

Microbial Respiration and Enzyme Activity Downstream from a Phosphorus Source in the Everglades, Florida, USA

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Abstract: Northeast Shark River Slough (NESS), lying at the northeastern perimeter of Everglades National Park (ENP), Florida, USA, has been subjected to years of hydrologic modifications. Construction of the Tamiami Trail (US 41) in 1928 connected the east and west coasts of SE Florida and essentially created a hydrological barrier to southern sheet flow into ENP. Recently, a series of bridges were constructed to elevate a portion of Tamiami Trail, allow more water to flow under the bridges, and attempt to restore the ecological balance in the NESS and ENP. This project was conducted to determine aspects of soil physiochemistry and microbial dynamics in the NESS. We evaluated microbial respiration and enzyme assays as indicators of nutrient dynamics in NESS soils. Soil cores were collected from sites at certain distances from the inflow (near canal, NC (0–150 m); midway, M (150–600 m); and far from canal, FC (600–1200 m)). Soil slurries were incubated and assayed for CO₂ emission and β-glucoside (MUFC) or phosphatase (MUFP) activity in concert with physicochemical analysis. Significantly higher TP contents at NC (2.45 times) and M (1.52 times) sites than FC sites indicated an uneven P distribution downstream from the source canal. The highest soil organic matter content (84%) contents were observed at M sites, which was due to higher vegetation biomass observed at those sites. Consequently, CO₂ efflux was greater at M sites (average 2.72 μmoles g dw^{−1} h^{−1}) than the other two sites. We also found that amendments of glucose increased CO₂ efflux from all soils, whereas the addition of phosphorus did not. The results indicate that microbial respiration downstream of inflows in the NESS is not limited by P, but more so by the availability of labile C.



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1. Introduction

Wetlands are considered to be both sources and sinks of terrestrial C and play an important role in the global C cycle. Wetlands are estimated to contain approximately 20–30% of the global C pool [1]. The total amount of C stored in wetlands in the conterminous US is about 11.52 Pg [2]. The nutrient dynamics and biogeochemistry of soils in wetlands are more complex than in uplands, specifically because of the intermittent anaerobic and semi-aerobic conditions. Microbial respiration and enzyme assays can be considered as potential indicators of soil health, nutrient enrichment, availability, and mineralization in wetlands [3–5]. Induced enzyme activity (EA) links environmental nutrient availability and microbial biomass stoichiometry [6]. For instance, higher EA in soil indicates low availability of labile nutrient contents. Additionally, small changes in the decomposition rate and enzyme activity will be able to alter both recalcitrant and labile C pools, impacting the long-term soil C stocks. Extracellular enzymes such as glucosidase and phosphatase are majorly studied for their ability to mineralize C from plant litter and P from nucleic acids, respectively, whereas the lability of soil organic matter (SOM) can be determined through the production of CO₂ in aerobic incubations [7]. These indicators are also helpful

in predicting nutrient status in soils under varying hydrologic conditions. This short-term research study was conducted in the Florida Everglades, the largest wetland in the USA, and is internationally recognized by several organizations including the United Nations Educational, Scientific, and Cultural Organization (UNESCO). Hydrologic modifications enabling anthropogenic development of South Florida have greatly reduced the water flow in Northeast Shark River Slough (NESS) at the northeastern border of Everglades National Park (ENP). The construction of the Tamiami Trail (US highway 41) in 1928 to connect the Florida Coasts created a significant hydrologic barrier (shorter hydroperiod) between NESS and the remaining Everglades to the north. Additionally, nutrient loading from the northern border of NESS resulted in a phosphorus gradient consistent with that seen in other areas of the Everglades in proximity to discharge canals [8]. Studies have suggested that native periphytons (calcareous, filamentous blue-green algal communities), primary producers of Everglades ecosystem, are highly sensitive to P concentrations and small changes in P loading can create rapid changes in the periphyton community [9]. Overall, the reduced hydroperiods in much of the Everglades as a result of water diversion have resulted in net soil C loss [10]. Altered hydroperiods are responsible for the replacement of native aquatic plant species with non-native species [11] and the shifting of native periphyton communities, consequently hampering the long-term ecological balance in the Everglades; however, limited infrastructure, including culverts under the Tamiami Trail, were constructed to allow water to flow southward into the NESS. Since the water flow through the culverts was not sufficient, two large bridges were constructed in 2012 and 2019 to increase water flow into the NESS and to partially restore the hydrology and ecological structure and function.

Historically, the Everglades has largely been a P-limited system [12]; however, nutrient runoff from agricultural and urban development areas may have changed the dynamics of nutrient availability in NESS soils, specifically in nutrient-rich pockets. We contend that using biological indicators in the NESS region will help identify potential ecological and biogeochemical imbalances. The results from this study will be able to provide insights about the nutrient dynamics of NESS specifically after the construction of new bridges. We hypothesized that microbial respiration and enzymatic assays in response to current hydrological changes will be able to indicate the lability of organic matter and P biogeochemistry at certain distances downstream in the NESS. The specific objectives of this study were: (a) to characterize total P (TP), total C (TC) contents and other physicochemical properties of NESS soils at locations with at certain distances from the inflow; (b) to evaluate the potential of microbial respiration (CO₂ efflux) and extracellular enzyme activity as predictors of nutrient status in the NESS.

2. Materials and Methods

2.1. Site Location and Sample Collection

Soils were collected from NESS sites at certain distances from the bridge (inflow) on the Tamiami Trail (Figure 1). Sample collection locations included near-canal ($n = 28$), midway ($n = 24$), and far from canal ($n = 24$) sites that were about 0–150, 150–600, and 600–1200 m downstream, respectively. Intact soil cores (0 to 10 cm) were collected by inserting plastic tubes of a known diameter (surface area) into the soil. Additional care was taken to minimize compaction during collection. Subsamples of known mass fresh soils were used in CO₂ efflux incubations and enzyme activity analysis. Additional subsamples were dried, ground, and used in physicochemical analysis. Water depths during each sampling were measured using a standard ruler (one meter stick) to the nearest cm as the depths between the surface of the water and the resistance of the soil.

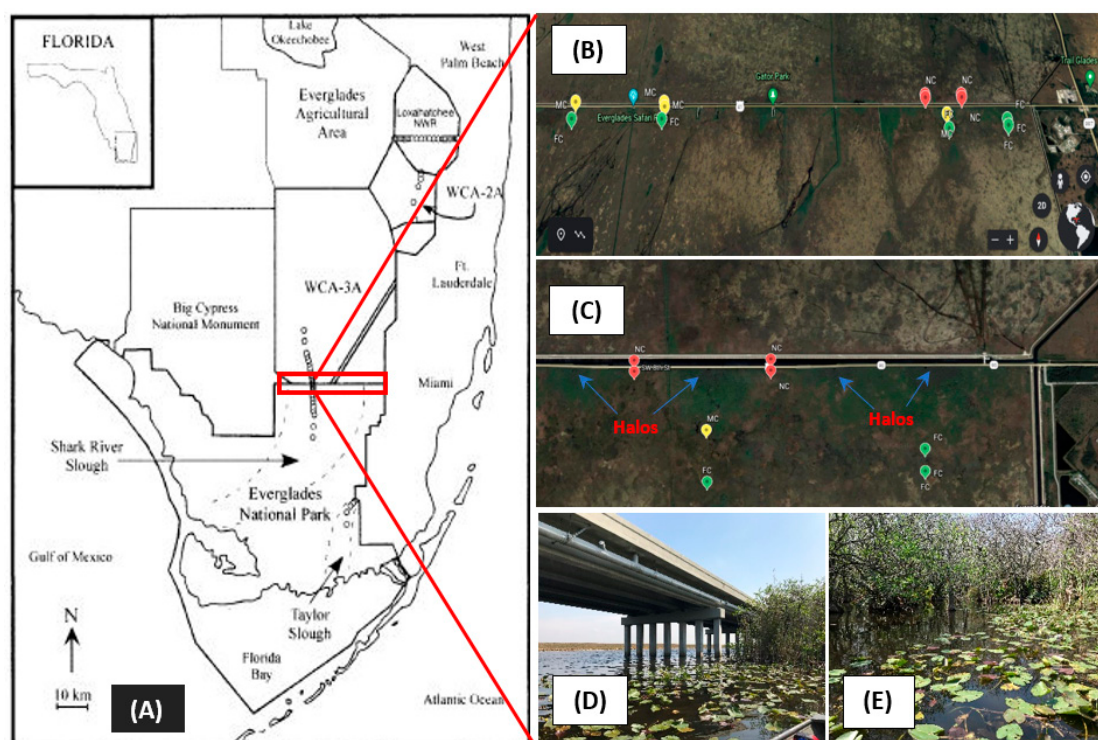


Figure 1. Locations of sampling sites in Northeast Shark Slough (NESS), Florida, USA (A), in proximity to a bridge constructed to partially restore southward water flow into Everglades National Park (ENP). Red tags are near-canal sites (0–150 m downstream of canal), yellow tags are midway (150–600 m), and green tags are far from the canal (600–1200 m) (B,C). Remnant vegetation “halos” (increased biomass and nutrients) are identified and show the locations of pre-bridge culverts (C). Google Earth images were used for context. Photographic images of the bridge (near-canal) (D) and sampling site (E).

2.2. Laboratory Analyses

The ability of soil microbes to generate CO_2 under controlled conditions was assessed by adding a known mass of fresh 1:1 soil slurries (soil/solution) to 20 mL CO_2 -free air purged from headspace incubation vials (triplicate) and subjected to dark incubation at 25 °C for 72 h. Gas samples collected from headspace vials were then injected into a HP 5890 Series II gas chromatograph equipped with a flame ionization detector and a Shimadzu MTN-1 methanizer. The measured CO_2 efflux was expressed as μmoles per unit mass of dry soil following the methods described by [7,13].

Four treatments were evaluated, including a non-amended control (C) and amendments of 1.2 mmol L^{-1} of glucose (G), 0.4 mmol L^{-1} of phosphorus (P), and a combination of phosphorus and glucose (P + G). Treatment strengths were calculated following the method proposed [7] and our previous experiments in NESS soil.

Enzyme activities were determined for each of the four treatments (C, G, P, and P + G) by adding moieties of the fluorogenic substrate, 4-methylumbelliferyl (MUF; either - β -glucoside (MUF-C) or MUF-phosphatase (MUF-P)), to soil solutions made by diluting slurries to a final 10^{-3} dilution. Diluted samples (200 μL) were pipetted into 8 wells of a 96-well plate, to which 50 μL of either MUF-P or MUF-C substrates were added to obtain final concentrations of 10 μM . Plates were then incubated in the dark for 2 h (MUF-P) or 24 h (MUF-C). Substrates were added to replicate wells after incubation and immediately before fluorometric analysis to determine the initial fluorescence (i.e., t_0). No substrate was added to rows or columns containing standards or background blanks. A Synergy HT microplate reader was used for fluorometric determination with enzyme activity regressed from standard curves.

Total carbon (TC) and total nitrogen (TN) levels of oven dried soil samples were analyzed using a Carlo-Erba NA-1500 CNS analyzer [14]. Determination of Total P was done via oxidation (dry combustion) and hydrolysis to soluble forms (soluble reactive, ortho-P; SRP) using $\text{MgSO}_4/\text{H}_2\text{SO}_4$ and HCl [15], followed by standard colorimetric analysis using USEPA method 365.1 [16]. Dry bulk density (g cm^{-3}) and organic matter (%) levels of soil were measured following the standard methods of ASTM D4531-86 and ASTM D2974-87, respectively.

We analyzed and cleaned the data (using interquartile range calculation) outliers before performing statistical analyses. One-way analysis of variance (ANOVA) was carried out independently on treatments at certain distances and four treatments were carried out between distances using the SAS9.4 PROC MIXED procedure. Tukey–Kramer post-hoc tests were conducted to compare mean separation levels at $p < 0.05$ among treatments and between distances. Pearson correlation coefficient analysis was performed on soil characteristics at $p < 0.05$ (two-tailed).

3. Results and Discussion

The construction of the Tamiami Canal and Highway bisected the natural north-to-south sheet flow of water into the ENP. The nutrient-enriched canal water that flowed southward was funneled through a series of culverts and established the long-term P gradient evident in our analysis, whereby soil TP content decreased with distance (Table 1). The soil TP from near-canal (NC) and midway (M) sites were significantly higher ($p < 0.05$) by about 2.45 and 1.52 times, respectively, than far from canal (FC) sites. Additionally, certain samples with high TP contents (as high as $2630 \mu\text{g g}^{-1} \text{ dw}$) in NC sites were proximal to “halos” (enriched pockets), areas of increased water depth, nutrient content, and plant biomass at the outfall of culverts (Figure 1). Soil organic matter (SOM) was highest (average 87%) at M sites, which resulted in lowest bulk density values (ranged from 0.11 to $0.16 \text{ g dw cm}^{-3}$) in those soils. A possible explanation would be the presence of the higher vegetation cover in M sites as compared to NC and FC sites. The Pearson analysis (Table 2) of selected soil physicochemical parameters indicated that SOM was highly correlated with TC and TN, which was expected for high organic “peaty” wetland soils [17,18] where soil nutrients are largely in organic forms. Consequently, TN and TC contents were also highest in midway (M) soil samples. In unimpacted Everglades soils (those not influenced by allochthonous sources of P), TP is often correlated with TC [19]; however, this was not the case in our analysis, indicating the possibility of an influence of external P loading (causing a biogeochemical disconnect) from agricultural areas [20].

Table 1. Physiochemical parameters (mean $\pm$ SD), including water depth, soil bulk density (BD), organic matter (SOM), total P (TP), total N (TN), and total C (TC), at increasing distances from a source canal in the NESS. Different lowercase letters indicate significant differences ($p < 0.05$) between sites.

Sites	<i>n</i>	Water Depth	BD	SOM	TP	TN	TC
		cm	g dw cm^{-3}	%	$\mu\text{g g}^{-1} \text{ dw}$	$\text{mg g}^{-1} \text{ dw}$	
Near canal	28	19 ± 8^a	0.140 ± 0.035^a	54 ± 19^b	1143 ± 273^a	2.2 ± 0.9^b	32 ± 6^c
Midway	24	12 ± 4^a	0.129 ± 0.029^a	84 ± 17^a	712 ± 151^b	3.4 ± 0.3^a	46 ± 8^a
Far from canal	24	8 ± 4^a	0.149 ± 0.052^a	67 ± 11^b	467 ± 87^c	2.8 ± 0.4^a	40 ± 9^b

Table 2. Pearson’s (two-tailed) correlation analysis between different physicochemical soil properties (bulk density, BD; total P, TP; total N, TN; total C, TC; soil organic matter, SOM) for all NESS sites combined.

Parameters	TP	Soil Moisture	TN	TC	SOM
BD	−0.33	−0.60 *	−0.35	−0.50	−0.51
TP		0.25	−0.28	−0.29	−0.21
Soil moisture			0.39 *	0.44 *	0.53 *
TN				0.95 *	0.95 *
TC					0.99 *

footerNote: * significant at $\alpha \leq 0.05$.

Soil organic matter accumulates in wetlands when C-fixation during photosynthesis exceeds decomposition through mineralization and respiration [21]. Several factors influence this balance, including those that affect fixation, decomposition, and the lability or stability of SOM [22,23]. Previous studies have suggested that decomposition of litter and belowground biomass in the oligotrophic Everglades is P-limited [24], as is the oft-cited P-limited productivity [12,25]. The hypothetical influence of P limitation and variations in SOM lability were studied in our CO₂ evolution experiment, where the CO₂ fluxes ranged from 0.18 in unamended controls to 5.62 ($\mu\text{mol CO}_2 \text{ g}^{-1} \text{ dw soil h}^{-1}$) under doubly-amended (P + G) treatments for all three locations along the gradient (Figure 2). The addition of P alone as an amendment did not significantly increase CO₂ emissions compared to the unamended controls at any location, suggesting that SOM respiration in these soils was not P-limited. Even in the FC sites that had the lowest TP contents (Table 1), where P addition might be suspected to have the greatest influence, P addition did not affect respiration. It should be noted that average TP contents at FC sites (467 mg g^{−1} dw) were much higher than the previously reported TP concentrations in unimpacted NESS soils (approximately 218 mg g^{−1} dw; 8). In unamended soils, CO₂ production was significantly greater ($p < 0.05$, as indicated by different capital letters) at the M sites than at the other sites. This could be reflective of the greater overall SOM content at this site rather than differences in the lability of the SOM. The addition of glucose (G) increased respiration relative to the unamended controls at the NC and M sites but not for FC sites (Figure 2). Adding a labile C source (glucose amendment) supports the hypothesis that the efflux of CO₂ from NESS soil is at least partially dependent on the SOM carbon quality. As might be expected, a major portion of the SOM present in the NESS soil is in more recalcitrant forms compared to labile glucose [7]. In a recent study conducted at Everglades National Park, [26] found that regardless of restoration activities, the decomposition and microbial respiration were limited by labile C rather than soil P. A potential ecological consequence of recalcitrant SOM under the influence of hydrologic restoration and concurrently longer hydroperiods would be an increase in peat accretion. An abundance of recalcitrant C indicates a healthy and productive wetland ecosystem [27]. In our study, across all treatments at all sites, the combination of phosphorus and glucose (P + G) produced significantly higher CO₂ than other treatments, suggesting readily available (labile) C and P increased microbial activity in the soils and caused a “priming effect”, as reported in other studies [7].

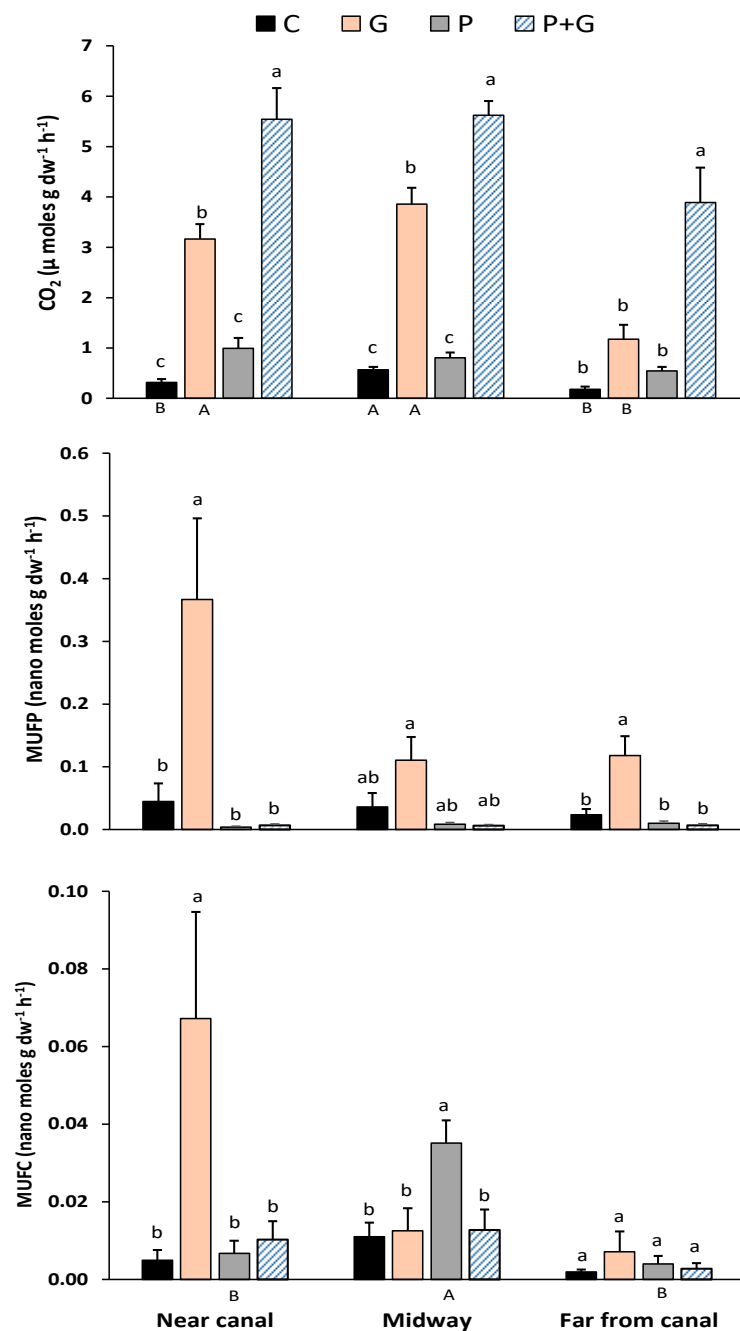


Figure 2. Carbon dioxide (CO₂) efflux and enzyme activity (phosphatase or MUFP and glucosidase or MUFC) analyses of incubated soil slurries collected at certain distances (near-canal, midway, and far from canal) downstream from the inflow in the Northeast Shark River Slough, ENP. Treatments included a control (C), glucose (G), phosphorus (P), and a combination of phosphorus and glucose (P + G). Lowercase letters indicate significant differences ($p < 0.05$) between treatments (within a distance), while uppercase letters indicate the treatment difference across distances (NC, M, and FC). Uppercase letters not used in this figure were for treatments with no significant difference between distances.

Enzyme assays were conducted to determine the activity levels of β -glucosidase and phosphatase enzymes in NESS soils. Soil microbes produce β -glucosidase or phosphatase enzymes to cleave the ester bond between complex organic molecules and the glucose or phosphate, respectively, allowing these compounds to become available for uptake and degrade organic compounds in the process. Availability of nutrients in the soil can

alter enzyme activity and soil respiration. In this study, the addition of G increased MUFP activity above the unamended controls at all sites (significantly so at NC and F sites), additionally indicating potential labile C limitation across all sites. Added labile C enhanced the microbial community to increase phosphatase production, likely as a response to a growing microbial population to take advantage of the added food source. In all sites, the MUFP responses were similar for each treatment, as they did not significantly differ for sites within treatments. Although not statistically significant, the addition of P appeared to reduce the phosphatase activity compared to controls at all sites, as would be expected for an inducible enzyme. The addition of glucose at the NC sites, where SOM was lowest and P content highest, significantly increased the MUFC activity over that of the controls, while P addition significantly increased this activity at the M sites, where SOM was highest relative to other locations. There were no other between-site differences regarding MUFC activity for any other treatments.

The biogeochemistry of P has major implications in controlling soil C dynamics in wetlands. The external addition of nutrients can potentially impact the enzyme activity and microbial respiration, specifically when those nutrients are not readily available in the soil; however, our soils had abundant P content (considering more than 450 mg g⁻¹ dw TP contents in FC sites), so phosphatase enzyme activity was negligible when additional P sources were applied. We also observed that heterotrophic microbial activity was more responsive to additional food sources; therefore, the outcomes of this experiment indicated that respiration and extracellular enzyme activity, at least downstream of inflows in the NESS, are not limited by P, but rather by the availability of labile C.

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