a 50-pmol μL^{-1} methyl-nonadecanoic acid (19:0) standard. The GC inlet was operated in the split-less mode at 290 °C with a septa purge time of 1.5 min. A 60-m Restek RTX-1 column (ID of 0.25 mm and a film thickness of 0.25 μm) was used with helium as the carrier gas at 1 mL min^{-1} at a column head pressure of 32 psi and no solvent delay during acquisition. The column was heated using a ramped program, starting at 110 °C held for 2 min, ramping at 10 °C min^{-1} to 150 °C with no hold time, next at 3 °C min^{-1} to 240 °C with no hold time, and finally ramped at 15 °C min^{-1} to 312 °C with a hold time of 9.2 min giving a total run time of 50 min. Components exited the column into the FID which was at a temperature of 300 °C, and profiles acquired with the HP GC Chemstation Software Rev. A.04.01.

FAME profiles were confirmed by mass spectrometry utilizing an HP5972 or HP5973 quadrupole mass selective detector coupled to an HP5890 GC or HP6890 GC, respectively. GC configurations were the same as above with the exception of a 13-min solvent delay. Following electron ionization, ions were scanned as the total ion current (range, 50–600 m/z at 1.53 scans s^{-1}). HP Chemstation G1701BA software was used for data acquisition and target lipid assessment.

A total of 53 PLFAs were identified for quantification of both total content (in nanomoles per gram dry weight of substrate) and concentration (in micromoles per square meter) [51]. Presentation of PLFA content enables comparison with other studies, but recently it has been argued that concentration should be used when making biogeochemical inferences [51]. We used 16 PLFAs as diagnostic of specific functional groups, accounting for on average over 50 % of the total detected lipids; the other PLFAs are considered general in their distribution amongst microorganisms [18, 54]. Functional groups were derived from the sum of PLFA biomarkers and comprised GPB (i14:0, i15:0, a15:0, and i16:0), GNB (18:1ω7, cy17:0, and cy19:0), fungi (saprotrophic (18:2ω6) and arbuscular mycorrhizal (16:1ω5)), and algae (green algae (18:3ω3) and diatoms (20:5ω3)) [3, 33, 50]. Caution has been urged when trying to interpret responses in the actionbacteria (actino) class due to overlapping PLFAs with SRB (e.g., 10Me16:0 [30]). In our samples, 10Me16:0 was the most abundant lipid within this group (10–50× greater relative abundance compared with 10Me18:0, 15:1ω6, and i17:1ω7), and when 10Me16:0 is in much greater abundance relative to 10Me18:0, anaerobic SRB are typically prominent [50, 59]. Therefore, actinobacteria and SRB were combined (actino_SRB; 10Me16:0, 10Me18:0, 15:1ω6, and i17:1ω7) [1, 50, 59].

Ratios of PLFAs were also used to infer physiological stress of GNB from the summation of the ratios cy17:0:16:1ω7 and cy19:0:18:1ω7 [9, 10]. Alterations in environmental condition (e.g., oxygen availability, substrate quality, and nutrient enrichment) were assessed with the ratios of fungi to bacteria (F/B) (Fungi/GPB+GNB) and GPB/GNB [3, 61]. A recent review by Frostegård et al. [21] suggested functional groups and ratios should be used with caution. However, the main goal of this work was to investigate broad changes and patterns in microbial biomass and functional groups to management that have previously been associated with environmental conditions, manipulations, and ecological functions in soils.

Statistical Analyses

The Everglades has a seasonality in its wet-and-dry periods. Therefore, to better assess trends in microbial dynamics among years, individual sampling events were grouped into water years (May–April) to factor out intra-annual variation. The eight sampling events fell within four water years (WY07, WY08, WY09, and WY10); however, WY07 was not included in the analysis as only one sampling event occurred. Water years were assumed to be independent since microbial turnover is greater than the sampling interval, and plots experienced wide-ranging hydrology (dry-downs and flooding) between water years.

Two-way analysis of variance (ANOVA) was used to determine if total PLFA content and concentration, and microbial functional groups within a region (i.e., regions E and T) differed significantly between control (C) and open (O) plots and with time. Two-way ANOVA was also used to determine if PLFA metrics differed among the open and reference plots (EO, TO, and UC). The treatment by time interaction was removed from the ANOVA model if it was not significant (α=0.05). Prior to analysis, PLFA content and concentration were $Log_{10}(n+1)$ transformed if needed to meet assumptions of normality and homoscedasticity. Tukey's HSD was used to identify significant pair-wise differences. Statistical analyses were performed with Systat v.11 (Chicago, IL).

Differences in plot functional group-based microbial composition (content and concentration) derived from PLFA

biomarkers were assessed using non-metric dimensional scaling (NMDS) with Bray–Curtis distanced matrix. Preliminary ordinations were conducted to determine the number of dimensions to include in the final analysis [37]. The criteria for the preliminary analysis consisted of stepping down from a six- to one-dimensional solution, with the instability criterion set at 0.0005, with 250 iterations, and 50 runs with real and randomized data. The final analysis was performed using the dimension solution obtained from the preliminary analysis, typically two dimensional, no-step down in dimensionality, and one real run. Rank-transformed multi-response permutation procedures with pair-wise comparisons (MRPP) using the natural weighting method and Bray–Curtis similarity index as the distance measure were used to determine if all measured microbial biomarkers differed between control, open, and reference plots [37]. NMDS and MRPP analyses were performed using PC-ORD, version 5.13 (MJM Software Design, Gleneden Beach, OR).

Results

PLFA content varied among control, open, reference plots, and sediment type (Fig. 2a, b). Average floc PLFA content (mean±SE) equaled 786±78, 1,521±168, 923±90, 1,026±103, and 445±34 nmol g^{-1} for EC, EO, TC, TO, and UC plots, respectively (Fig. 2a). Soil (0–5 cm) PLFA contents were lower than floc, averaging 294±41 for EC, 378±51 for EO, 276±51 for TC, 331±46 for TO, and 97±27 nmol g^{-1} for UC plots (Fig. 2b).

Treatment effects on PLFA content differed between regions (Figs. 3 and 4a, b, and e). Within the E plots, removal of emergent vegetation resulted in significantly greater PLFA content in floc (Table 1; Fig. 3a) but not in soil (Table 2; Fig. 4a); however, content in soil did vary with time (Table 2; Fig. 4a). In contrast, vegetation removal in the T region did not significantly influence PLFA content of floc (Table 1; Fig. 3b) or soil (Table 2; Fig. 4b); however, soil content did significantly vary with time (Table 2; Fig. 4b). Compared with reference plots (reference sloughs), floc and soil PLFA contents were significantly greater for the created openings in the E and T regions (Figs. 3e and 4e; Tables 3 and 4). Average floc and soil PLFA contents for EO and TO were approximately three and two times greater than UC, respectively.

Average floc PLFA concentration (mean±SE) equaled 436±60, 1,139±216, 484±70, 809±187, and 611±172 μmol m^{-2} for EC, EO, TC, TO, and UC plots, respectively (Fig. 2c). Soil PLFA concentrations averaged 982±140 for EC, 1,403±241 for EO, 888±184 for TC, 1,190±208 for TO, and 591±194 μmol m^{-2} for UC plots (Fig. 2d).

Treatment by water year interactions for floc PLFA concentrations in both regions were significant (Table 1; Fig. 3c, d). Soil PLFA concentrations differed significantly among water years but not between treatment and control plots (Table 2; Fig. 4c, d). For floc, treatment effects were greater for the E region (Fig. 3c) than T region (Fig. 3d). Although concentrations varied with time, consistent trends were not evident between floc and soils nor within experimental open and control plots (Figs. 3c, d and 4c, d).

Between created open and reference plots, floc PLFA concentrations followed similar patterns to content, with significantly lower values for UC than TO and EO (Table 3; Fig. 3f). Sediment PLFA concentration were also significantly lower at UC, and varied significantly through time (Table 4; Fig. 4f).

Floc microbial composition based on functional group contents (NMDS final stress 5.55; MRPP A=0.17) were significantly different between EO and EC plots (MRPP P<0.001) and differed for TO and TC plots (MRPP P=0.08) (Fig. 5). Open plots (EO and TO) differed between each other (MRPP P=0.06) and significantly with UC (MRPP P<0.001). Open plot distributions were positively associated with functional group content (Fig. 5). Ordination based on functional group concentrations followed a similar pattern (NMDS final stress 18.2, MRPP A=0.09), with the exception that TO was not significantly different from UC (MRPP P=0.14).

Floc functional group contents and concentrations were greater in open plots than controls (Fig. 6a, c). Content of GNB, fungi, and algae were significantly greater in EO than EC (Table 1; Fig. 6a). No significant treatment effects were observed in T plots, and there were no significant time effects for E or T plots (Table 1). Functional group contents between EO, TO, and UC had a significant treatment by water year interaction (Table 3). There were significant treatment by water year interactions for floc functional group concentrations for EO-EC and TO-TC (Table 1). Functional group concentrations were significantly greater for EO and TO relative to UC (Fig. 6c), and varied significantly with time (Table 3). Changes in functional group content and concentration between WYs followed patterns observed in the overall total PLFA mass.

Soil microbial community composition based on group contents did not differ significantly between open and control plots (NMDS final stress=26.2; MRPP A=0.20, P>0.05), but EO and TO were significantly different from UC (MRPP P<0.001). Functional group concentrations followed a similar pattern (NMDS final stress=35.6, MRPP A=0.08), with the exception that composition was marginally non-significant between TO and TC (MRPP P=0.08). All P-enriched regions were significantly different from the reference (MRPP P<0.001).

Changes in functional group contents and concentrations in sediment open plots relative to controls were less than

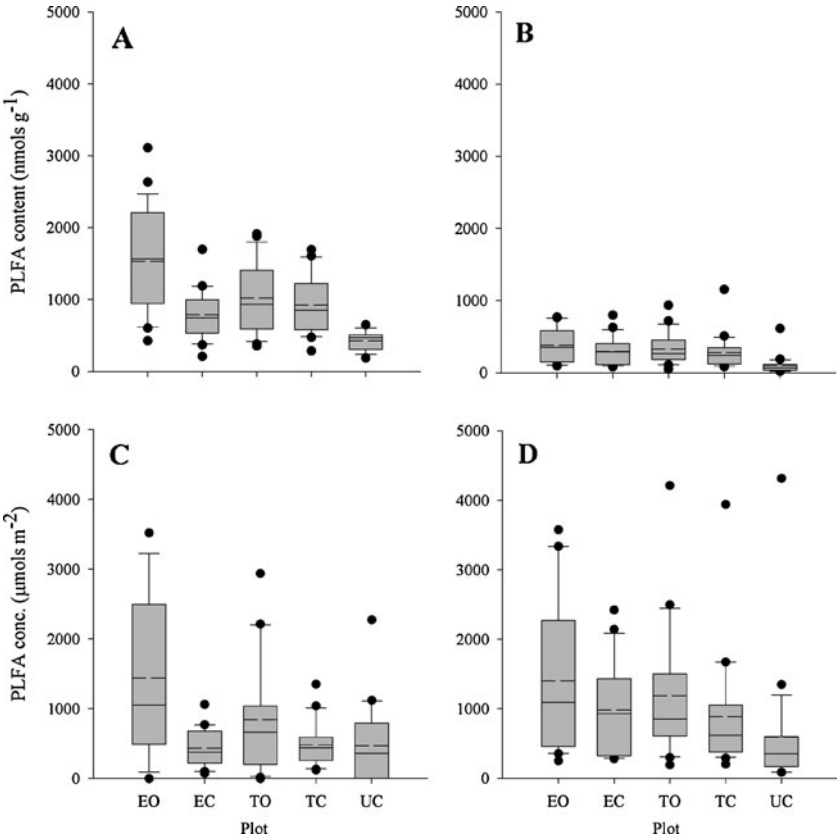

Figure 2 Mean (*dashed line*), median (*solid line*), 25th and 75th percentiles represented by *box limits*, 10th and 90th percentiles by *error bars*, and *points outside of error bars* indicate data outliers of PLFA contents and concentrations in floc (**a**, **c**) and soils (**b**, **d**) for the entire sampling period. Floc content/concentration N=19/19 (EC), 18/20 (EO), 20/20 (TC), 17/19 (TO), and 11/18 (UC); soil content/concentration N=20/20 for all plots. *E* enriched, *T* transitional, *C* control, *O* open, *UC* reference

that observed in floc (Fig. 6). Greater content of GNB in EO was marginally non-significant relative to EC (Table 2; Fig. 6b). Content of all functional groups significantly varied with time (Table 2). Concentrations of GNB in EO were significantly greater than EC (Table 2; Fig. 6d). In TO, greater GNB average concentrations were marginally non-significant (Table 2; Fig. 6d). All functional group concentrations significantly varied through time (Table 2). EO and TO microbial group contents and concentrations were significantly greater than UC (Fig. 6b, d) and varied significantly through time (Table 4).

In floc, ratios of GNB stress, GPB/GNB, and F/B declined in open plots, EO having significant reductions relative to EC in the former two (Table 5). Significant changes through time were observed for stress and F/B ratios in the E region, and the F/B ratio in the T region. All ratios were greater at EO and TO relative to UC; however, F/B and GPB/GNB had significant treatment by water year interactions (Table 5).

Soil GNB stress and F/B ratios were significantly lower in EO than EC, and F/B varied significantly with time (Table 5). Stress and F/B ratios were not significantly different in TO than TC, but there were significant changes in F/B and GPB/GNB through time (Table 5). Ratios of stress and F/B were significantly greater in EO and TO than UC, whereas the GPB/GNB ratio was significantly greater in UC than EO and TO (Table 5).

Discussion

Expression of Microbial Biomass as Concentration Rather than Content

All sediment PLFA studies to date express patterns in terms of relative abundance (percent detected) or the closely related unit content (mass per unit mass). Recently, the use of content to express sediment biogeochemical properties has been criticized because content is decoupled from the actual amount (e.g., of microbial biomass) present and can lead to erroneous conclusions of ecological cause and effect [51]. At issue, specifically, are that biogeochemical measures of content may not be independent and are confounded by spatial and temporal variations in actual sediment mass within a unit area. Therefore, it is argued that sediment biogeochemical properties be expressed as concentrations (mass per unit volume or area) in order to avoid confounding effects and mistaken inferences [51]. For example, the amount of floc is strongly influenced by drying-rewetting and compaction. Expression of microbial biomass per gram of floc could provide similar *contents* in wetted and dried floc, but the former would actually have less amount of material due to the large amount of water present, which would be more accurately reflected as a lower biomass *concentration* (e.g., Fig. 3). In addition, caution is stressed when comparing studies because the terms are often confused (e.g., [49, 50]). For example, Steger et al.

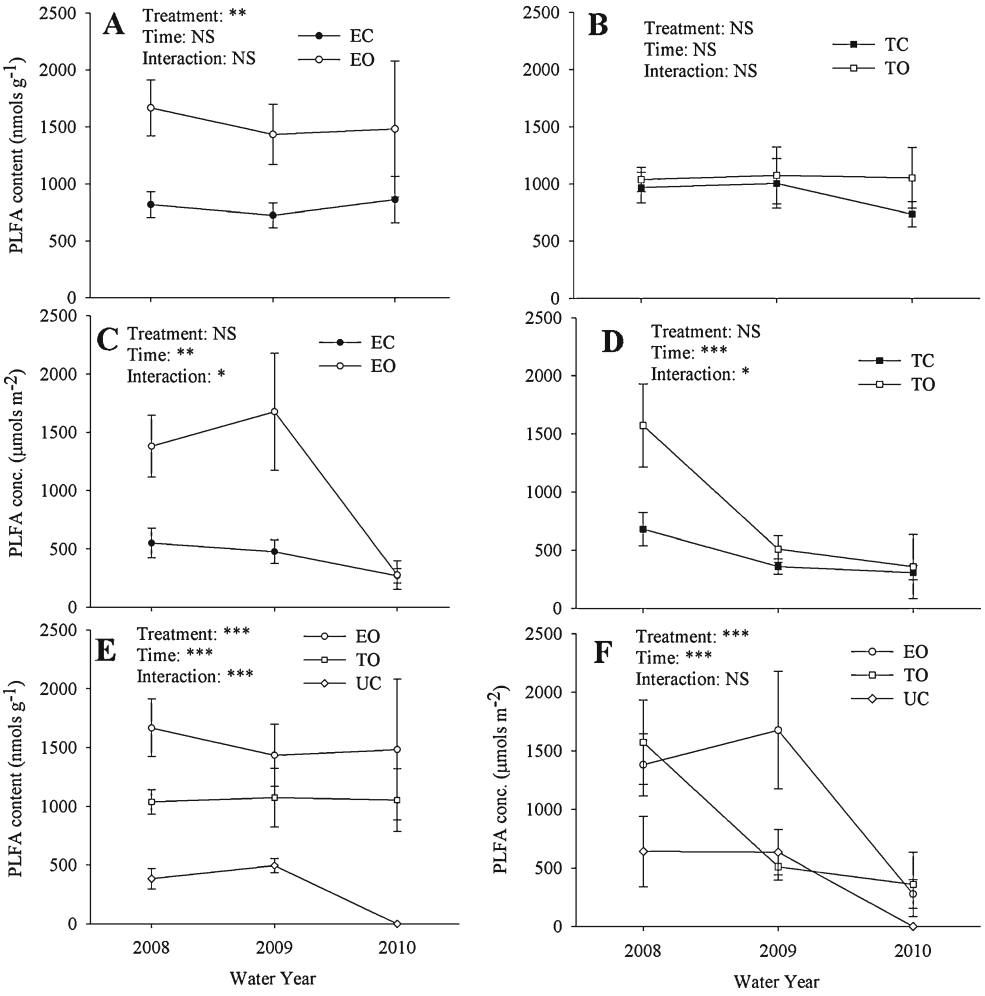

Figure 3 Floc PLFA contents and concentrations at the enriched (**a**, **c**) transitional (**b**, **d**) open (*O*) and control (*C*) plots, and comparing open and reference (*UC*) (**e**, **f**) plots grouped into water years. Two-way ANOVA results are included, which refer to Tables 1 and 3 for *F*-statistics. Note different responses of PLFA mass to treatment and time when expressed as content or concentration. No floc was present at UC sites in WY2010. *NS* not significant. **P*<0.05; ***P*<0.01; ****P*<0.001, significant values. Data presented as means and standard errors

[49] incorrectly use concentration to refer to sediment PLFA data with the units of mass per unit mass (i.e., content). We presented here sediment PLFA patterns in both terms of content and concentration, our intent being to enable comparison across systems based on PLFA contents, but we restrict ecological assessment/significance to the more relevant and pertinent concentration of PLFAs.

Drivers of Microbial Responses in the Created Environment

Floc and soil microbial biomass, whether expressed as content or concentration, was generally greater for the created openings than for the dense emergent macrophyte controls (Fig. 2). Total PLFA contents observed here were within the 1–4,500 nmol g^{-1} range reported for floodplain, flooded upland, temperate and boreal wetlands, and lake sediments [10, 12, 36, 44, 49]. Microbial biomass is regulated by the physiochemical environment (e.g., temperature and oxygen) and substrate quality (e.g., lignin and cellulose content and nutrient stoichiometry), and nutrient availability has been positively associated with microbial biomass due to high physiological P demands of bacteria [38, 42, 45].

Physicochemical

The physicochemical environment may be directly affected by hydrology, influencing microbial concentrations and community structure by regulating the aerobic environment of soils through duration of inundation [36, 38, 44]. Persistently flooded conditions typically result in lower DO concentrations due to diffusive constraints and biological activity, resulting in a microbial community reflective of reducing conditions [16, 35]. However, in this study removal of dense vegetation did not affect water depth or hydroperiod between created open and macrophyte control plots. Thus, intra-annual hydrologic effects alone are less likely to account for observed differences in the microbial community between experimental plots. Rather, hydrology effects and associated microbial community responses and functions are linked to drying–rewetting events [46, 48], as evidenced by the large inter-annual variation in PLFA concentrations (Figs. 3 and 4). Independent of treatment or region, significant changes in floc PLFA concentration were observed across all plots between water years. During the drought of WY2010, a complete dry-down was observed which significantly reduced or eliminated the floc layer via compaction into

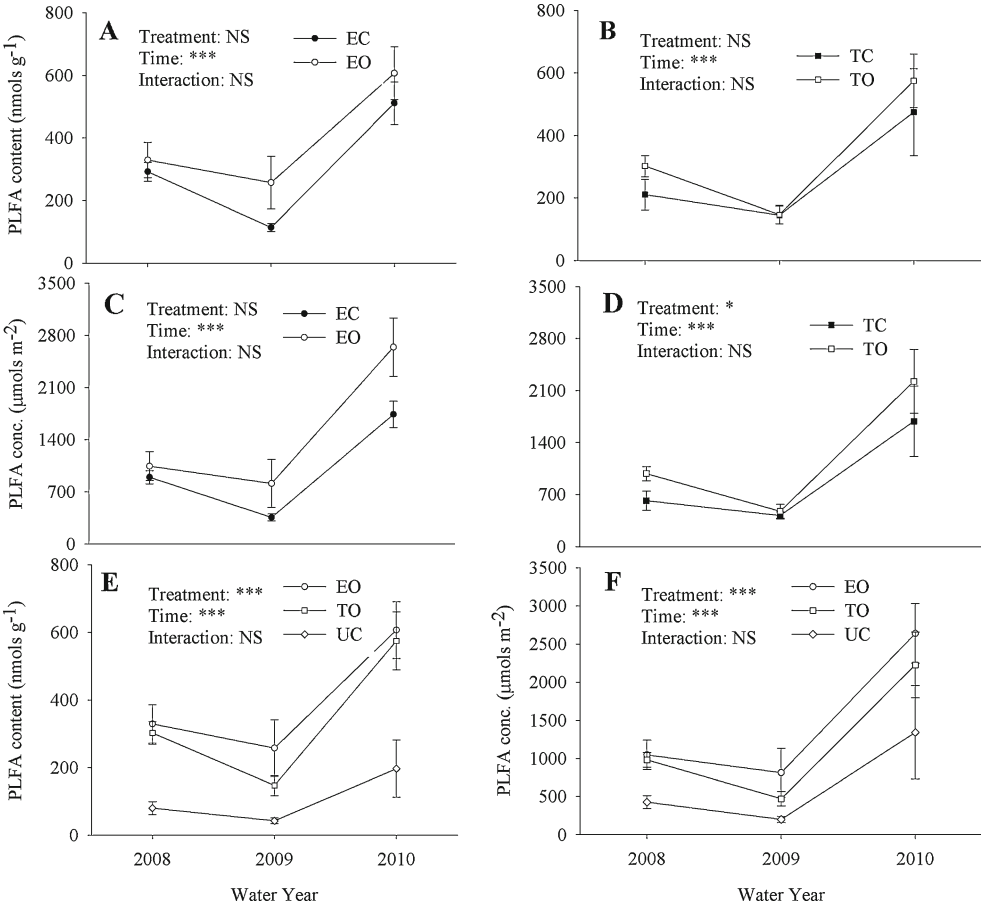

Figure 4 Soil PLFA contents and concentrations at the enriched (**a**, **c**) transitional (**b**, **d**) open (*O*) and control (*C*) plots and comparing open and reference (*UC*) (**e**, **f**) plots grouped into water years. Two-way ANOVA results given; refer to Tables 2 and 4 for *F*-statistics. Note that for soil, responses of PLFA mass to treatment and time when expressed as content or concentration were similar. *NS* not significant. **P*<0.05; ***P*<0.01; ****P*<0.001, significant values. Data presented as means and standard errors

the surfical peat. However, reduction of the floc layer had minimal impacts on PLFA content but significantly reduced PLFA concentration biomass (Fig. 3). These intra- and inter-annual differences highlight uncertainty associated with expression of microbial biomass as contents rather than concentrations. Depending on data expression, discussion about microbial biomass, and subsequently potential rates of microbial activity, would be dramatically different.

Instead, greater microbial concentrations and community structure differences between created open and macrophyte control plots are a function of physicochemical changes in the availability of DO related to the altered aquatic plant environment [11, 44]. Whereas surface water temperature differences between plots averaged less than 2 °C, the DO concentrations for the created openings (average, >3.9 mg/L) were greater than the dense emergent macrophyte controls (<1.9 mg/L), driven by aquatic primary production and wind mixing [46]. Oxygen availability affects microbial biomass by changing the supply of electron acceptors [38] giving a competitive advantage to aerobic organisms [41]; thus, the lower biomass within control plots may be due to the dependence on less energetically rewarding alternative electron acceptors to sustain microbial processes given the prevalence of anaerobic conditions.

In response to the altered oxic environment, PLFA concentrations indicative of GNB were greater for created open plots relative to macrophyte controls (Fig. 6). Monounsaturated fatty acids have typically been used as indicators of aerobic bacteria, notably GNB, and greater contents have previously been related to oxic environment [6, 10, 30, 39]. For example, the mass of GNB was found to more than double in oxic floodplain soils relative to anoxic, riverine soils [47]. The greater metabolic energy yield associated with aerobic catabolism [41] supports our observations of significantly greater concentrations of monounsaturated PLFAs in created open plots. Conversely, terminally branched PLFAs, which include GPB and few GNB, typically have greater biomass when soils are flooded and having low oxygen concentrations [11, 30, 61]. While we did observe increased concentrations of GPB, the ratio GPB/GNB significantly declined, further reflecting greater oxygen availability [11, 25, 33]. Overlaps in changes to both aerobic and anaerobic microbial indicators in response to site condition alterations have been attributed to greater microsite variations (i.e., habitat heterogeneity) supporting a richer microbial community and one more tolerant to an improved oxic environment [50].

Substrate Quality

In addition to oxygen, greater PLFA biomass concentrations in created open plots may reflect the altered resource quality (SAV)

Table 1 Two-way ANOVA (treatment×time effects) results for floc content (top rows) and concentration (bottom rows) of total measured PLFAs and functional groups

	EC-EO				TC-TO			
	Treatment ($F_{1, 33}$)	Time ($F_{2, 33}$)	Tukey's HSD	Interaction ($F_{2, 31}$ and ($F_{2, 33}$)	Treatment ($F_{1, 33}$)	Time ($F_{2, 33}$)	Tukey's HSD	Interaction ($F_{2, 33}$)
Total content	***F*$_{(1, 33)}$=11.73****	*F*=0.35; NS		*F*=0.14; NS	*F*=0.51; NS	*F*=0.31; NS		*F*=0.22; NS
GPB	*F*=1.34; NS	*F*=0.26; NS		*F*=0.21; NS	*F*=0.15; NS	*F*=0.32; NS		*F*=0.12; NS
GNB	***F*=14.63****	*F*=0.33; NS		*F*=0.07; NS	*F*=0.69; NS	*F*=0.39; NS		*F*=0.44; NS
Actino_SRB	*F*=3.38; NS	*F*=0.76; NS		*F*=0.26; NS	*F*=0.72; NS	*F*=0.31; NS		*F*=0.06; NS
Fungi	***F*=5.52***	*F*=0.71; NS		*F*=0.29; NS	*F*=0.02; NS	*F*=0.29; NS		*F*=0.40; NS
Algae	***F*=23.05*****	*F*=1.09; NS		*F*=1.05; NS	*F*=3.16; NS	*F*=0.47; NS		*F*=0.62; NS
Total concentration	*F*=0.27; NS	***F*=8.00****	2008=2009>2010	***F*=3.87***	*F*=0.31; NS	***F*=8.37*****	2008>2010	***F*=3.30***
GPB	*F*=0.09; NS	***F*=9.73*****	2008=2009>2010	***F*=3.91***	*F*=0.58; NS	***F*=9.16*****	2008>2010	***F*=3.31***
GNB	*F*=1.02; NS	***F*=9.27*****	2008=2009>2010	***F*=3.50***	*F*=0.02; NS	***F*=7.72****	2008>2010	*F*=2.69; NS
Actino_SRB	*F*=0.72; NS	***F*=9.89*****	2008=2009>2010	***F*=3.80***	*F*=0.74; NS	***F*=9.93*****	2008>2010	***F*=3.41***
Fungi	*F*=1.74; NS	***F*=7.61****	2008=2009>2010	***F*=4.00***	*F*=0.17; NS	***F*=10.37****	2008>2010	***F*=3.38***
Algae	***F*=9.33****	***F*=5.21***	2008=2009>2010	***F*=5.17***	*F*=1.07; NS	***F*=9.73*****	2008>2010	*F*=3.15; NS

Left columns represent comparison between highly enriched open and control plots; the right columns between the transitional open and control plots. Significant differences are set in bold. Tukey's HSD denotes significant pair-wise differences where applicable

NS not significant, *GPB* Gram-positive bacteria, *GNB* Gram-negative bacteria, *Actino_SRB* sum abundance of actinobacteria and sulfate-reducing bacteria

*P<0.05; **P<0.01; ***P<0.001, significant values

[11, 52]. The resultant macrophyte community shift caused by the removal of emergent vegetation altered the supply and quality of organic matter substrates [46]. Created openings were dominated by periphyton, SAV, and the macroalga *Chara* sp. Algae, SAV, and floating macrophytes have lower fiber components (complex polymers like lignin or cellulose) than emergent macrophytes [17, 22]. Substrate quality differences between plots were also reflected in the carbon and nutrient

Table 2 Two-way ANOVA (treatment×time effects) for soil content (top rows) and concentration (bottom rows) of total measured PLFAs and functional groups

	EC-EO			TC-TO		
	Treatment ($F_{1, 36}$)	Time ($F_{2, 36}$)	Tukey's HSD	Treatment ($F_{1, 36}$)	Time ($F_{2, 36}$)	Tukey's HSD
Total content	*F*=2.48; NS	***F*=21.93*****	2009<2008<2010	*F*=3.25; NS	***F*=22.04*****	2009<2008<2010
GPB	*F*=0.44; NS	***F*=14.51*****	2009<2008<2010	*F*=0.66; NS	***F*=15.42*****	2009<2008<2010
GNB	*F*=3.25; NS	***F*=19.71*****	2009<2008<2010	*F*=1.31; NS	***F*=12.50*****	2008=2009<2010
Actino_SRB	*F*=0.12; NS	***F*=22.16*****	2009<2008<2010	*F*=1.08; NS	***F*=23.34*****	2009<2008<2010
Fungi	*F*=0.40; NS	***F*=25.11*****	2009<2008<2010	*F*=0.84; NS	***F*=24.41*****	2009<2008<2010
Total concentration	*F*=3.76; NS	***F*=28.85*****	2009<2008<2010	*F*=5.94*	***F*=29.21*****	2009<2008<2010
GPB	*F*=0.21; NS	***F*=18.88*****	2009<2008<2010	*F*=0.86; NS	***F*=19.64*****	2009<2008<2010
GNB	*F*=4.54*	***F*=26.98*****	2009<2008<2010	*F*=3.11; NS	***F*=19.96*****	2009<2008<2010
Actino_SRB	*F*=0.05; NS	***F*=28.23*****	2009<2008<2010	*F*=1.57; NS	***F*=29.22*****	2009<2008<2010
Fungi	*F*=0.05; NS	***F*=31.56*****	2009<2008<2010	*F*=1.16; NS	***F*=30.55*****	2009<2008<2010

Left columns represent comparison between highly enriched open and control plots; the right columns between the transitional open and control plots. Significant differences are set in bold. Tukey's HSD denotes significant pair-wise differences where applicable. No significant treatment by time interaction effects were observed in soils

NS not significant, *GPB* Gram-positive bacteria, *GNB* Gram-negative bacteria, *Actino_SRB* sum abundance of actinobacteria and sulfate-reducing bacteria

*P<0.05; **P<0.01; ***P<0.001, significant values

Table 3 Two-way ANOVA (treatment×time effects) for floc comparing open (EO and TO) and reference (UC) plot content (top rows) and concentration (bottom rows) of total PLFAs and functional groups

	Treatment ($F_{2, 45}$ and $F_{2, 53}$)	Tukey's HSD	Time ($F_{2, 45}$ and $F_{2, 53}$)	Tukey's HSD	Interaction ($F_{4, 45}$ and $F_{4, 49}$)
Total Content	**F=66.22*** **	EO = TO > UC	**F=22.26*** **	2008=2009>2010	**F=20.93*** **
GPB	**F=50.06*** **	EO = TO > UC	**F=18.75*** **	2008=2009>2010	**F=15.13*** **
GNB	**F=71.28*** **	EO = TO > UC	**F=19.96*** **	2008=2009>2010	**F=18.94*** **
Actino_SRB	**F=70.89*** **	EO = TO > UC	**F=15.95*** **	2008=2009>2010	**F=12.77*** **
Fungi	**F=59.38*** **	EO = TO > UC	**F=9.53*** **	2008=2009>2010	**F=10.99*** **
Algae	**F=42.85*** **	EO = TO > UC	**F=7.34** **	2008=2009>2010	**F=11.47*** **
Total Concentration	**F=9.99*** **	EO = TO > UC	**F=24.49*** **	2008=2009>2010	F=2.039; NS
GPB	**F=6.94** **	EO = TO > UC	**F=27.52*** **	2008=2009>2010	F=1.53; NS
GNB	**F=10.21*** **	EO = TO > UC	**F=25.14*** **	2008=2009>2010	F=1.61; NS
Actino_SRB	**F=9.58*** **	EO = TO > UC	**F=27.04*** **	2008=2009>2010	F=1.18; NS
Fungi	**F=10.66*** **	EO = TO > UC	**F=25.63*** **	2008=2009>2010	F=1.58; NS
Algae	**F=8.26** **	EO = TO > UC	**F=23.65** **	2008=2009>2010	F=2.12; NS

Significant differences are set in bold. Tukey's HSD denotes significant pair-wise differences where applicable. *F* values for content on left, concentration on right

NS not significant, *GPB* Gram-positive bacteria, *GNB* Gram-negative bacteria, *Actino_SRB* sum abundance of actinobacteria and sulfate-reducing bacteria

*$P<0.05$; **$P<0.01$; ***$P<0.001$, significant values

concentrations and stoichiometry of the aboveground macrophyte and periphyton biomass [46]. Relative to the created openings, dense emergent macrophyte control plots not only had significantly greater C, N, and P concentrations but also greater molar C/P and C/N. For example, the C/P, C/N, and N/P of live and dead emergent macrophytes ranged from 1,478 to 6,600, 67 to 85, and 22 to 78, respectively. In contrast, ratios for periphyton, SAV, and floating macrophytes ranged from 696 to 1,597 for C/P, 16 to 26 for C/N, and 33 to 92 for N/P. Bacterial growth efficiency is tightly coupled to substrate stoichiometry, with lower efficiencies associated with higher ratios driven by resource supply departures from bacterial resource requirements [23, 32]. Thus, the contribution of higher C/P and C/N of emergent macrophyte litter may account for the lower microbial biomass in control plots by constraining bacterial growth and contributing to reduced decomposition rates [41].

In macrophyte control plots, our observed lower fungal biomass concentrations relative to created openings could be related to the abundance of *Typha*, independent of substrate stoichiometry. Cornwell et al. [14] observed no

Table 4 Two-way ANOVA (treatment×time effects) for soils comparing open and reference plot content (top rows) and concentration (bottom rows) of total measured PLFAs and functional groups

	Treatment ($F_{2, 55}$)	Tukey's HSD	Time ($F_{2, 55}$)	Tukey's HSD
Total content	***F*=42.66*** **	EO=TO>UC	***F*=22.12*** **	2008=2009<2010
GPB	***F*=5.87** **	EO=TO>UC	***F*=14.95*** **	2008=2009<2010
GNB	***F*=41.84*** **	EO=TO>UC	***F*=18.93*** **	2008=2009<2010
Actino_SRB	***F*=27.99*** **	EO=TO>UC	***F*=15.61*** **	2008=2009<2010
Fungi	***F*=53.11*** **	EO=TO>UC	***F*=22.85*** **	2008=2009<2010
Total concentration	***F*=18.35*** **	EO=TO>UC	***F*=31.50*** **	2009<2008<2010
GPB	***F*=5.87** **	EO=TO>UC	***F*=22.47*** **	2009<2008<2010
GNB	***F*=19.29*** **	EO=TO>UC	***F*=28.88*** **	2009<2008<2010
Actino_SRB	***F*=18.95*** **	EO=TO>UC	***F*=27.50*** **	2009<2008<2010
Fungi	***F*=27.77*** **	EO=TO>UC	***F*=31.06*** **	2009<2008<2010

Significant differences are set in bold. Tukey's HSD denotes significant pair-wise differences where applicable. No significant treatment by time interaction effects were observed in soils

NS not significant, *GPB* Gram-positive bacteria, *GNB* Gram-negative bacteria, *Actino_SRB* sum abundance of actinobacteria and sulfate-reducing bacteria

*$P<0.05$; **$P<0.01$; ***$P<0.001$, significant values

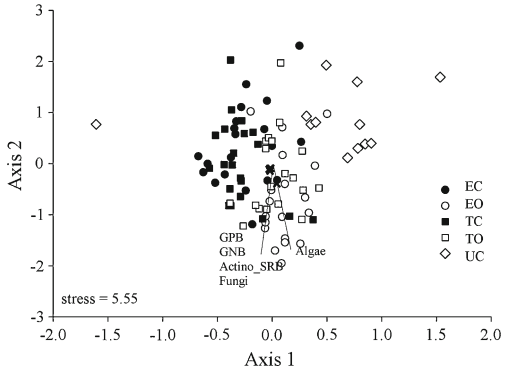

Figure 5 NMDS ordination based on floc PLFA functional group contents for plots across all water years. Distribution of PLFA functional groups correlated with axis 2 (r^2>0.5), indicating greater mass associated with created open plots ordinated negatively along axis 2. Axes 1 and 2 account for >97 % of the distance matrix variance. *E* enriched, *T* transitional, *C* control, *O* open, *UC* reference, *GPB* Gram-positive bacteria, *GNB* Gram-negative bacteria, *Actino_SRB* sum abundance of actinobacteria and SRB

mycorrhizal fungal growth associated with *Typha latifolia* and overall associations with wetland monocots were extremely low. Conversely, our observed reduced fungal biomass concentrations in all plots, and notably in our floc, may be related to the substrates' state of decomposition. During our sampling of floc, large leafy detrital material was removed in the field, leaving smaller, more degraded plant litter material. Significant fungal growth has been found associated with standing dead macrophytes in other wetlands [34, 52, 41]; thus, a significant amount of fungal biomass may have been removed with the larger detrital material prior to our analyses. The significantly greater fungal biomass concentrations in created open plots could be related to greater abundances of dicots (SAV) in addition to the altered aerobic conditions [10, 12, 36]. Fungal contents have been shown to be greater under drained, aerobic conditions than for flooded (reduced oxygen) sites [10, 39]. The enzyme phenol oxidase, a corollary of fungal biomass and metabolic activity [56], is oxygen sensitive, and increased enzymatic rates have been observed in aerobic or drained detrital and surface soil layers [19, 41]. As such, our sampling during times of inundation of the benthic floc and soils may be suppressing fungi, even in the relatively oxygen-rich created open plots [1, 9, 29, 30].

Actinobacteria and SRB represent two very ecologically and functionally distinct groups, but their identification based on PLFAs tends to be ambiguous, and many authors will combine the two when using PLFAs to assess changes in the microbial community (e.g., [11, 59, 61]). We observed increased mass of actinobacteria+SRB concentrations in open plots, both potentially responding to the altered detrital quality (i.e., C/P ratios) [6, 12]. However, each group would be expected to have different responses to the improved oxygen environment of the created open plots. Actinobacteria primarily function as aerobes [6, 12], though a few species are also

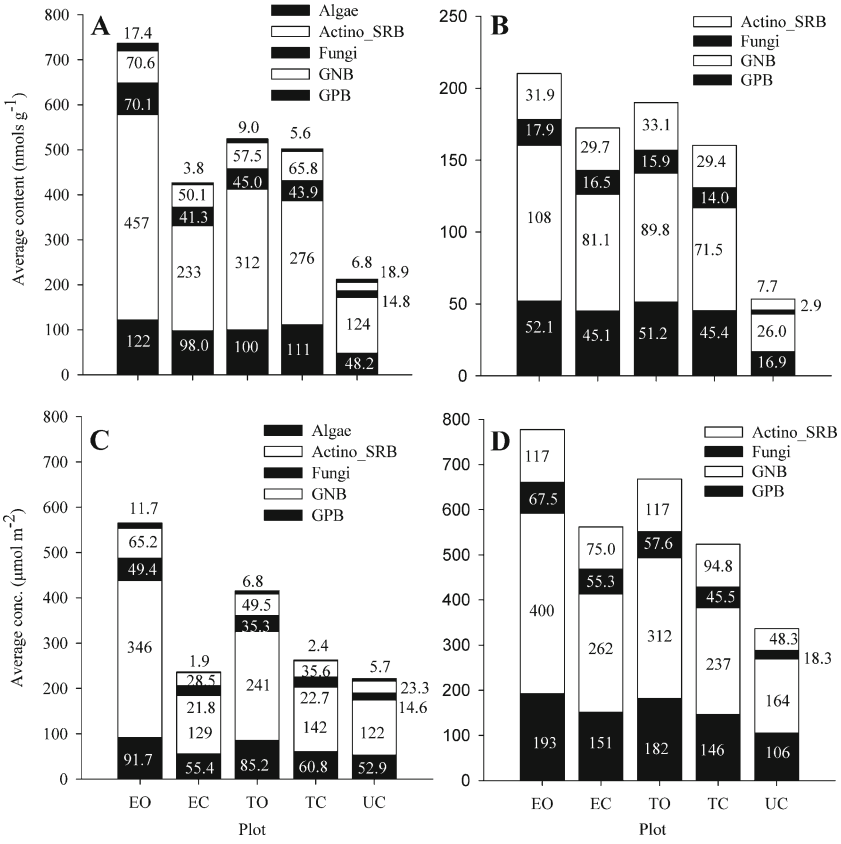

Figure 6 Microbial functional group contents (*top panels*) and concentrations (*bottom panels*) of floc (**a**, **c**) and soils (**b**, **d**). *E* enriched, *T* transitional, *C* control, *O* open, *UC* reference, *GPB* Gram-positive bacteria, *GNB* Gram-negative bacteria, *Actino_SRB* sum abundance of actinobacteria and SRB. Mean values for replicate plots across WYs for each functional group included within the stacked bars. See Tables 1, 2, 3, and 4 for statistical outputs comparing functional group mass among plots and water years and Table 6 for proportional change due to management

Table 5 Mean ± standard errors of ratios, analyzed with two-way ANOVA (treatment×time effects) in floc and soils

	Plot	Mean ± Std. Error	Plot	Mean ± Std. Error	2-Way ANOVA	Tukey's HSD
Floc						
GNB stress ratio	EO	0.22±0.01	EC	0.28±0.02	**Treatment $F_{(1,33)}$=6.05*** **Time $F_{(2,33)}$=4.25***	2008<2010
	TO	0.27±0.02	TC	0.35±0.03	Treatment F=3.68; NS Time F=2.88; NS	
	UC	0.10±0.01			**Treatment $F_{(2,45)}$=22.20***** Time $F_{(2,45)}$=1.42; NS	EO=TO>UC
Fungi/ Bacteria	EO	0.11±0.03	EC	0.12 ±0.03	Treatment F=0.85; NS **Time F=8.38****	2009<2010
	TO	0.11±0.03	TC	0.12±0.03	Treatment F=0.48; NS **Time F=4.01***	2009<2010
	UC	0.06±0.02			**Treatment F=19.85***** **Time F=5.84**** **Interaction $F_{(4,45)}$=11.90*****	EO=TO>UC 2008>2010
GPB/ GNB	EO	0.27±0.06	EC	0.42±0.10	**Treatment F=29.38***** Time F=0.18; NS	
	TO	0.37±0.09	TC	0.44±0.10	Treatment F=2.07; NS Time F=0.20; NS	
	UC	0.28±0.07			**Treatment F=3.87*** **Time F=12.50***** **Interaction F=9.66*****	EO < TO 2008=2009>2010
Soil						
GNB stress ratio	EO	0.55±0.03	EC	0.67±0.03	**Treatment $F_{(1,36)}$=16.77***** Time $F_{(2,36)}$=1.81; NS	
	TO	0.62±0.04	TC	0.69±0.04	Treatment F=1.60; P=0.22 Time F=2.38; P=0.11	
	UC	0.32±0.02			**Treatment $F_{(2,55)}$=26.22***** Time $F_{(2,55)}$=2.34; NS	EO = TO > UC
Fungi/ Bacteria	EO	0.11±0.02	EC	0.12±0.03	**Treatment F=5.10*** **Time F=21.53*****	2009<2008<2010
	TO	0.11±0.03	TC	0.11±0.02	Treatment F=0.01; NS **Time F=3.81***	2009<2010
	UC	0.06±0.01			**Treatment F=14.94***** Time F=2.92; NS	EO = TO > UC
GPB/ GNB	EO	0.46±0.10	EC	0.52±0.11	Treatment F=2.04; NS Time F=1.34; NS	
	TO	0.54±0.12	TC	0.59±0.13	**Interaction $F_{(2,36)}$=3.88*** Treatment F=0.60; NS **Time F=4.78***	2008=2010>2009
	UC	0.67±0.15			**Treatment F=10.55***** Time F=0.69; NS	EO = TO < UC

Treatment and time effects were determined within a region (E and T; O and C) and between O and UC regions. Significant differences are set in bold. Tukey's HSD denotes significant pair-wise differences where applicable. Interaction effects included only if significant

NS not significant, *E* enriched, *T* transitional, *C* control, *O* open, *UC* reference, *GPB* Gram-positive bacteria, *GNB* Gram-negative bacteria, *Actino_SRB* sum abundance of actinobacteria and sulfate-reducing bacteria

*P<0.05; **P<0.01; ***P<0.001, significant values

facultative anaerobes [24, 30]. Soil actinobacteria have been noted as being sensitive to water-logged soils due to reductions in oxygen availability [24]. Actinobacteria, like fungi, are important for their ability to decompose complex OM, notably higher plant cell wall compounds, and it is possible that our sampling has similarly missed a significant amount of their biomass. Alternately, SRB function under anaerobic conditions, utilizing sulfate as an electron acceptor during reduction of OM. In Everglades' floc and soils, we believe that SRB are of greater abundance relative to actinobacteria

due to the typically lower sustained DO concentrations and the abundance of sulfate. Sulfate concentrations average >20 mg L^{-1} throughout WCA-2A, and we observed significantly greater 10Me16:0 relative to 10Me18:0, suggesting SRB are the dominant organisms [60]. More detailed analyses using molecular markers (e.g., [13, 31]) are needed to better define the true abundances and relative functional importance of these two groups.

In contrast to the rapid and significant changes in the overlying floc layer, we observed few significant responses in soil microbial communities to the created openings (Table 2; Figs. 2 and 6). This is likely related to the removal of macrophytes having a lesser effect on the physicochemical and substrate quality of soil. For example, surface soils suffer legacy effects of abundant recalcitrant OM (high C/P) derived from below ground roots/rhizomes and structurally complex emergent macrophyte leaves (e.g., lignin-rich residue in surface soils) that accumulated prior to management [16]. In a managed Canadian wetland, minimal differences in PLFA composition in peat across vegetation classes were attributed to a similar parent material of soils among sites [1], and rates of change by soil microbial communities after management may be highly variable [55]. Smaller changes may also be related to sampling depth of this study. Total PLFA mass generally declines rapidly from the soil surface (e.g., by more than half within the top 10 cm), and functional group abundances shift in dominance between soil layers [11, 50], factors which could be influencing our observed responses in surface soils.

Nutrient Availability

Whereas the physiochemical environment and substrate quality account for microbial community differences between created open and emergent macrophyte control plots, the effect of nutrient supply is limited because of the similarity in water column TP concentrations [46]. However, nutrient supply may be a critical factor accounting for the significantly greater microbial concentrations in the P-impacted open plots relative to oligotrophic reference plots [43, 49]. In the eutrophic and transitional region, water column TP was two to five times greater, and floc and soil TP contents were approximately five times greater, compared with oligotrophic regions. The influence of nutrients on the microbial community between regions was also reflected in PLFA ratios. In the reference region the GNB stress ratio was lower, but the GPB/GNB ratio was significantly greater than values observed in created open plots (Table 5), suggesting that substrate quality (i.e., stoichiometry) and nutrient (TP) availability, and not DO concentrations (1.5× greater at reference sites), were influencing the microbial community structure. Additionally, PLFA concentrations were also likely related to differences in organic

Table 6 Summary of the percent changes in average microbial group contents and concentration in response to open plot creation relative to macrophyte controls, and relative to reference oligotrophic sloughs

Substrate	% change in average content					% change in average concentration			
	Group	EO-EC	TO-TC	EO-UC	TO-UC	EO-EC	TO-TC	EO-UC	TO-UC
Floc	GPB	24.1	-9.0	152.3	107.7	65.5	40.1	73.3	61.1
	GNB	96.0	13.1	269.6	152.7	168.4	69.7	183.9	97.1
	Fungi	69.7	2.5	373.6	204.1	126.6	55.5	238.4	141.8
	Actino_SRB	40.9	-12.6	273.5	204.2	128.8	39.0	179.8	112.4
	Algae	357.9	60.7	155.9	32.4	515.8	183.3	105.3	19.3
	GNB stress	-21.4	-27.0	120.0	170.0				
	Fungi:Bacteria	-8.3	-8.3	83.3	83.3				
	GPB:GNB	-35.7	-20.5	-3.6	25.0				
Soil	GPB	15.5	12.8	208.3	203.0	27.5	24.4	82.6	72.3
	GNB	33.5	25.6	316.5	245.4	52.9	31.5	143.5	89.7
	Fungi	8.5	13.6	517.2	448.3	22.1	26.6	268.9	214.8
	Actino_SRB	7.4	12.6	314.3	329.9	56.4	23.4	142.9	142.2
	GNB stress	-17.9	-10.1	71.9	93.8				
	Fungi:Bacteria	-8.3	0.0	83.3	83.3				
	GPB:GNB	-11.5	-8.5	-31.3	-19.4				

Positive values indicate an increase in mass or ratio in the created open plot relative to either macrophyte controls or reference plots, while negative values indicate an overall decline

E enriched, *T* transitional, *C* control, *O* open, *UC* reference, *GPB* Gram-positive bacteria, *GNB* Gram-negative bacteria, *Actino_SRB* sum abundance of actinobacteria and sulfate-reducing bacteria

carbon (OC) contents, as positive correlations have been observed between OC and PLFA contents in terrestrial soils [40]. In reference region floc and soils, OC is only approximately half of the total carbon (TC) content due to marl inputs from periphyton, whereas OC is 95 % of the TC content in P-impacted regions. Abundances of fungi have been shown to be reduced in mineral soils because of P-limitations ([7, 20] but see [3]). Here, we observed significant reductions in the F/B ratio in reference region floc and soils relative to EO or TO plots.

Functional Links to PLFA Ratios and Concentrations

While the functions of the microbial community (e.g., decomposition and nutrient cycling) may be similar regardless of the species present, rates of metabolic activity and degradative pathways are regulated by microbial community structure (composition and concentration), and physiological state (e.g., logarithmic vs. stationary growth) [15, 38, 43, 54] (Table 6 summarizes microbial structure and state responses due to management). We observed lower ratios of cyclopropyl/precursor lipids, an indicator of GNB community growth state, in open plots suggesting greater potential metabolism of OM by rapidly growing oxidizers ([10, 11, 39, 54, 60] but see [21]). White et al. [59] noted a ratio of 0.05 associated with log growth of bacteria, whereas ratios up to 2.5 were associated with stressed (slower) metabolism. Ratios in floc were typically half those of soils, but even in the latter ratios were between 0.55 and 0.70, suggesting rapid growth potentials for GNB. Significant reductions in EO plots nonetheless indicate alterations in external conditions conducive to further stimulating GNB growth.

Greater PLFA concentrations in created open plots suggest that metabolic rates should generally be greater relative to macrophyte controls, but may be highly variable between years. However, further study is needed to link enzyme activity rates and litter decomposition to microbial biomass concentrations as has been done with PLFA content. Greater potential OM processing in created open plots should be expected not only as a function of the overall greater biomass, but specifically as a result of greater abundances of the aerobic groups GNB and fungi. Biomarkers for GNB were the most abundant among all microbial groups in substrates from the Everglades, similar to finding elsewhere [30, 49], and decomposition rates have been positively related to GNB biomass in soils [28, 39]. Despite relatively low biomass concentrations, fungi and actinobacteria are recognized as important aerobic decomposers of complex OM, and early conditioners of litter for colonization by bacteria [7, 41, 52, 53]. Conversely, decomposition rates have been negatively correlated with GPB [36], and decomposition is known to occur slowly during anoxia, related to lower microbial biomass and less efficient energy yields during OM reduction [41]. While the greater concentrations of oxidizing microorganisms can translate to increased processing rates of OM, the importance of anaerobes in wetland biogeochemical cycles cannot be discounted [9], functioning during decomposition and nutrient cycling through methanogensis, denitrification, and sulfate reduction [41]. The greater concentration of microbial groups associated with reductive pathways in created open plots suggest these processes should likewise be altered as a result of management. While PLFA analysis may be limited in the details (e.g., species diversity) about the microbial community provided, we have predictably linked an altered physicochemical environment with changes in functional microbial groups, concentrations, and relative ratios (Table 6), which should translate into a mechanistic understanding of altered processes mediated by the microbial community in created open plots.

Acknowledgments This work was funded by the South Florida Water Management District. The authors would like to thank Robert Shuford, Kristin Seitz, Michael Manna, Megan Jacoby, Patty Holowecky, and contractors that assisted with sample collection, and James Beerens for producing the site map. The authors would also like to thank the two anonymous reviewers whose comments helped improve the quality of this manuscript.

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