Soil microbial eco-physiological response to nutrient enrichment in a sub-tropical wetland

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Abstract

Eutrophication in subtropical wetland ecosystems can lead to extensive displacements of vegetative communities and as a result changes in overall environmental conditions (loss of indigenous habitat, substrate quality, etc.). This has generated a demand for a set of sensitive indicator(s) that prelude these structural changes. The functional response of bacterial communities may indicate the effect and extent of the impact on the overall system. The effects of nutrient enrichment on the microbial community and its ecophysiology were measured in a subtropical marsh (Water Conservation Area 2a) in the northern Everglades, USA. We investigated the microbially mediated organic matter decomposition processes and nutrient cycling in three areas of the marsh, a nutrient enriched site, an intermediate site and a unimpacted (oligotrophic) site. We chose measures associated to the hydrolytic enzyme activities of alkaline phosphatase, β-glucosidase and aminopeptidase. We also monitored microbial biomass carbon (C), nitrogen (N) and phosphorus (P) and the associated elemental turnover rates (C, N and P). We found a significant (α = 0.05) spike in microbial biomass C, N, and P in the intermediate site. The elemental turnover rates (C, N and P) where significantly higher in the impacted and intermediate site when compared to the unimpacted site. The enzymatic profiles at the unimpacted site illustrate a system regulated for optimal use of P. In the intermediate zone between the overall P-limited and P-impacted areas, the nutrient inputs alleviates the stress imposed by the P-limitation. Microbial biomass increased dramatically without a decrease in the overall microbial metabolic efficiency. The metabolic coefficients (particularly q-Potentially Mineralizable P – qPMP and qCO2) indicated that after the disturbance, the impacted areas in the Everglades are characterized by relatively open, inefficient nutrient cycles. The nonlinear shifts (threshold behavior) in microbial parameters indicate that microbial indicators function effectively as early warning signals.

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Keywords: Cattail; Ecosystem responses; Eutrophication; Everglades; Microbial activities; Microbial ecophysiological indicators; Nutrient enrichment; Sawgrass; Water Conservation Area 2a

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1. Introduction

Ecosystem evaluation and management consistently necessitates measures that monitor the actual state of the system and characterize its rate of change. Microbial communities play a pivotal role in nutrient cycling and organic matter degradation in terrestrial, wetland, and aquatic systems. Inversely, environmental and resource conditions form the fundamental forces that control the microbial community size and its physiology. Monitoring the variables associated with the microbial eco-physiology both in response to exogenous disturbances, as well as establishing the baseline endogenous environment could provide the necessary information for management of biological systems.

The Florida Everglades represents one of the largest and most distinct freshwater marshes in North America and is unique in that its formation is the result of the accumulation of organic matter over a limestone depression (Gleason et al., 1984). The allochthonous system is one adapted to low nutrient content (oligotrophic), particularly P (McCormick et al., 1996), which in addition to fire and hydrological conditions (Newman et al., 1996) have resulted in endogenous communities characterized by strands of sawgrass (Cladium jamaicense) and open slough areas. Recent autochthonous nutrient (P and N) inputs into the northern areas of the Everglades have resulted in significant alterations to the indigenous system with large incursions of cattail (Typha domingensis). Extensive documentation of the temporal and spatial distribution of the nutrients across the northern marshes of the Everglades has established areas of nutrient enrichment (Davis, 1991; Reddy et al., 1993; DeBusk et al., 1994), associated with changes in the predominant plant communities. The combination of nutrient availability and changes in the litter source has resulted in a shift in litter quality and quantity (Davis, 1991; DeBusk and Reddy, 1998), with concomitant increases in organic matter mineralization rates (Davis, 1991; Qualls and Richardson, 2000) and significant increases in carbon (C) (DeBusk and Reddy, 1998), nitrogen (N) (White and Reddy, 2000; Newman et al., 2001) and P mineralization rates (Newman et al., 2001). The nutrient enriched areas have also been associated with significant alterations in overall microbial community size (DeBusk and Reddy, 1998; White and Reddy, 2001) and physiology (Castro et al., 2002). In essence, the nutrient influx has directly affected the microbial community eco-physiology by alleviating nutrient limitations (McCormick et al., 1996) and indirectly through changes in the quality of soil litter and organic material (DeBusk and Reddy, 1998).

To the extent that the Everglades has been studied, it is an ideal system to further test whether the information generated by microbial community response measures together with the characterization of their physico-chemical environment, i.e., collectively; the microbial eco-physiology (Mamilov and Dilly, 2002), provides the insights into the ecosystem structure and functioning (Scholter et al., 2003) commensurate to the importance of microbial communities (Bentham et al., 1992; Eckerschmitt and Griffiths, 1998). To address this, we sampled three sites in a subunit of the Everglades, sampling areas that have been mapped previously as nutrient impacted, intermediate and original (unimpacted) Everglades ecosystems (DeBusk et al., 1994). We selected a set of microbial community response measures known to be sensitive to nutrient enrichment in aquatic systems, such as extracellular enzyme activities (Prenger and Reddy, 2004), respiratory activities (Qualls and Richardson, 2000), microbial biomass C, N and P, and microbially mediated N and P turnover rates (Reddy et al., 1999). These measures, as well as their derivatives or simple combinations, have been used (Sinsabaugh et al., 1997; Anderson and Domsch, 1990) to each individually characterize the microbial community physiological response to changes in its environment. The objectives of this study are to generate a fairly complete overall assessment of the effectiveness of the microbial community and its eco-physiology as indicators of ecological perturbation generated by nutrient enrichment, with a specific emphasis on the microbially mediated organic matter decomposition and associated nutrient cycling.

2. Materials and methods

The Water Conservation Areas are sections of the original Everglades that were impounded in the 1960s for flood control and water supply. Water
Conservation Area 2A covers 54,700 hectares (ha) and the inflow water is mostly introduced through four control structures along the northern edge of the area (S-10A, S-10C, S-10D and S-10E). The majority of this water originates in the Everglades Agricultural Area (EEA, 58%) as drainage water that often exceeds 100 µg P L⁻¹ (McCormick et al., 1996); an additional significant source of nutrient laden water results from the flood control in the EEA in response to storm events (2.28 × 10⁵ kg P year⁻¹ and 8.17 × 10⁶ kg N year⁻¹; DeBusk et al., 1994). The nutrient gradients (Davis, 1991; Reddy et al., 1993; DeBusk et al., 1994) produce a patterned response within the wetland, T. domingensis Pers (Cattail) characterizes areas close to the inflow (Fig. 1), with concomitant high levels of water column and soil P content. The nutrient levels decrease to background levels (water column levels <10 µg TP L⁻¹) gradually into the marsh interior, which is P-limited. This area can be described as a native Everglades ridge and slough ecosystem consistent of C. jamaicense Crantz (Sawgrass) and open slough communities typified by Nymphaea odorata. The soils present in the WCA-2A are of the order of Histosols,
with high C contents, low ash content and low bulk densities.

Soil and litter were collected monthly in areas characterized as impacted (highly P-enriched), intermediate (moderately enriched) and an unimpacted zone (Table 1) every two months over a period of about 1 year (2001–2002). The impacted site (F1) was selected to represent the *Typha* sp. dominated eutrophic area, and an unimpacted (U3) site was selected that best reflected the historical ecosystem. The intermediate site (F4) was selected in an area that consisted of a mix of *Typha* sp. and *Cladium* sp. It was hypothesized that when nutrient loading into a severely P-limited system occurs, the increase in nutrients initially fuels the existing ecosystem components such as the soil microbial community activities. Once the amount of P exceeds a given threshold value, this will result in structural changes in the ecosystem. The intermediate site was chosen to approximate the geographic location of this P-front.

Table 1
Geographic coordinates of the sampling stations and selected physico-chemical properties of the soils collected in the impacted (F1), intermediate (F4) and unimpacted (U3) areas in Water Conservation Area 2a (n = 36, averages over floc and 0–10 cm of soil)

<table>
<thead>
<tr>
<th></th>
<th>F1</th>
<th>F4</th>
<th>U3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coordinates</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Latitude</td>
<td>26° 21’ 35.3 N</td>
<td>26° 19’ 01.3 N</td>
<td>26° 17’ 16.3 N</td>
</tr>
<tr>
<td>Longitude</td>
<td>80° 22' 12.2 W</td>
<td>80° 23’ 06.2 W</td>
<td>80° 24’ 40.2 W</td>
</tr>
<tr>
<td>Soil physiochemical properties</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bulk density (g cm⁻³)</td>
<td>0.08 (0.003)</td>
<td>0.08 (0.004)</td>
<td>0.08 (0.003)</td>
</tr>
<tr>
<td>LOI (%)</td>
<td>81.7 (4.1)</td>
<td>72.5 (16.3)</td>
<td>67.8 (20.8)</td>
</tr>
<tr>
<td>TN (g kg⁻¹)</td>
<td>25.1 (0.7)</td>
<td>24.7 (1.0)</td>
<td>26.8 (0.9)</td>
</tr>
<tr>
<td>TC (g kg⁻¹)</td>
<td>419 (2.6)</td>
<td>377 (7.8)</td>
<td>358 (10.0)</td>
</tr>
<tr>
<td>TP (mg kg⁻¹)</td>
<td>1355 (45.0)</td>
<td>707 (36.3)</td>
<td>305 (11.2)</td>
</tr>
</tbody>
</table>

2.1. Analytical methods

Soil and detrital samples were homogenized in a grinder after removal of any visible live plant material, soil bulk density (BD) was determined on a dry weight basis (70 °C) after grinding. Loss on ignition (LOI) was determined as the mass lost after ashing at 550 °C. Total P (TP), C (TC) and N (TN) concentrations were determined on a Carlo-Erba NA 1500 CNS Analyzer (Haake-Buchler Instruments, Saddlebrook NJ), while TP was determined by the TP ashing method (Andersen, 1976) and analyzed by the ascorbic acid colorimetric procedure (Kuo, 1996; Technicon Autoanalyzer II, Terrytown, NY).

Microbial biomass carbon (MBC) was determined by a chloroform fumigation extraction procedure coupled to a 0.5 M K₂SO₄ extraction (White and Reddy, 2001). The extracted dissolved organic C (DOC) was determined on a Shimadzu total organic
carbon analyzer (TOC-5050A). Microbial biomass C was calculated using the extraction efficiency factor $k_{EC} = 0.37$ as the difference between treated (fumigated) and untreated soils, the untreated extract C concentrations were reported as TOC–K2SO4. Microbial biomass phosphorus (MBP) was similarly determined by fumigation extraction, using 25 mL 0.5 M NaHCO3 extractant. The difference in TP between the treated and untreated sample constituted MBP, no extraction efficiency factor was used (Ivanoff et al., 1998).

Potential mineralizable nitrogen (PMN) was measured using a 10-day anaerobic incubation, followed by extraction with 0.5 M K2SO4 (White and Reddy, 2000). Extractions were analyzed for NH4-N using an automated colorimetric analysis, EPA365.1, Technicon Autoanalyzer). Potential mineralizable phosphorus (PMP) was also determined using a 10-day anaerobic incubation (Chua, 1999). Equivalent of 0.5 g dry weight soil samples were placed in 50 mL serum bottles and mixed with 5 mL of distilled deionized water, capped and purged with O2 free N2. The samples were subsequently incubated in the dark at 40 °C for 10 days, after which, 20 mL of 1.0 M HCl was injected into the serum bottle and after 3 h samples were extracted, filtered (0.45 μm) and the filtrate stored at 4 °C until analysis. A second set of samples of equivalent weights (controls) was directly extracted with 25 mL (versus 20 mL) of 1.0 M HCl as described previously. The HCl extract was analyzed on a Technicon Autoanalyzer (Terrytown, NY) by the ascorbic acid colorimetric procedure (Kuo, 1996). The difference in HCl–extractable P over the 10-day incubation period constitutes PMP (mg P kg\(^{-1}\) d\(^{-1}\)), the control was reported as TPi.

The enzyme activities (EA) of β-1,4-glucosidase (βG; EC 3.2.1.21), alkaline phosphatase (AP; EC 3.1.3.1) and aminopeptidase (AM; EC 3.4.11.10) were assayed using a fluorescent enzyme specific artificial substrate methyl-umbelliferone (MUF-phosphate, MUF-β-d-glucoside and MUF-guanidinobenzoate, respectively). Repression or production of alkaline phosphatase by microbial communities has been shown to correspond to the relative availability of P (Wright and Reddy, 2001). Aminopeptidase (AM) activity has been shown to represent the general proteolytic activity (Hoppe et al., 1988), β-glucosidase is an exemplar of a C-acquiring enzyme (Sinsabaugh and Moorhead, 1994). For dehydrogenase activity the substrate used was 5-cyano-2,3-ditolyl tetrazolium chloride (CTC). Dehydrogenase measures intracellular catalysis and more is likely to be correlated to the activity of the cells compromising the microbial community is present in all microorganisms (Mersi and Schinner, 1991) and an accurate measure of the total oxidative capacity of soil. The EA assays were executed on a 1 g to 20 mL soil slurry homogenized using a Tissue Tearor (Fisher Scientific). Subsequently, 200 μL of a 1/100 dilution of this soil slurry was transferred to eight wells of a 96-well microtiter plate and 50 μL of substrate added to four wells (and four blank). Plates were incubated at room temperature (25 ± 2 °C) for 2 h for phosphatase, and for 24 h for β-glucosidase. Enzyme activity was expressed as the mean difference in fluorescence reading (Bio-Tek FL600 fluorometric plate reader, Bio-Tek Instruments Inc.) between the blank and sample over the incubation period (Prenger and Reddy, 2004).

The aerobic and anaerobic metabolic activities were measured as described in Wright and Reddy (2001). The aerobic assays consisted of 10 g of moist soil placed in Schott media bottles fitted with a NaOH trap, sealed in an atmosphere with 21% O2. The anaerobic treatment differed in that the headspace consisted entirely of N2. After 2 h of incubation, the CO2 in the NaOH traps was released by the injection of HCl through the septa to produce a pH below 2. Headspace CO2 was measured through thermal conductivity (TCD detector temperature at 30 °C; Shimadzu 8AIT GC) and in the anaerobic treatments, the headspace CH4 was analyzed by means of flame ionization detection (FID, detector temperature at 110 °C; Shimadzu 8AIF GC) as described in D’Angelo and Reddy (1999).

2.2. Data analysis

The rates of CO2 and CH4 production were analyzed as zero order kinetic reactions and estimated as the coefficient of simple linear regression (Excel version 2000). Enzyme activities were normalized by the highest value obtained for a particular enzyme (Sinsabaugh et al., 1997) resulting in $E_P$ for acid phosphatase and $E_C$ for β-glucosidase.
Subsequent contrasts and comparisons were executed in JMP (JMP version 4.0.2) and SAS (version 8.2), using either a general linear model or a mixed model. Simple contrasts were executed as t-test, the Tuckey–Kramer adjustment (Kramer, 1956) was used for multiple comparison of means (all at \( \alpha = 0.05 \) unless stated otherwise). As all above procedures carry the normality assumption, the data was examined for normality and homoscedacity of variance, outliers were identified as observations that fell beyond \( \pm 1.5 \) interquartile range. Nonparametric pair-wise correlations executed as Spearman’s Rho correlation coefficients (JMP version 4.0.2).

3. Results

We found that total P levels showed a significant gradient in values, with the impacted site exhibiting the highest TP levels 1355 (±45) mg P kg\(^{-1}\) the intermediate site 707 (±36.3) mg P kg\(^{-1}\) and the unimpacted (U3) site 305 (±11.2) mg P kg\(^{-1}\) (Table 1). The concentration of TC in these soils decreased from 418 g kg\(^{-1}\) (impacted, U3), 362 g kg\(^{-1}\) (intermediate, F4) to 291 g kg\(^{-1}\) (unimpacted, F1). Soil TN concentrations did not vary much across the three sites, with 26.8 g kg\(^{-1}\) (impacted, U3), 24.7 g kg\(^{-1}\) (intermediate, F4) and 25.1 g kg\(^{-1}\) (unimpacted, F1), respectively. There was a significant difference in C/N ratios over the sites, the impacted (F1) site and intermediate (F4) site had similar ratios (17:1) whereas the C/N ratio was significantly lower at the unimpacted (U3) site, averaging a 30 (±3) \( \mu \)g MUF g\(^{-1}\) h\(^{-1}\). We found no significant difference in AM activities across the sites, with 33 (±4), 46 (±6) and 39 (±6) \( \mu \)g MUF g\(^{-1}\) h\(^{-1}\) for the impacted (F1), the intermediate (F4) and unimpacted (U3) sites, respectively. The total levels of dehydrogenase activity varied little across all soils, 540 (±50), 452 (±100) and 467 (±29) \( \mu \)g MUF g\(^{-1}\) h\(^{-1}\) for the impacted (F1), intermediate (F4) and unimpacted (U3) sites, respectively.

The intermediate site exhibited the largest levels of microbial biomass P (237 mg kg\(^{-1}\)) when compared to the impacted (159 mg kg\(^{-1}\)) and unimpacted (73 mg kg\(^{-1}\)) sites, possibly a result of the immediate alleviation of the P limitation in the case of the intermediate site (Table 2). Likewise, microbial biomass C and N where all to contain the largest concentrations (12 g kg\(^{-1}\) and 1709 mg kg\(^{-1}\), respectively) in the intermediate (F4) site. The lowest concentrations where found in the unimpacted (U3) site (9 g kg\(^{-1}\) and 897 mg kg\(^{-1}\), respectively), followed by the impacted (F4) site with 7.5 g kg\(^{-1}\) and 1019 mg kg\(^{-1}\), respectively. The levels of microbial biomass P, C and N in these soils were of a similar order of magnitude as found by other authors for similar soils (White and Reddy, 2001; Wright and Reddy, 2001; DeBusk and Reddy, 2003).

### Table 2

Analytically determined microbial measures (microbial biomass C, N, and P; MBC, MBP, MBN; potential mineralizable P, N; PMP and PMN, total organic C; TOC, values denote means and standard error in parentheses, \( n = 36 \), averages over floc and 0–10 cm of soil)

<table>
<thead>
<tr>
<th></th>
<th>F1</th>
<th>F4</th>
<th>U3</th>
</tr>
</thead>
<tbody>
<tr>
<td>MBC (g kg(^{-1}))</td>
<td>7.5 (0.7)</td>
<td>12 (1.3)</td>
<td>9 (1.0)</td>
</tr>
<tr>
<td>MBP (mg kg(^{-1}))</td>
<td>159 (9)</td>
<td>237 (20)</td>
<td>73 (4)</td>
</tr>
<tr>
<td>MBN (mg kg(^{-1}))</td>
<td>1019 (91)</td>
<td>1709 (228)</td>
<td>897 (99)</td>
</tr>
<tr>
<td>PMP (mg kg(^{-1}) d(^{-1}))</td>
<td>13.5 (2.8)</td>
<td>5.8 (0.19)</td>
<td>1.9 (0.18)</td>
</tr>
<tr>
<td>PMN (mg kg(^{-1}) d(^{-1}))</td>
<td>42.2 (1.2)</td>
<td>51.4 (2.4)</td>
<td>32.3 (1.1)</td>
</tr>
<tr>
<td>TOC–K(_2)SO(_4) extractable (mg/kg)</td>
<td>2784 (132)</td>
<td>3042 (130)</td>
<td>2610 (90)</td>
</tr>
<tr>
<td>Aerobic CO(_2) production (( \mu )g g(^{-1}) d(^{-1}))</td>
<td>299 (15)</td>
<td>430 (64)</td>
<td>226 (17)</td>
</tr>
<tr>
<td>Anaerobic CH(_4) production (( \mu )g g(^{-1}) d(^{-1}))</td>
<td>63 (12)</td>
<td>113 (9)</td>
<td>58 (9)</td>
</tr>
</tbody>
</table>
The overall rates of P mineralization in this system were significantly higher (13.50 mg kg\(^{-1}\) d\(^{-1}\)) in the impacted (F1) site and decreased rapidly from intermediate (F4; 5.76 mg kg\(^{-1}\) d\(^{-1}\)) to the unimpacted site (U3; 1.90 mg kg\(^{-1}\) d\(^{-1}\)), all differences significant. The unimpacted (U3) soils contained significantly lower concentrations of PMN (Table 2); 32.3 mg kg\(^{-1}\) d\(^{-1}\), compared to the largest values that were found in the intermediate (F4) site at 51.4 mg kg\(^{-1}\) d\(^{-1}\), and those found at the impacted (F1) site at 42.2 mg kg\(^{-1}\) d\(^{-1}\). Methanogenesis was significantly higher in the soils from the P-enriched site (F1), 63 μg g\(^{-1}\) d\(^{-1}\), when contrasted to the unimpacted (U3) site at 58 μg g\(^{-1}\) d\(^{-1}\) (Table 2), which was generally consistent with earlier research (Wright and Reddy, 2001). Anaerobic microbial activities at the intermediate (F4) site were higher, 113 μg g\(^{-1}\) d\(^{-1}\), than activities at the impacted (F1) site and unimpacted sites. We also found this pattern of microbial activity in the aerobic respiration rates, with the highest activity recorded for the intermediate (F4) site at 430 μg g\(^{-1}\) d\(^{-1}\), the lowest, 226 μg g\(^{-1}\) d\(^{-1}\) at the unimpacted (U3) site and intermediate activities, 299 μg g\(^{-1}\) d\(^{-1}\), at the impacted (F1) site.

4. Discussion

The C/N/P ratios of the unimpacted (U3), intermediate (F4) and impacted (F1) soils; 1228:90:1, 620:36:1 and 331:20:1, respectively, suggest that the system was P limited at the intermediate and unimpacted sites (Reddy et al., 1993). Substrate quality will become a limiting factor when the substrate C:P ratios are at 1200:1 or more (Amador and Jones, 1997), which is approximated at the unimpacted site (U3, 1395:1). The nutrient enrichment induced changes in substrate type and quality (DeBusk and Reddy, 1998) can result in significant changes in the microbially mediated degradation rates.

4.1. Enzymatic activities (EA) as a fingerprint of the microbial community responses

Due to the complex nature of plant and soil matter, degradation requires the concerted activity of multiple classes of enzymes, the combined relative activity of the enzymes has been suggested by Sinsabaugh et al. (1997) and as a model for microbial response to environmental conditions. We found that AP activity was a significant response variable when describing the P dynamics in this system, protease and dehydrogenase activities showed little response and β-glucosidase activity was higher in the intermediate site (Fig. 2).

Analysis of the combination of enzymatic activities can be obtained through meta-analysis (Saiya-Cork et al., 2002), in which the independent variables are combined to generate a single test (Gurevitch and Hedges, 2001). This analysis indicated that the overall EA was different if the benchmark was set to the unimpacted site (Table 3), the largest mean difference was found when contrasting the intermediate (F4) site to the unimpacted (U3) site, not when contrasting the P-enriched, impacted (F1) site to the P-limited, unimpacted (U3) site. The overall EA profiles would (Fig. 2) indicate that phosphatase dominates the response profile, as a result the largest difference was expected in the impacted contrast versus the intermediate contrast. However, the meta-analysis results indicate that the change in alkaline phosphatase activity seems to be offset by the change in β-glucosidase and amino-peptidase.

An alternative approach is the optimal resource allocation model, in which the relative EA should give an indication of the needs and demands by the microbial communities. To that end Sinsabaugh and Moorhead (1994) constructed the microbial allocation...
of resources among community indicator enzymes (MARCIE) model. The model equates some response variable, such as litterbag mass loss or bacterioplankton production, to enzyme activity through a first order model that includes specific C, P and N allocation factors. Subsequent evaluation of the model (Sinsabaugh et al., 1997) resulted in two parameters of interest $E_C/E_N$ and $E_C/E_P$ or the ratio between the amounts of enzyme activity associated to C acquisition ($E_C$) to the enzymes involved in N ($E_N$) and P ($E_P$) acquisition, respectively, which is illustrated in Fig. 3. The microbial environment shifts from a primarily P-limited system (lower left panel) to a possibly an N-limited system (top left panel). Cross classification by sites illustrates the gradual shift in nutrient limitation as 75% and 60% of the unimpacted (U3) and intermediate (F4) observations, respectively, fell in the lower left panel, whilst 75% of the observations from the impacted area (F1) fell in the upper left panel. The $E_C/E_P$ ratios encountered in this study for the unimpacted area were significantly lower than those encountered elsewhere (Sinsabaugh et al., 1997), this discrepancy was resolved for the impacted ratios. The proportion of N and C acquiring enzymes did vary across the marsh with the lowest levels of both enzymes found at the unimpacted site and increasing as a result of the P inputs. Interpretation of the $E_C/E_N$ ratios alone would indicate that the unimpacted (U3) area was N limited, while the intermediate (F4) and impacted (F1) showed this limitation was somewhat relieved, yet these values were probably an extension of the overwhelming P-limitation in that both carbon and nitrogen acquiring enzyme production was limited.

In evaluating the model, as there was no coherent response variable in this study, the approach used in contrasting the two models is necessarily qualitative. In comparing: (i) the productivity is proportional to total enzyme activity $E_T(\sum(E_N, E_C, E_P))$ versus (ii) the microbial productivity is a function of specific nutrient acquiring enzymes $E_T/(1+E_N/E_C + E_P/E_C)$, thus illustrating specific resource allocation versus generic resource allocation. There where the correlation

Table 3
Comparison of overall enzyme activities in the impacted and intermediate site vs. the unimpacted site by meta-analysis

<table>
<thead>
<tr>
<th>Metric</th>
<th>$D$</th>
<th>$n$</th>
<th>$\text{Var}(d)$</th>
<th>$\alpha$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unimpacted vs. intermediate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\beta$-Glucosidase</td>
<td>-2.65</td>
<td>36</td>
<td>0.17</td>
<td></td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>0.02</td>
<td>36</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td>Dehydrogenase</td>
<td>-0.15</td>
<td>36</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td>Peptidase</td>
<td>-0.78</td>
<td>36</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>0.90</td>
<td>36</td>
<td>0.02</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Impacted vs. unimpacted</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\beta$-Glucosidase</td>
<td>-1.19</td>
<td>36</td>
<td>0.09</td>
<td></td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>0.68</td>
<td>36</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td>Dehydrogenase</td>
<td>0.11</td>
<td>36</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td>Peptidase</td>
<td>-0.28</td>
<td>36</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>0.57</td>
<td>36</td>
<td>0.02</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

The size of the effect ($d$) is units standard deviation, $\text{Var}(d)$ is the associated variance, $n$ is the number of samples in the analysis, and $\alpha$ is the probability that the overall microbial enzymatic responses were not different in the two contrasting sites.

![Fig. 3. Microbial resource allocation according to the MARCIE model (Sinsabaugh and Moorhead, 1994). The model assumes a trade-off between the production of N-, P- and C-acquiring enzymes by the microbial consortia, the y-axis represents the relative P availability ($E_C/E_P$) vs. relative N availability ($E_C/E_N$), log transformed for visual comparison (values taken over floc and 0–10 cm soil).](image-url)
coefficient approaches 1, there is no difference in resource allocation (i.e. relative quantities of enzyme production), whilst weak to no correlations would indicate the prevalence of one or more enzymes. The Pearson correlation coefficients (Table 4) indicated that in terms of EA, the microbial communities were generalists in the nutrient impacted areas, and becoming increasingly specialists towards the interior of the marsh.

This study only included one enzyme representative of the C, N and P cycles each, microbial responses may have varied based on the litter quality (lignen to cellulose content), the type of electron acceptors present (oxidases versus hydrolases) and actual microbial community composition (fungal phytases versus bacterial and plant phosphatases). A more complete suite of enzyme analysis (for example: Alvarez and Guerrero, 2000) along with a finer sampling scale along the gradient associated with a clear response variable would better elucidate microbial resource allocation beyond the overwhelming P-limitation in the water conservation area.

4.2. Microbially mediated C, N and P turnover rates and biomass patterns

At the edge of the eutrophication front, the P limitation is presumably sufficiently relieved for the microbial communities to dramatically increase their P content; we found that the intermediate (F4) site had the overall the highest levels of MBP (236.77 mg kg\(^{-1}\)). Similarly, Qualls and Richardson (2000) found increases in MBP as a result of direct P additions to an unenriched area in the WCA-2A. Biomass C to P ratios have been found to range from 25 (Singh and Singh, 1993) to ratios of 79 and 279 in humus rich soils (He et al., 1997). A broad study done on soils under a beech forest resulted in biomass C:N ratios ranging from 17.3 to 4.5 (Joergensen et al., 1995a,b) and biomass C:P ratios ranging from 5.1 to 26.3 (Joergensen et al., 1995a,b), generally the microbial C:P ratio in soils has been suggested in the range 10:1 to 35:1 (He et al., 1997, and references therein). In general terms, all soils (Joergensen et al., 1995a,b; He et al., 1997) reflected higher C:P ratios in P-limited soils. Adjustment of our ratios for a conversion (extraction factor) of 0.4, showed they were consistent with the above suggested range (Table 5), and the microbial biomass at unimpacted soils at (U3) had significantly higher C:P ratios than that at the intermediate site (F4) and the impacted site (F1). The biomass C:P ratios were not significantly different between the intermediate (F4) and impacted (F4) sites. Biomass C:N ratios did not vary significantly between sites.

Nutrient enrichment has been noted to generally increase organic matter mineralization rates in wetland soils (Davis, 1991; Qualls and Richardson, 2000),

<table>
<thead>
<tr>
<th>Table 4</th>
<th>Microbial allocation of resources among community indicator enzymes (MARCIE, Sinsabaugh and Moorhead, 1994) over the three everglades sites</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F1</td>
</tr>
<tr>
<td>(E_C/E_N)</td>
<td>1.00 a</td>
</tr>
<tr>
<td>(E_C/E_P)</td>
<td>2.06 a</td>
</tr>
<tr>
<td>(E_C + E_N + E_P = E_T)</td>
<td>0.37 a</td>
</tr>
<tr>
<td>(E_T/(1 + E_N/E_C + E_P/E_C))</td>
<td>0.10 a</td>
</tr>
<tr>
<td>Pearson’s correlation coefficient: (E_T) to (E_T/(1 + E_N/E_C + E_P/E_C))</td>
<td>0.69</td>
</tr>
</tbody>
</table>

Test of the assumption that microbes must undergo trade-off in enzyme production, a strong positive correlation coefficient indicates no preferential resource allocation (adaptation from Sinsabaugh et al., 1997). Different letters denote significant differences; the bolded correlation coefficients are significant.

<table>
<thead>
<tr>
<th>Table 5</th>
<th>Microbial biomass ratios and potential P and N mineralization (PMP, PMN) as a function of total P and N (cumulative potential P, N turnover rates) and as a function of microbial biomass P and N (PMP, PMN quotient) in WCA-2A (values denote means and standard error in parentheses, (n=36), taken over the floc and 0–10 cm of soil)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F1</td>
</tr>
<tr>
<td>Phosphorus</td>
<td></td>
</tr>
<tr>
<td>MBC/MBP</td>
<td>38.5 a</td>
</tr>
<tr>
<td>PMP quotient ((d^{-1}))</td>
<td>0.94 a</td>
</tr>
<tr>
<td>Cumulative potential P turnover rates (mg mineralized P g(^{-1}) TP)</td>
<td>0.11 b</td>
</tr>
<tr>
<td>Nitrogen</td>
<td></td>
</tr>
<tr>
<td>MBC/MBN</td>
<td>6.4 a</td>
</tr>
<tr>
<td>PMN quotient ((d^{-1}))</td>
<td>0.45 ab</td>
</tr>
<tr>
<td>Cumulative potential N turnover rates (mg mineralized N g(^{-1}) TN)</td>
<td>15.0 a</td>
</tr>
</tbody>
</table>

Different letters denote significant differences.
including the mineralization of P. Both the cumulative potential P turnover rates as the PMP-quotient were significantly higher in the soils from impacted (F1) site when compared to those from the unimpacted (U3) site (Table 5). At the intermediate (F4) site, increases in P mineralization rates were commensurate with increases in microbial biomass, resulting in a PMP quotient similar to the soils from the unimpacted (U3) site. However, the cumulative P turnover rates were similar to the impacted (F1) site. When comparing the intermediate (F4) site to the unimpacted (U3) site, the increases in cumulative P turnover indicated that a larger pool of P was being mobilized in excess of the increase in soil P content. No increase beyond the intermediate (F4) site reflects an increase in P mineralization that narrowly responds to the increase in P pools due to P inputs. Inversely, the microbial communities in the P-impacted (F1) site were relatively inefficient with the P obtained from organic matter mineralization, as qPMP levels were significantly higher in the soils from the impacted (F1) site. The combination of qPMP, and cumulative P turnover rates described a very efficient microbial community that initially responded to the increases in P availability by increasing in biomass (P immobilization) and extending P mineralization to pools previously unattainable.

There were no significant differences in qPMN over the three sites or the cumulative potential N turnover pool, despite a significantly higher N mineralization potential in the impacted (F1) and intermediate (F4) sites contrasted with the unimpacted (U3) site. The variability in the levels of PMN probably reflected increases in microbial biomass as a result of the alleviation of the P-limitation, they are highly correlated (Pearson r = 0.70; p < 0.001), as a result qPMN and the cumulative potential N turnover rates were not significantly different across the system. Nitrogen turnover (potential) in this system indicates an overall N turnover in the intermediate (F4) site that reflects higher MBN and higher aminopeptidase activities, probably contemporaneous to the overall increase in microbial biomass as a result the alleviation of the P-limitation.

The proportion of aerobic basal respiration (CO₂ production) to microbial biomass carbon, i.e. metabolic coefficient qCO₂ (Anderson and Domsch, 1990) has been identified as a sensitive response variable to soil organic matter quality (Kaiser and Heinemeyer, 1993). No significant changes in the levels of qCO₂ (Fig. 4; qCO₂ at F1, F4 and U3; 0.057, 0.045, 0.048, respectively) were found. In the intermediate (F4) sites, the proportion of microbial biomass in soil C was significantly higher, yet the relative metabolic activity of this biomass (qCO₂) did not change, indicating that what C was introduced into this system was efficiently cycled into biomass. In the soils from the impacted (F1) site, on the other hand, the nutrient influx has resulted in a dramatic increase in primary productivity (Davis, 1991), a greater deposition of C and a concomitant increase in microbial biomass C while maintaining the same metabolic efficiency as in the P-limited system. Measures associated with the C cycle describe a system that originally was a closed, efficient C cycle. The nutrient influx altered this system to a highly active, younger microbial system with rapid C turnover (Saggar et al., 2001). In terms of its global C pools, the P-impacted site reflected the greater primary productivity prevalent, however, in terms of its metabolic activities, anaerobic or aerobic, the system possibly reflected the imposition of a new limitation on microbial heterotrophic activity.

4.3. Elemental cycling and microbial responses measures, what do they tell us about WCA-2A?

Microbial activities and their respective community responses are considered a measure of ecosystem stability (Ohtonen, 1994) and an indicator of ecosystem perturbation (Anderson and Domsch, 1978). The rates of microbially mediated organic matter degradation not only play a pivotal role in nutrient
regeneration (Newman et al., 2001), but are also known to respond to numerous environmental perturbations. The original Everglades system is significantly P limited, which has been well established throughout the literature (Reddy et al., 1993; Richardson and Vaithayanathan, 1995; Newman et al., 2001), the combination of organic P fraction distribution (Ivanoff et al., 1998), repressed heterotrophic aerobic and anaerobic activities (DeBusk and Reddy, 1998; Wright and Reddy, 2001), and the overall enzymatic profiles clearly illustrate a system constructed to make optimal use of what little P is available. Microbial community dynamics in the intermediate zone between the overall P-limited and P-impacted areas illustrate an immediate response as the disturbance alleviates the stress imposed by the nutrient limitation. We found increased concentrations of microbial biomass without the projected decrease in microbial metabolic activity to microbial biomass ratios (Anderson and Domsch, 1985; Wardle, 1993), i.e. loss in the system efficiency. There were little corresponding increases in the metabolic coefficients as all activity changes were generally commensurate with the increase in biomass.

In the impacted regions of the Everglades, the ecosystem succession is expressed as changes in plant communities in response to the nutrient enrichment, establishing an altered microbial environment. This system is relatively young (<50 years) and comparison of the metabolic coefficients (particularly \(q_{PMP}\) and \(q_{CO_2}\)) in nutrient enriched sites and the original P-limited system confirm the general postulations by Ohtonen (1994), Anderson and Domsch (1985), Wardle (1993) and ultimately Odum (1969) in that after a disturbance an ecosystem such as the impacted areas in the Everglades are characterized relatively open, inefficient nutrient cycles. The impacted areas did indicate that in some of the parameters, particularly the EA profiles, the system may be N-limited.

The concept of utilizing microbial eco-physiological measures as indicators of disturbance is particularly reinforced by the spiked microbial response characteristic of the intermediate site. Soil chemical characteristics changed gradually as a result of the influx of nutrients, soil microbial measures exhibited a threshold type (step) response. These distinct, abrupt changes in microbial parameters compared to the more progressive change in the soil chemical characteristics indicates that microbial indicators function effectively as early warning signals. A composite analysis in which all measures, biotic and abiotic, are scrutinized collectively and a more quantitative direct comparison of the aptitude of the individual measures to distinguish impacted, intermediate and unimpacted, might cement microbial eco-physiological measures as useful indicators of ecological perturbation.

In summary, at the three sites in this system, we were able to effectively describe microbial eco-physiology in response to the environmental conditions present. Profiles of the relative enzyme activity resulted in insights in the initial microbial response to the changes in environmental conditions. Subsequent analysis of the measures associated with microbial biomass and associated nutrient turnover rates illustrated the changes in microbial physiological responses to an altered physico-chemical environment. The information generated by microbial community response measures and characterization of the physico-chemical environment in which these communities are embedded provided significant insights into ecosystems such as the Everglades.

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**References**


