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Patterns of heterotrophic microbial activity in eutrophic and oligotrophic peatlands

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ABSTRACT

Nutrient enrichment of peatlands may alter patterns of heterotrophic microbial activity (HMA) and organic matter cycling. The utilization of C-substrates by heterotrophic microbial communities in response to changes in environmental conditions may serve as sensitive indicators of changes in the trophic state of wetlands. The objective of this study was to measure the response of heterotrophic microbial communities to added C-substrates in the plant detritus layer and underlying soil (0–10 cm) for eutrophic, transitional, and oligotrophic sites along a nutrient enrichment gradient in the Florida Everglades, USA. The short-term response to C-substrates (alcohols, amino acids, carboxylic acids, and polysaccharides) was measured as CO_2 production. The nutrient gradient was characterized by decreasing P concentrations and microbial biomass from eutrophic to oligotrophic sites. Basal respiration was 73% higher at eutrophic than oligotrophic sites, and 41% higher in detritus than underlying soil. Heterotrophic microbial activity varied along the gradient with greater C-substrate utilization at the eutrophic site resulting from higher levels of microbial biomass and inorganic nutrients compared to the oligotrophic site. The C-substrates enhanced CO_2 production at all sites suggesting that labile organic C was a limiting factor to HMA in these peatlands. Substrate-induced respiration (SIR) of detritus was 25, 45, and 42% greater for polysaccharides than other C-substrates at the eutrophic, transitional, and oligotrophic sites, respectively. Likewise, SIR at the eutrophic, transitional, and oligotrophic sites was 0, 40, and 39%, respectively, greater for detritus amended with carboxylic acids than with amino acids and alcohols. Polysaccharides dominated HMA profiles at all sites along the nutrient gradient in detritus. The transitional site was characterized by carboxylic acids and alcohols, while the HMA profile at the oligotrophic site was dominated by carboxylic acids. Patterns of HMA along the nutrient gradient provided insight into the microbial response to changes in tropic status, indicating the heterotrophic microbial community was more sensitive with increasing eutrophication.

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1. Introduction

The Florida Everglades wetlands developed as nutrient-poor and supported vegetation adapted to these conditions [1]. In the past century, the Everglades was drained and separated into hydrologic units where water movement and storage were regulated, including the Everglades Agricultural Area (EAA) and the Water Conservation Areas. The compartmentalization of the Everglades into distinct hydrologic units altered traditional flow paths through the landscape, modifying the hydroperiod and increasing nutrient enrichment [2]. Runoff from agricultural soils in the EAA and altered hydrologic conditions have impacted Everglades wetlands by increasing soil nutrient levels, particularly P, which promoted shifts in vegetation patterns [1,2]. The ecosystem change most evident is the replacement of sawgrass (Cladium jamaicense) and slough communities by cattail (Typha domingensis) in northern Everglades wetlands [1–3].

In addition to contributing to changes in vegetation patterns in the Everglades, P loading has altered soil biogeochemical processes [4–8]. The impacts of anthropogenic nutrient loading to the Everglades is well documented in the distribution of floodwater and soil total P from nutrient-impacted peripheral areas of wetlands extending into the oligotrophic interior [9–11]. The addition of limiting nutrients to ecosystems often results in enhanced productivity of vegetation and stimulation of the microbial community. However, the microbial community may be more sensitive or respond more readily to changes in trophic status than vegetation. Changes in vegetation patterns due to nutrient loading may take years to be observed [12,13], while microbial processes display effects after a short exposure to elevated nutrient levels [4]. Thus, microbial processes and patterns may be used as sensitive indicators of eutrophication or changes in environmental conditions.

Organic matter decomposition and HMA in wetland soils depend on many factors, including microbial biomass, available nutrients, organic substrates, and temperature, pH, and redox potential [14,15]. In wetland soils, the availability of electron acceptors is often considered the primary limiting factor to organic matter decomposition and HMA [14]. However, bioavailable organic C may also limit microbial activity in Everglades soils [6,16]. Carbon in wetland soils is predominantly present as ligno-cellulose, lignin, or other fractions with varying degrees of recalcitrance [17]. Decomposition often undergoes an initial short-term rapid breakdown of labile portions of the dissolved organic matter pool, followed by a longer-term degradation of more recalcitrant fractions [18]. Decomposition of lignin, ligno-cellulose and other plant residues produces polysaccharides and amino acids which are utilized in microbial respiratory pathways [18]. Decomposition of these compounds by fermentative microorganisms produces alcohols, carboxylic acids, and inorganic nutrients. Thus, the heterotrophic microbial community in peatlands is typically exposed to a wide variety of organic C-substrates.

The exposure of the heterotrophic microbial community to broad classes of substrates enables characterization of microbial ecophysiology using measurements of their short-term response to C-substrate addition [15,19,20]. Substrate-induced respiration is used as a measure of microbial ecophysiology, as the response of HMA to C-substrates indicates the catabolic diversity of soil heterotrophs [19,21]. Characterization of the metabolic activities of the heterotrophic microbial communities has been successfully utilized in understanding C flow in natural ecosystems [8,15,20]. Wetland soils at different trophic states may exhibit variable HMA patterns, which ultimately influences organic matter dynamics. The objectives of this study were therefore to determine patterns in the response of heterotrophic microbial communities to addition of C-substrates in relation to nutrient enrichment for a subtropical Everglades peatland.

2. Materials and methods

2.1. Site description and sampling

The study was conducted in WCA-2a (44,700 ha) of the northern Florida Everglades. This wetland was historically P-limited and vegetated by Cladium, periphyton, and open-water sloughs [1]. External nutrient loading increased total P concentrations in the soil and water column and led to the development of distinct gradients in soil total P from water inflow points extending into the interior of the wetland [2,9]. Nutrient loading, particularly P, has been implicated in causing ecosystem shifts, including changes in vegetation patterns, organic matter accumulation, water quality, and biogeochemical processes [2,22].

Detritus and soil samples (0–10 cm) were collected from WCA-2a at three sites along a nutrient enrichment gradient 2.3 (eutrophic), 5.1 (transitional), and 10.2 (oligotrophic) km south of the S10-C water-control structure. Sampling sites encompassed a wide range of soil P concentrations and vegetative zones, from Typha in eutrophic areas to Cladium in oligotrophic areas in the interior [2,23]. Detritus consisted of recently deposited and partially decomposed plant material that accumulated on the soil surface, while soil consisted of consolidated, more decomposed organic matter. Triplicate cores (15 cm diameter) were collected at each site along the gradient, and all samples were stored at 4 °C until analysis, which began within a week of sample collection.

2.2. Biogeochemical analysis

Bulk density was measured for soil [24]. Total P was determined by ashing at 550 °C [25] and $NaHCO_3$-Pi by extraction with 0.5 M $NaHCO_3$, followed by colorimetric analysis [26]. Loss on ignition was determined as the mass loss of soil after ashing at 550 °C. Extractable organic C was measured by extraction with 0.5 M K_2SO_4 [8] and analysis with a Dohrmann total organic C analyzer (Rosemount Analytical, Santa Clara, CA). Total C was measured with a Carlo-Erba NA-1500 CNS Analyzer (Haak-Buchler Instruments, Saddlebrook, NJ). Microbial biomass C was measured by fumigation-extraction [27] with an extraction efficiency factor of 0.37 [28]. Microbial biomass P was calculated as the difference between the total P

of 0.5 M NaHCO$_3$ extracts of chloroform-fumigated and unfumigated samples [29]. Data for detritus and soil characterization is provided in Table 1.

2.3. Basal and substrate-induced respirations

Experiments were designed to provide estimates of short-term responses of heterotrophic microbial communities to addition of C-substrates. The C-substrates were selected based on their presence in soils and prior utilization for measurement of catabolic diversity [19,21]. A listing of C-substrates tested is provided in Tables 2 and 3, which included various amino acids, alcohols, carboxylic acids, and polysaccharides. All C-substrates were dissolved in water, adjusted to the pH of the soil (pH = 7), and applied on a C-equivalent basis (10 mg C g^{-1} soil) to soil pre-incubated at 30 °C for 3 d. Approximately 10 g of soil were incubated in the dark with C-substrates under N$_2$ in 120-mL glass bottles with 20-mL vials containing 3 mL of 0.5 M NaOH. Vials containing NaOH were removed at 4, 24, and 48 h, followed by measurement of CO$_2$ production. For CO$_2$ analysis, 0.5 mL of 3 M HCl was added to enclosed vials and resulting headspace CO$_2$ quantified by gas chromatography (Shimadzu GC-8A, thermal conductivity detector at 25 °C, Porapak N column at 20 °C). Incubations were carried out at 30 °C and rates were calculated for cumulative CO$_2$-C production during the 2-d incubation using measurements taken at 4, 24, and 48 h. Carbon dioxide production was linear for this incubation period.

Table 1 – Biogeochemical indicators at eutrophic, transitional, and oligotrophic sites in Water Conservation Area-2a.

Indicator	Units	Eutrophic	Transitional	Oligotrophic
Detritus				
Total P	mg P kg^{-1}	1890a	1050b	693c
NaHCO$_3$-Pi	mg P kg^{-1}	15a	0b	0b
Loss on ignition	%	88a	85a	82a
Total C	g C kg^{-1}	439a	419a	412a
Extractable organic C	mg C kg^{-1}	3403a	3028a	3183a
Microbial biomass C	mg C kg^{-1}	3620b	8758a	4331b
Microbial biomass P	mg P kg^{-1}	344a	289b	151c
Soil (0–10 cm)				
Total P	mg P kg^{-1}	796a	688a	336b
NaHCO$_3$-Pi	mg P kg^{-1}	2.9a	2.0a	0.2b
Loss on ignition	%	89a	88a	85a
Total C	g C kg^{-1}	461a	443a	437a
Extractable organic C	mg C kg^{-1}	3900a	2580b	2290b
Microbial biomass C	mg C kg^{-1}	2680a	2530a	733b
Microbial biomass P	mg P kg^{-1}	45b	107a	48b

Values in rows followed by the same letter were not significantly different at P < 0.05.

Table 2 – Basal and substrate-induced CO$_2$ production (μg CO$_2$-C g^{-1} h^{-1}) in detritus and 0–10 cm soil amended with amino acids and alcohols for eutrophic, transitional, and oligotrophic sites in Water Conservation Area-2a.

Treatment	Strata	Eutrophic	Transitional	Oligotrophic	LSD
Basal	Detritus	19	11	11	4
	Soil	8	12	9	
Amino acids					
Alanine	Detritus	39	14	19	11
	Soil	26	32	33	
Cysteine	Detritus	40	19	20	11
	Soil	25	26	22	
Aspartate	Detritus	36	16	18	9
	Soil	15	16	12	
Glutamine	Detritus	34	17	13	8
	Soil	25	26	26	
Methionine[a]	Detritus	24	13	14	7
	Soil	23	16	15	
Histidine	Detritus	37	15	23	10
	Soil	21	21	25	
Lysine	Detritus	31	12	14	7
	Soil	21	23	19	
Proline	Detritus	28	13	14	7
	Soil	21	19	22	
Tyrosine	Detritus	27	13	14	6
	Soil	15	19	19	
Alcohols					
Glycerol[a]	Detritus	29	15	16	9
	Soil	21	18	18	
Mannitol	Detritus	37	13	17	13
	Soil	23	34	25	

ANOVA results were obtained for a model with the term site (DF = 2), nested depth within site (DF = 3) with residual DF = 12. The LSD was determined at α = 0.05.

a The site term was significant at P < 0.05. The LSD for the site was 3.2 for methionine and 3.9 for glycerol.

Incubations were also carried out in the absence of C-substrates to determine basal CO$_2$ production rates. Substrate-induced respiration represented the response of the heterotrophic microbial community to C-substrates above basal CO$_2$ production rates.

2.4. Data analysis

A nested experimental design was utilized with factors being sampling site and soil depth. Responses to single treatment addition were analyzed using a nested two-way ANOVA model,

$$y_{ijk} = \mu + \alpha_i + \beta_{ij} + \varepsilon_{ijk} \tag{1}$$

where y_{ijk} is the HMA response to the i-th site and j-th depth, μ = overall mean, α_i = effect due to the i-th site effect and assumed to be normally distributed with mean zero and standard deviation σ_α. β_{ij} is the nested effect due to the j-th depth and assumed to be normally distributed with mean zero and standard deviation $\sigma_{\beta \subset \alpha}$. ε_{ijk} is the residual effect which was normally distributed with mean zero and standard deviation σ_ε. A nested model rather than a full factorial with interaction was selected because detrital material at the eutrophic site was different (nutrient-enriched decaying plant

Table 3 – Substrate-induced CO₂ production (μg CO₂-C g⁻¹ h⁻¹) in detritus and 0–10 cm soil amended with carboxylic acids and polysaccharides for eutrophic, transitional, and oligotrophic sites in Water Conservation Area-2a.

C-substrate	Strata	Eutrophic	Transitional	Oligotrophic	LSD
Carboxylic acids					
Acetate[a]	Detritus	40	23	29	12
	Soil	26	22	25	
Formate[b]	Detritus	26	19	18	6
	Soil	20	24	22	
Oxalate[b]	Detritus	35	28	38	12
	Soil	29	30	30	
Butyrate	Detritus	35	19	19	10
	Soil	19	21	19	
Malate	Detritus	36	22	29	11
	Soil	23	23	18	
Propionate[a]	Detritus	23	12	12	6
	Soil	19	18	17	
Valerate	Detritus	31	17	16	7
	Soil	15	14	17	
Polysaccharides					
Glucose	Detritus	39	27	33	15
	Soil	18	23	19	
Maltose	Detritus	43	20	20	10
	Soil	15	21	25	

The ANOVA results were obtained for a model containing the term site (DF = 2), nested depth within site (DF = 3) with residual (DF = 12). The LSD was determined at $\alpha = 0.05$.

a The site term was significant at $P < 0.05$. The LSD for the site was 5.2 for acetate and 4.2 for propionate.

b Neither site nor depth within site was significant at $P < 0.05$.

residues) from the oligotrophic site (nutrient-poor plant residues and periphyton).

The analysis was extended to cover the multivariate HMA responses by executing MANOVAs for each of the main groups; alcohols, amino acids, carboxylic acids, and polysaccharides. Within the MANOVA, Eq. (1) was revalued with y_{ijk} as a p by 1 vector of responses on the i-th site and j-th depth, with p the number of HMAs within each group under consideration. The terms α_i and β_{ij} are p by 1 vectors and ε_{ijk} is a p by 1 random vector, assumed to have mean zero and variance–covariance matrix Σ. The significance of the model terms was evaluated using Wilks' Lambda (Λ).

The full multivariate HMA responses were evaluated using canonical variate or canonical discriminant analysis. The objective of this analysis was to generate a set of variates maximizing differences between groups (sampling sites along the gradient). These canonical variates were linear combinations of the original variables in which the coefficients (loadings) indicate the relative importance of each variable to the discrimination efforts. When original data was reintroduced into the canonical variates, resultant values were denoted as the canonical scores and were typically presented graphically. Given three groups, a single biplot sufficed (Fig. 1). A more detailed discussion on the analysis can be found in Khattree and Naik [30]. All statistical analysis was executed using Genstat (Version 8.1, 2005, Lawes Agricultural Trust) on natural log transformed data (JMP version 4.0.2).
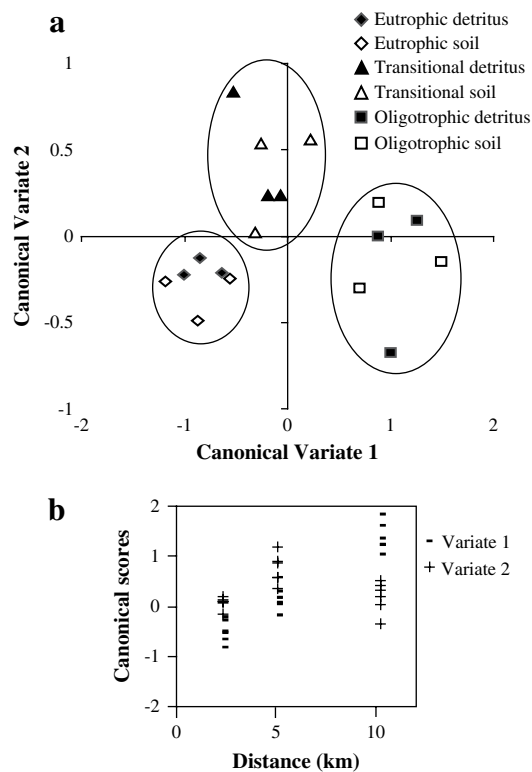

Fig. 1 – The biplot of canonical scores is presented in (a), whereas the canonical variate 1 represented the percentage of the variation in HMA profiles and canonical variate 2 represented the remaining percentage of the variation. The insert (b) displays the relative importance of the canonical variates as a function of distance from the S10-C inflow structure along the nutrient enrichment gradient.

3.   Results

3.1.   Biogeochemical properties of detritus and soil

Soil and detritus biogeochemical properties were presented in Table 1. Detritus consisted of recently deposited plant material, mainly *Typha* residue at the eutrophic site and *Cladium* residue plus periphyton at the oligotrophic site. Detritus at the transitional site was a mixture of residues of the two adjacent sites. Soil at all sites represented a more decomposed, consolidated organic material having approximately 87% organic matter. No differences in soil bulk density were observed across sites along the gradient (data not shown), which averaged (0.10 g cm⁻³). The sampling sites were characterized by detritus total P ranging from 1890 mg kg⁻¹ at the eutrophic site to 693 mg kg⁻¹ at the oligotrophic site. Total P and NaHCO₃-Pi concentrations were approximately twice as high for detritus than underlying 0–10 cm soil. Corresponding to the gradient in total P and NaHCO₃-Pi, detritus microbial biomass P was highest at the eutrophic site near the water inflow point into WCA-2a and decreased toward the oligotrophic interior of the wetland. Likewise, total and extractable organic C tended to be higher at eutrophic than transitional

and oligotrophic sites. While P levels and microbial biomass C and P were higher for detritus than soil, total and extractable C did not vary between strata.

3.2. Basal and substrate-induced respirations

Basal CO_2 production was highest at the eutrophic site and lowest at the oligotrophic site for detritus but not soil (Table 2). These differences were not significant between sites, but the depth nested within the site was significant ($P = 0.006$). Basal respiration was 137% higher in detritus than soil at the eutrophic site, but no differences between detritus and soil occurred at transitional and oligotrophic sites. Detritus CO_2 production was higher than soil basal and SIR at the eutrophic site for all amino acids except methionine, and most alcohols, carboxylic acids, and polysaccharides (Tables 2 and 3). For all amino acids and alcohols, SIR of detritus was significantly higher at the eutrophic than transitional and oligotrophic sites. In contrast, half of the carboxylic acids and polysaccharides did not enhance SIR of detritus at the eutrophic compared to the oligotrophic site. For all C-substrates, SIR of detritus did not differ between transitional and oligotrophic sites.

In contrast to detritus, SIR of soil did not vary along the nutrient gradient for any C-substrate except when amended with methionine. Addition of C-substrates increased CO_2 production above basal rates for most sites and strata, indicating that labile organic C was limiting in this peatland. This result was not expected because detritus and soil along the gradient had at least 2.2 g extractable organic C kg^{-1} (Table 1), indicating that much of the organic C pool in this ecosystem was not readily available to soil heterotrophs.

Short-term responses of heterotrophic microbial communities were evident by the stimulation of CO_2 production by added C-substrates in detritus and soil along the nutrient gradient. Carboxylic acids and polysaccharides provoked the greatest increase in detrital CO_2 production of all C-substrates, with significant site (depth) terms at $P = 0.0002$ and $P = 0.002$, respectively. However, there were relatively few differences in CO_2 production between C-substrate treatments in the underlying soil. The C-substrates contributing organic N produced no significant responses beyond those contributing only organic C, and no amino acid MANOVA model terms were significant at $P < 0.01$ (Table 4).

3.3. Analysis of heterotrophic microbial community response to C-substrates

All added C-substrates enhanced SIR in the short-term incubation for both detritus and soil compared to basal respiration, suggesting a prior exposure to a wide range of C-substrates produced during the breakdown of plant and periphyton residues. Among the C-substrates, the carboxylic acids and polysaccharides generated the greatest overall HMA response. Substrate-induced respiration for detritus was 25, 45, and 42% greater for polysaccharides than other substrates at the eutrophic, transitional, and oligotrophic sites, respectively. Likewise, SIR at the eutrophic, transitional, and oligotrophic sites was 0, 40, and 39%, respectively, greater for

detritus amended with carboxylic acids than amino acids or alcohols. In soil, however, the response of HMA was similar for all C-substrates.

When analyzed across all C-substrates, the HMA responses were moderately successful at distinguishing the sites (Fig. 1). The HMA profiles for the eutrophic site generated a distinct and tight cluster of points in the lower left hand corner of the graph, and were characterized by negative scores in both canonical variates. Variables with large negative loadings in both or either canonical variate were the carboxylic acids butyric and aspartic acids, and the amino acids histidine, lysine, tyrosine, glutamine and proline (Table 5). The transitional site was characterized by generally negative scores for

Table 4 – Multivariate analysis of variance (MANOVA) of substrate-induced CO_2 production ($\mu g\ CO_2$-C $g^{-1}\ h^{-1}$) in detritus and 0–10 cm soil from eutrophic, transitional, and oligotrophic sites in Water Conservation Area-2a.

C-Substrate	Wilks' Lambda	F	Numerator DF	Denominator DF	P value
Amino acids					
Site	0.0213	2.60	18	8	0.085
Site (depth)	0.000646	5.19	27	12	0.002
Alcohols					
Site	0.454	2.66	4	22	0.060
Site (depth)	0.304	2.98	6	22	0.028
Carboxylic acids					
Site	0.0660	2.48	14	12	0.060
Site (depth)	0.00481	4.59	21	18	0.001
Polysaccharides					
Site	0.506	2.23	4	22	0.099
Site (depth)	0.112	7.27	6	22	0.002

Table 5 – Canonical loadings for C-substrates.

C-substrate	$v1$	$v2$
Alanine	0.89	−0.14
Cysteine	−0.96	0.52
Aspartate	−1.05	−0.68
Glutamine	−0.36	−0.03
Methionine	0.10	0.29
Histidine	−1.06	0.64
Lysine	−0.97	−0.53
Proline	−0.24	0.03
Tyrosine	−0.77	1.05
Glycerol	0.18	−0.14
Mannitol	−0.81	−0.01
Acetate	−0.31	−0.81
Formate	−0.36	−0.30
Oxalate	0.11	0.18
Butyrate	−1.71	0.13
Malate	0.73	0.32
Propionate	1.65	−0.24
Valerate	0.86	−0.16
Glucose	0.74	−0.85
Maltose	0.23	0.11

The resultant scores from canonical variate 1 ($v1$) and canonical variate 2 ($v2$) were depicted in Fig. 1.

canonical variate 1 (v1) and strong positive scores for canonical variate 2 (v2). Variables with strong negative loadings in v1 and positive loadings in v2 were carboxylic acids (butyric acid) and amino acids (histidine and cysteine), as well as alcohols (mannitol). The oligotrophic site was characterized primarily by strong positive scores on v1, which encompassed polysaccharides, carboxylic acids (maleic, valeric, and proprionic acid) and an amino acid (alanine). The HMA profiles over the different sites along the gradient were therefore substantially different.

4.   Discussion

Heterotrophic microbial activity at all sites along the nutrient enrichment gradient was limited by organic C, as evidenced by the rapid stimulation of CO_2 production following addition of C-substrates. Additions of simple organic substrates have been shown to enhance organic matter turnover in other studies of Everglades wetlands [6,15,16]. The basal respiration rates were generally higher under eutrophic than oligotrophic conditions. Both basal and SIR are often higher in nutrient-enriched soils [6,15,16] due to higher microbial biomass P than oligotrophic soils (Table 1). In detritus, greater levels of microbial biomass P, in addition to increased P availability, were factors likely contributing to the higher basal and SIR rates occurring under eutrophic compared to oligotrophic conditions. Basal and SIR were higher for detritus than soil at the eutrophic site because of high plant production caused by the influx of inorganic nutrients, which stimulated plant growth and contributed to detritus accumulation [2]. The transitional and oligotrophic sites in the interior of the wetland received significantly less external nutrient loading, thus vegetative growth was limited by inorganic nutrients [2]. Subsequently lower plant production resulted in less deposition of detritus and slower turnover times, hence no differences in basal and SIR between detritus and underlying soil at these sites.

The greater sensitivity to C-substrates of detritus compared to soil indicates that detritus is more responsive to changes in the ecosystem. The higher turnover rates of C-substrates for detritus reflect an environment more conducive for microbial activity, including higher nutrient concentrations, O_2 availability, and organic matter deposition [5,31]. Thus, the response of the heterotrophic microbial community to alteration served as an excellent indicator of ecosystem changes. The high levels of extractable organic C seemed to indicate that organic C would not be limiting to the microbial community. However, since C-substrates stimulated heterotrophic microbial activity, the response of the microbial community was a better indicator than chemical parameters.

A major factor stimulating detritus production and HMA is external nutrient loading [6,8,15], which differentiates the eutrophic from the transitional and oligotrophic sites. Continued external nutrient loading, or internal nutrient loading caused by turnover of organic matter within the peatlands, may increase the extent of nutrient impacts in the future and leads to development of eutrophic conditions in former oligotrophic areas.

Short-term incubations employed in this study measured the ability of soil microorganisms to rapidly utilize C-substrates, indicating the presence of the appropriate enzyme systems within the soil microbial communities [19,21]. The HMA profiles differed considerably between eutrophic and oligotrophic sites. Some carboxylic acids and a slew of amino acids drove the HMA profiles at the eutrophic site. A carboxylic acid (butyric acid) and an alcohol seemed to characterize the transitional site, while the HMA profile at the oligotrophic site was dominated by the carboxylic acids. Although not all of these results were directly interpretable (e.g. mannitol at the transitional site), they do suggest that microbial communities were exhibiting an affinity for N-containing substrates at the eutrophic site and a preference for fermentation products at the oligotrophic site. In fact, N limitations to HMA and organic matter decomposition have been observed in P-enriched areas of this peatland [8,31]. These results illustrate the effectiveness of the short-term assays in describing catabolic diversity of the heterotrophic microbial community. In this study nutrient availability was the driving factor affecting catabolic diversity along the nutrient enrichment gradient. It is likely that nutrient loading caused shifts in the composition of the microbial community [32,33] which initiated differences in the response to C-substrates.

The relative importance of the discriminatory power of variates is summarized in Fig. 1, where the canonical scores are plotted as a function of distance from the water inflow point into WCA-2a. Overall low scores for both variates characterized the eutrophic site, indicating less variation in HMA than the oligotrophic site. A general tendency to more positive scores was found for the transitional site, indicating an overall increase in the relative importance of variables with positive loadings in both variates. Finally, v1 continued to increase along the gradient from eutrophic to oligotrophic conditions indicating greater variability in HMA profiles. v2 underwent an abrupt change, as variables with negative loadings gained prominence. However, utilization of the canonical variates as comprehensive descriptors of the C cycling processes in this wetland may require a finer sampling of soils along the nutrient gradient.

We concluded that detritus and soil from eutrophic, transitional, and oligotrophic sites possessed the ability for utilization of a wide variety of C-substrates. The patterns of substrate utilization were different depending on trophic state. Heterotrophic microbial activity was enhanced by addition of C-substrates, suggesting that labile organic C was a limiting factor to HMA, even under oligotrophic conditions. Utilization rates of C-substrates varied between eutrophic and oligotrophic sites, suggesting that external nutrient loading altered metabolic pathways of organic matter decomposition. The patterns of HMA in relation to nutrient enrichment provided insight into the microbial response to changes in environmental conditions. Stimulation of HMA by C-substrates, and resulting increases in organic matter decomposition, have important implications for management of the Everglades ecosystem. External nutrient loading and internal nutrient cycling stimulate HMA and ultimately increase organic matter decomposition and nutrient regeneration to floodwater, thus further contributing to the eutrophication of WCA-2a.

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