Influence of Phosphorus Loading on Organic Nitrogen Mineralization of Everglades Soils

J. R. White and K. R. Reddy*

ABSTRACT

There have been recent concerns about the anthropogenic phosphorus (P) loading to the naturally oligotrophic Everglades ecosystem. We investigated the effect of P loading on the biogeochemical cycling of nitrogen (N). We investigated the distribution of the potentially mineralizable N (PMN) rate as an indicator of the influence of P loading on selected microbial activities in soil and detritus layers. Soil characteristics measured included bulk density; total C, N, and P; microbial biomass C; and N and extractable NH₄⁺. PMN rates ranged from 10.4 to 325 mg N kg⁻¹ d⁻¹. The highest values of microbial biomass C and N, total P, extractable NH₄⁺, and PMN were observed in the detrital layer, and rates decreased with increasing soil depth. An increase in the size of the microbial pool and heterotrophic activity (organic N mineralization) was found to be related to the P-loading rate and related to the distribution of total P content in the soil and detrital layers. Extractable NH₄⁺ was a good indicator of PMN rates and total P was found to be significantly correlated to microbial biomass C and N. The stimulatory effect of P enrichment on microbial activity, overall size of the microbial biomass pool, and the PMN rate has led to an increased availability of inorganic N, which could potentially affect macrophyte growth and water quality of the northern Everglades system.

Organic N mineralization (ammonification) in wetland soils is an important process regulating water column inorganic N concentrations and providing a steady N supply to aquatic vegetation. Ammonification, or the net release of ammonium N (NH₄⁺-N) is a continuous decomposition process by which high molecular weight organic N compounds are sequentially hydrolyzed into simpler compounds by extracellular enzyme activity (Sinsabaugh et al., 1991), followed by the breakdown of dissolved amino acid compounds and release of NH₄⁺-N (Fuhrman and Bell, 1985; Gardner et al., 1989). The rate limiting step can occur anywhere along the decomposition continuum, but the process is generally limited by the rate of hydrolysis of the larger organic compounds (Stanford and Smith, 1972).

Ammonification is dependent on a number of factors including the C:N ratio of the soil organic matter (SOM) and detrital tissue (Amador and Jones, 1997), temperature (Reddy, 1982; Addiscott, 1983), O₂ status (Gale and Gilmour, 1988; Humphrey and Pluth, 1996), size and activity of the microbial pool (Perucci, 1990; Wardle, 1992; Amador and Jones, 1993), and limiting nutrients (Munevar and Wollum, 1977; Damman, 1988; Nair, 1996). Net N mineralization has been observed in flooded peat soils with C:N ratios of > 24:1 (Williams and Sparling, 1988), 45:1 (Humphrey and Pluth, 1996), and 80 to 100:1 (Damman, 1988). Therefore, there is little evidence that a specific C:N ratio in peat soil can be applied to predict field anaerobic organic N mineralization rates (Williams, 1984).

The microbial pool sequesters N in organic forms (proteins, amino acids) which are released upon cell death. Inorganic N, released from the organic N pool via ammonification, accumulates in wetland soils as NH₄⁺ rather than NO₃⁻, because of the anaerobic status of the flooded soil system (Reddy and Patrick, 1984) and diffusion limitations (Reddy et al., 1980). The high soil moisture content of peat soil restricts the supply of O₂, leading to decreased organic matter decomposition rates (Humphrey and Pluth, 1996; Amador and Jones, 1997).

The availability of inorganic N in peat soils is mediated to a great extent by heterotrophic microbial activity. The soil microbial biomass has been significantly correlated with N mineralization rates in studies of wetland soils (Williams and Sparling, 1988; McLatchey and Reddy, 1998). The size and activity of the microbial pool can be regulated by the availability of nutrients. It is well established that the size of the soil microbial biomass is dependent upon the C content of soils and

Abbreviations: CFE, chloroform-fumigation extraction; PMN, potentially mineralizable nitrogen; SINM, substrate induced nitrogen mineralization, SFWMD, south Florida water management district; SOC, soil organic carbon; WCA-2A, Water Conservation Area-2A.
additions of readily hydrolyzable C sources results in increased microbial growth and activity (Anderson and Domsch, 1985; Schnurer et al., 1985). However, relationships between microbial biomass and soil organic carbon (SOC) have been shown to be strongest in soils with less than 2.5% organic C (Anderson and Domsch, 1989; Wardle, 1992) and might not be applicable to high organic matter soils (Histosols). Stimulatory responses to P additions on either microbial pool size or activity (represented by C or N mineralization rates) have been reported for a variety of ecosystems (Munevar and Wotton, 1977; Biederbeck et al., 1984; Prescott et al., 1992; Hosseini et al., 1995; DeBusk and Reddy, 1998) while other studies have shown no response to P additions (Tate et al., 1991; Ross et al., 1995). Problems may exist in assessing the effect of added P on microbial properties in upland agricultural sites due to the simultaneous additions of N and P and extensive soil fertilization histories which can mask the overall effect of P addition (Wardle, 1992). All the aforementioned regulators, in concert with field scale soil heterogeneity, can make reliable bulk soil net N mineralization difficult to estimate.

**Study Area**

The Florida Everglades are currently affected by nutrient loading from urban and agricultural surface water runoff. Most notably, this impact can be seen in the Water Conservation Areas, one of the major hydrologic units of the Everglades (DeBusk et al., 1994). Water Conservation Area 2A (WCA-2A) has been receiving nutrient-laden (N and P) drainage waters for the past 40 yr. Peat accretion has increased over historical rates in areas receiving surface drainage water compared with unimpacted sites in the marsh interior (Koch and Reddy, 1992; Craft and Richardson 1993). Most notably, the impact of anthropogenic nutrient loading is reflected in the spatial distribution of surface soil (0–10 cm) total P. Concentrations of surface soil total P concentrations range from ~1600 mg kg⁻¹ at the surface water inflow points to background concentrations of ~400 mg kg⁻¹ in unimpacted areas, in the interior of the marsh (Koch and Reddy, 1992; Reddy et al., 1993). A gradient of N and P in surface water and periphyton tissue has also been documented along the identical transect in WCA-2A (McCormick and O’Dell, 1996).

Historically, WCA-2A consisted of a P-limited sawgrass (Cladium jamaicense Crantz) marsh. The vegetation began a shift towards a dominant cattail (Typha domingensis Pers.) community proximal to all surface water inflow points (Davis, 1991; Craft and Richardson, 1997). The replacement of the natural marsh vegetative community, consisting of stands of sawgrass separated by shallow, open sloughs, by a dense cattail community could potentially affect ecosystem function.

The objectives of this study were to determine (i) the potential N mineralization rates in detritus and soil under anaerobic conditions, (ii) the relationship between soil characteristics and short-term mineralization rates under drained and flooded conditions, and (iii) the effect of added P on the size and activity of the soil microbial biomass and potential N mineralization rates in a P limited wetland soil.

**MATERIAL AND METHODS**

**Experimental Design**

Eight stations were located along a 10 km north-south transect in WCA-2A (Fig. 1). The study transect spanned the marsh from a primary water control inflow structure (S-10C), southward across the dominant cattail vegetation and terminated approximately 10 km into the native (unaffected) marsh characterized by sawgrass separated by shallow sloughs dominated by floating and attached cyanobacterial mats. Sampling stations were located at distances of 1.4, 2.3, 3.3, 4.2, 5.1, 7.0, 8.4, and 10.1 km from the S-10C water control inflow structure (Fig. 1). Water depths varied seasonally from <2 cm to ~2 m along the transect length [South Florida Water Management District (SFWMD), 1996, unpublished data].

Sampling along the transect was not designed to identify differences between individual stations, but rather to investigate the gradient or trends in soil characteristics, including organic N mineralization rates with distance. Soils were collected three times along the transect over a 1-yr period (February and August 1996, and March 1997). Data from the three sampling periods were combined to produce mean values and to provide a measure of variability about the mean.

Soil sampling was also conducted in conjunction with a mesocosm nutrient-dosing study in WCA-2A. The SFWMD established 21 circular enclosures of 1.8 m² each, and three open control plots in an unimpacted sawgrass-periphyton-slush (McCormick and O’Dell, 1996). The mesocosm site was located approximately 11 km SW from the S-10C inflow water control structure (Fig. 1). The enclosures were installed entirely within a shallow slough that contained no stands of sawgrass within the study site proper. The soil surface was dominated by floating and benthic cyanobacterial (periphyton) mats, purple bladdernose (Utricularia purpurea Walt.), and water lily (Nymphaea odorata Ait.) (McCormick and O’Dell, 1996). At the experiment start, three replicate tanks were selected at random and spiked with various amounts of NaH₂PO₄, mixed with slough water to achieve loading rates of 0, 0.4, 0.8, 1.6, 3.2, 6.4, and 12.8 P g⁻² m⁻² yr⁻¹. The enclosures were perforated, which allowed exchange with the surrounding water, and equipped with sliding collars which could be moved over the holes in the side of the tanks to prevent exchange during dosing. The enclosures remained closed for 24 h after P spiking, then were subsequently opened to permit exchange with the surrounding water. These systems were dosed with P at respective levels starting in June 1995, and continued weekly for a period of 17 mo. This experiment was part of a larger ecological study conducted by McCormick and others at SFWMD (McCormick, P., 1997, personal communication).

**Soil Sampling**

A minimum of four soil cores were collected and composited within 5 m at each station along the transect by driving a 10-cm-diam. aluminum irrigation pipe into the soil. A probe was inserted into each core to verify that negligible (<5%) compaction had occurred during coring. Cores were sealed, removed from the ground, immediately extruded and separated into discrete soil intervals (0–10 and 10–30 cm) in the field. Each interval was well mixed to yield a representative and homogenous sample from each station. The February 1996 samples were transported to the laboratory on ice, transferred into 2-L polyethylene containers within 24 h of collec-
In order to investigate spatial variability of organic N mineralization rates, three stations (2.3, 7.0, and 10.1 km from the inflow) were sampled for detritus and 0- to 10-cm soil depths along a short, east-west transect, normal to the direction of the major sampling transect, on 13 Oct. 1997. Five cores were taken at 10-m intervals, sectioned in the field, stored in plastic bags and placed on ice until return to the laboratory the following day for soil characterization and determination of the potential net N mineralization rate.

Soils were collected on 21 Nov. 1996 from the experimental mesocosms by driving a 10-cm diameter polyethylene tube into the soil. A single core was taken from each of the triplicate enclosures for all 7 P-dosing levels and three additional cores were taken from within the slough to serve as open controls (total of 24 cores). The periphyton-floc layer was poured off into separate sampling containers. The top soil interval (0-3 cm) was then extruded, stored in plastic bags and placed on ice until returning to the laboratory where samples were stored at 4°C.

**Soil Characterization**

Bulk density was calculated for each soil layer on a dry weight basis. Bulk density was not determined for detritus. Total C and N content of detritus and soils was determined on dried, ground samples using a Carlo-Erba NA-1500 CNS Analyzer (Haak-Buchler Instruments, Saddlebrook, NJ). Total P concentrations were determined on sub-samples by nitric-perchloric acid digestion (Kuo, 1996), followed by analysis of P by an automated ascorbic acid method (Method 365.4, USEPA, 1983).

Extractable NH$_4$-N was determined by shaking triplicate soil samples with 25 mL of 2 M KCl at a ratio of approximately 1:40 (g dry soil:extractant) for 1 h on a longitudinal shaker. Samples were centrifuged for 10 min and vacuum-filtered through Whatman #42 filter paper. The supernatant was analyzed colorimetrically for NH$_4$-N (Method 351.2, USEPA 1983).

Microbial biomass C was determined by the 24 h chloroform fumigation-extraction (CFE) technique after Vance et al.
were added to 250-mL Nalgene (Nalge Nunc International, Rochester, NY) polyethylene bottles for aerobic incubations containing 400 mg L−1 alanine (C₃H₇NO₂) and 10 g of field moist soil in 120 mL media. To each bottle, 40 mL of distilled deionized water was added and mixed well with the soil. The following treatments were evaluated: (i) control-no addition and (ii) NaH₂PO₄ added (0.1, 1.0, 5.0, 10 mg P L⁻¹ final concentration in the porewater). Each treatment was performed in triplicate. Bottles were capped and purged with O₂-free N₂ gas to create anaerobic conditions. Samples were incubated in the dark at 30°C for 20 d and were shaken by hand for 30 s each day. Triplicate soil controls were spiked with distilled de-ionized water. The 20-d pre-incubation period allowed the microbial community time to react to added P. At the terminus of the 20-d incubation, 20 mL of soil-water slurry was collected from each bottle by pipette, and extracted with 20 mL of 2 M KCl to determine the extractable NH₄⁺ (Method 351.2, USEPA 1983). An additional 10 mL were placed in air-tight serum bottles under a O₂-free N₂ headspace for incubation at 40°C for 10 d to determine the effect of differential P additions on PMN rates.

RESULTS AND DISCUSSION

Soil Characterization

The organic soils were characterized by high moisture contents (90–95% w/w) and low dry weight bulk densities averaging 0.059 ± 0.008 (mean ± standard devia-
Table 1. Select physiochemical properties of detritus and soils collected from along the transect in WCA-2A. Data are mean values (n = 3) with 1 standard deviation in parentheses. Samples were collected February and August, 1996 and March 1997.

<table>
<thead>
<tr>
<th>Distance</th>
<th>Depth</th>
<th>Bulk density</th>
<th>Total C</th>
<th>Total N</th>
<th>Total P</th>
</tr>
</thead>
<tbody>
<tr>
<td>km</td>
<td></td>
<td>g cm^{-3}</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.4</td>
<td>0-10 cm</td>
<td>n.d.</td>
<td>0.049 (0.006)</td>
<td>0.040 (0.009)</td>
<td>0.036 (0.009)</td>
</tr>
<tr>
<td>2.3</td>
<td>0-10 cm</td>
<td>n.d.</td>
<td>0.050 (0.016)</td>
<td>0.042 (0.013)</td>
<td>0.039 (0.011)</td>
</tr>
<tr>
<td>3.3</td>
<td>0-10 cm</td>
<td>n.d.</td>
<td>0.052 (0.011)</td>
<td>0.043 (0.011)</td>
<td>0.040 (0.011)</td>
</tr>
<tr>
<td>4.2</td>
<td>0-10 cm</td>
<td>n.d.</td>
<td>0.070 (0.015)</td>
<td>0.063 (0.012)</td>
<td>0.059 (0.011)</td>
</tr>
<tr>
<td>5.1</td>
<td>0-10 cm</td>
<td>n.d.</td>
<td>0.067 (0.011)</td>
<td>0.061 (0.010)</td>
<td>0.058 (0.010)</td>
</tr>
<tr>
<td>6.1</td>
<td>0-10 cm</td>
<td>n.d.</td>
<td>0.080 (0.009)</td>
<td>0.073 (0.010)</td>
<td>0.069 (0.009)</td>
</tr>
<tr>
<td>7.0</td>
<td>0-10 cm</td>
<td>n.d.</td>
<td>0.086 (0.016)</td>
<td>0.078 (0.012)</td>
<td>0.073 (0.011)</td>
</tr>
<tr>
<td>8.4</td>
<td>0-10 cm</td>
<td>n.d.</td>
<td>0.074 (0.009)</td>
<td>0.066 (0.009)</td>
<td>0.061 (0.009)</td>
</tr>
<tr>
<td>10.1</td>
<td>0-10 cm</td>
<td>n.d.</td>
<td>0.094 (0.007)</td>
<td>0.087 (0.006)</td>
<td>0.081 (0.006)</td>
</tr>
<tr>
<td>1.4</td>
<td>10-30 cm</td>
<td>n.d.</td>
<td>0.096 (0.018)</td>
<td>0.087 (0.013)</td>
<td>0.081 (0.012)</td>
</tr>
<tr>
<td>2.3</td>
<td>10-30 cm</td>
<td>n.d.</td>
<td>0.104 (0.015)</td>
<td>0.096 (0.010)</td>
<td>0.090 (0.009)</td>
</tr>
<tr>
<td>3.3</td>
<td>10-30 cm</td>
<td>n.d.</td>
<td>0.096 (0.003)</td>
<td>0.087 (0.002)</td>
<td>0.082 (0.002)</td>
</tr>
<tr>
<td>4.2</td>
<td>10-30 cm</td>
<td>n.d.</td>
<td>0.102 (0.006)</td>
<td>0.094 (0.005)</td>
<td>0.088 (0.005)</td>
</tr>
<tr>
<td>5.1</td>
<td>10-30 cm</td>
<td>n.d.</td>
<td>0.076 (0.012)</td>
<td>0.067 (0.011)</td>
<td>0.060 (0.011)</td>
</tr>
<tr>
<td>6.1</td>
<td>10-30 cm</td>
<td>n.d.</td>
<td>0.096 (0.007)</td>
<td>0.087 (0.006)</td>
<td>0.081 (0.006)</td>
</tr>
<tr>
<td>7.0</td>
<td>10-30 cm</td>
<td>n.d.</td>
<td>0.108 (0.004)</td>
<td>0.099 (0.003)</td>
<td>0.093 (0.003)</td>
</tr>
<tr>
<td>8.4</td>
<td>10-30 cm</td>
<td>n.d.</td>
<td>0.080 (0.001)</td>
<td>0.072 (0.001)</td>
<td>0.066 (0.001)</td>
</tr>
<tr>
<td>10.1</td>
<td>10-30 cm</td>
<td>n.d.</td>
<td>0.074 (0.009)</td>
<td>0.066 (0.006)</td>
<td>0.060 (0.006)</td>
</tr>
</tbody>
</table>

n.d. = not determined.

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In the experimental mesocosms, soil bulk density did not vary significantly among treatments (P > 0.9) and averaged 0.093 ± 0.012 g cm^{-3} for the 0- to 3-cm soil depth (Table 2). Total C and N values did not vary significantly among treatments and were significantly correlated with each other (P < 0.01; r = 0.86). The mean C:N ratio was 13.1 ± 0.54 for the 0- to 3-cm surface soil. Total P of soil was significantly correlated (P < 0.01; r = 0.9) to the experimental P loading rate indicating that P was not completely scavenged by the benthic periphyton-floc layer (Table 2). A significant decrease in the N:P ratio of the 0- to 3-cm soil was observed with increased P loading, ranging from a mean 75:1 in the no dose treatment to 40:1 in the highest P dose treatment. Extractable NH\textsubscript{4}+ was significantly correlated (r = 0.55) to soil total P in the mesocosm field study further suggesting a possible relationship between N and P.

**Microbial Biomass**

Microbial biomass C (r = -0.91) and N (r = -0.78) in the detritus were each negatively correlated (P < 0.01) with distance from the inflow point along the transect. A significant correlation was observed for microbial biomass C and N vs total P (r = 0.65; 0.41) for the detrital layer, suggesting that P-loading at the inflow point, might have some relationship to the increased size of the microbial biomass.

Microbial biomass C and N both decreased with depth along the transect (P < 0.01; r = 0.69; 0.67, respectively) pooling data for all stations with respect to depth. Microbial biomass C averaged 13.3, 4.8 and 1.6 g kg^{-1} and microbial biomass N averaged 1090, 347, and 109 mg
Table 2. Selected physiochemical properties of the 0-3 cm soil interval from the mesocosm experiment in WCA-2A. Data are mean values (n = 3) with 1 standard deviation in parentheses. Samples were collected October 1997.

<table>
<thead>
<tr>
<th>P-loading rate</th>
<th>Bulk density</th>
<th>Total C</th>
<th>Total N</th>
<th>Total P</th>
</tr>
</thead>
<tbody>
<tr>
<td>g m⁻² y⁻¹</td>
<td>g cm⁻³</td>
<td>mg g⁻¹</td>
<td>mg g⁻¹</td>
<td></td>
</tr>
<tr>
<td>0 (oc)</td>
<td>0.081 (0.008)</td>
<td>392 (9.3)</td>
<td>30.2 (3.1)</td>
<td>492 (56)</td>
</tr>
<tr>
<td>0.4</td>
<td>0.094 (0.024)</td>
<td>404 (25)</td>
<td>31.8 (4.6)</td>
<td>427 (41)</td>
</tr>
<tr>
<td>1.6</td>
<td>0.113 (0.023)</td>
<td>345 (65)</td>
<td>26.6 (6.5)</td>
<td>475 (65)</td>
</tr>
<tr>
<td>3.2</td>
<td>0.101 (0.032)</td>
<td>349 (11)</td>
<td>26.4 (2.0)</td>
<td>471 (71)</td>
</tr>
<tr>
<td>6.4</td>
<td>0.074 (0.015)</td>
<td>424 (4.2)</td>
<td>32.9 (0.5)</td>
<td>380 (55)</td>
</tr>
<tr>
<td>12.8</td>
<td>0.096 (0.033)</td>
<td>366 (48)</td>
<td>25.4 (2.2)</td>
<td>460 (34)</td>
</tr>
<tr>
<td>6.4</td>
<td>0.093 (0.011)</td>
<td>383 (26)</td>
<td>29.5 (3.8)</td>
<td>594 (48)</td>
</tr>
<tr>
<td>12.8</td>
<td>0.093 (0.034)</td>
<td>355 (62)</td>
<td>27.6 (7.6)</td>
<td>688 (200)</td>
</tr>
</tbody>
</table>

(oc) = open control.

kg⁻¹ for detritus, 0 to 10 cm and 10 to 30 cm, respectively (Fig. 2). DeBusk (1996) found that the lignin/cellulose increased with depth in these soils which suggests the decrease in microbial biomass C and N with depth is likely due to the lower availability of C to support microbial populations in the subsurface and is similar to results found by others (Williams and Sparling, 1988; Franzluebbers et al., 1995; DeBusk and Reddy 1998).

Relative availability of C and N to the microbial pool can be assessed by comparing the microbial biomass C and N pool sizes to soil total C and N concentrations (Anderson and Domsch, 1989). Microbial biomass C as a percentage of total C averaged 3.11 (S.E. = 0.27), 1.10 (S.E. = 0.15), and 0.36 (S.E. = 0.04) and microbial biomass N averaged 4.70 (S.E. = 0.52), 1.23 (S.E. = 0.52), and 0.37 (S.E. = 0.04) as a percentage of total N for detritus, 0- to 10-cm, and 10- to 30-cm depths, respectively. These results suggest that detritus is the most microbiologically active portion of the wetland soil profile and is likely to be responsible for the greatest amount of nutrient turnover-release.

A significant correlation was observed between microbial biomass C and N from the transect study (r = 0.86, P < 0.01) with the C:N ratio averaging 12.3, 13.9 and 14.7 for detritus, 0- to 10-cm, and 10- to 30-cm soil depths, respectively. A regression of microbial C vs N yielded an average C:N ratio of 11.4 for the microbial pool (R² = 0.72; P < 0.01).

The mean microbial biomass C of the 0- to 3-cm depth soil from the P-dosing study was 7.1 g kg⁻¹ while microbial biomass N averaged 519 mg N kg⁻¹. A strong correlation between microbial biomass C and N was observed (r = 0.78; P < 0.01) and the slope of the regression returned an average C:N ratio of 9.9 for the microbial pool. The similarity in average C:N ratio of microbial pools from the transect and mesocosm studies does not address the differences in the relative, functional, microbial pool composition along the transect (Drake et al., 1996). Mean microbial biomass C as a percentage of total C and microbial biomass N as a percentage of total N in the P-dosing study were 2.0 (S.E. = 0.20) and 1.8% (S.E. = 0.34), respectively. These values were higher than the values for the 0- to 10-cm depth but lower than from the detrital layer along the transect.

Potentially Mineralizable Nitrogen Rate

Potentially mineralizable nitrogen rates from along the transect were highest in the detrital layer, decreasing with depth averaging 126, 35.8, and 18.2 mg N kg⁻¹. The similarity in average C:N ratio of microbial pools from the transect and mesocosm studies does not address the differences in the relative, functional, microbial pool composition along the transect (Drake et al., 1996). Mean microbial biomass C as a percentage of total C and microbial biomass N as a percentage of total N in the P-dosing study were 2.0 (S.E. = 0.20) and 1.8% (S.E. = 0.34), respectively. These values were higher than the values for the 0- to 10-cm depth but lower than from the detrital layer along the transect.

Microbial biomass N was significantly correlated with P-loading rate (r = 0.50) for the 0- to 3-cm soil depth. Both microbial biomass C and N were positively, significantly correlated with soil total P (r = 0.50; 0.70, respectively) providing evidence that P was likely the limiting nutrient to the microbial biomass in natural Everglades peat soils.

Potentially mineralizable nitrogen rates from along the transect were highest in the detrital layer, decreasing with depth averaging 126, 35.8, and 18.2 mg N kg⁻¹.
d\(^{-1}\) for detritus, 0- to 10- and 10- to 30-cm soil depth, respectively (Fig. 3). A similar pattern of decreasing N mineralization with depth has been observed by others in aerobic soils (Franzluebbers et al., 1996; Hossain et al., 1995). There existed a significant negative correlation of PMN rate with distance from inflow for all sample intervals combined (\(P < 0.05, r = -0.28\)), as well as for each depth interval taken separately, with the most significant effect seen in detritus samples (\(r = -0.57\)).

The results of the spatial study conducted in November, 1997 yielded PMN rates averaging 112 for detritus and 33.6 mg N kg\(^{-1}\) d\(^{-1}\) for the 0- to 10-cm soil interval. Microbial processes have been shown to be highly variable in the field with coefficient of variations (CV) on the order of 100 to 200% (Duncan and Groffman, 1994; Velthof, et al., 1996). The average CV for the PMN rate of detritus and 0- to 10-cm samples was 28% for triplicate cores taken at each station. The CV for subsample incubation PMN averaged 7.8%.

Overall, PMN rates were significantly correlated with several soil properties including microbial biomass C (\(r = 0.81\)) and N (\(r = 0.85\)), total P (\(r = 0.65\)) and extractable NH\(_4^+\) (\(r = 0.78\)) with significant negative correlations with total N (\(r = -0.48\)). These relationships might be useful in assisting in the development of diagnostic biogeochemical indicators, however care should be taken to examine each relationship before proceeding from correlation to causation (regression). The organic rich Everglades soils have a low redox potential and contain a thin (2-4 mm) oxidized layer due to high available C coupled with high microbial activity (DeBusk, 1996). The low O\(_2\) status of the soil can result in the near complete inhibition of the autotrophotrophic conversion of NH\(_4^+\) to NO\(_3^-\). Therefore, the concentration of extractable NH\(_4^+\) might provide a good indication of in situ N mineralization rates in flooded soils (Ross et al., 1995; Williams and Sparling, 1988).

The strong relationship of PMN rate with the size of the microbial pool is likely one of causation. Mineralization is a microbial-mediated process and given a substrate (SOM) with a similar C:N ratio, one could expect differences in total active microbial biomass to influence the rate at which inorganic N is liberated from the organic fraction.

The mesocosm experiment provided an excellent opportunity for a separation of effects in the field, as P was loaded at several rates to soil at the same station containing similar vegetative characteristic and presumably, similar microbial populations. Unlike the transect study, where vegetation type and density as well as functional microbial communities varied (Drake et al., 1996), any differences in soil characteristic or microbial processes should be directly attributed to P enrichment.

Total P was significantly, positively correlated with both microbial biomass C and N in the P-dosing study, suggesting a P limitation to the microbial pool. In addition, total P was significantly (\(P < 0.01\)) correlated with PMN rate indicating an increase in inorganic N release from soil with increasing total P. These results suggest that total P was a reliable indicator of microbial activity. A similar P limitation to organic N mineralization was found for a volcanic ash (Inceptisol) soil (Munevar and
Wollum, 1977) and a peat soil (Histosol) from the Everglades National Park (Nair, 1996).

Combining all the data from the transect and mesocosm studies, the best fit regression model of PMN vs total P was significant at P < 0.01 (Fig. 4). These results suggest that organic N mineralization rates in WCA-2A were likely controlled by availability of P to the microbial pool.

For the combined data from the transect and mesocosm studies, there existed a significant relationship between PMN rate and extractable NH$_4^+$ (Fig. 5). This result supports the assertion that extractable NH$_4^+$ is a valid indicator of potential N mineralization. Microbial biomass C and N pools demonstrated significant regression with PMN rates, demonstrating the influence of the size or the activity of the microbial pool on potential N mineralization rates in these wetland soils (Fig 6). Additionally, our results support the use of the CFE method in wetland soils, sometimes criticized because it does not measure the active microbial pool but simply measures cellular products (primarily cytoplasm) of cells, whether they are active or not. However, our findings suggest that for anaerobic incubations of highly organic wetland soils, the relative size of the microbial biomass determined by CFE was a suitable predictor of potential ammonification rates over the short term (few weeks).

**Phosphorus Addition—Laboratory Study**

Surface soil (0–10 cm) samples spiked and incubated with various levels of PO$_4^-$-P demonstrated a significant difference in PMN from the control over the entire range of P additions (Table 3). A similar result was found in bottle incubations under three levels of P-loading for a peat soil from the Everglades National Park (Nair, 1996). There were no significant differences in total microbial biomass C among the treatments. This result suggests that increased availability of P increased the heterotrophic activity, but not total microbial biomass, at least in the short term. The effect of continual P loading over longer terms (many months to years), seen in the results of the P-dosing field study, not only resulted in increased microbial activity but also an increased size of the microbial pool. It seems that inorganic P concentrations had a direct influence on increasing the specific heterotrophic microbial activity responsible for net N mineralization of native soil organic matter.

### Table 3. Potentially mineralizable nitrogen (PMN) rates for soil (0–10 cm) utilized in the nutrient addition study. Letters following rates depict significant differences (same letter = not significant).

<table>
<thead>
<tr>
<th>Porewater concentration</th>
<th>PMN rate mg N kg$^{-1}$ d$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>14.5a</td>
</tr>
<tr>
<td>0.1 P</td>
<td>18.2a</td>
</tr>
<tr>
<td>1.0 P</td>
<td>21.2b</td>
</tr>
<tr>
<td>5.0 P</td>
<td>21.3b</td>
</tr>
<tr>
<td>10.0 P</td>
<td>21.6b</td>
</tr>
</tbody>
</table>

The SINM technique was used to measure the relative activity of the portion of the microbial pool responsible for N mineralization in soil. Detritus and soil samples under drained conditions exhibited higher ammonification rates than samples under anaerobic, flooded condition, with the highest rates found in the detrital layer. Under flooded conditions, the detrital layer, exhibited higher ammonification rates than the 0- to 10- and 10- to 30-cm soil depths, averaging 36.8, 22.8, and 21.7 mg N kg$^{-1}$ h$^{-1}$ respectively. Mean ammonification rates of drained samples of detritus, 0- to 10- and 10- to 30-cm soil depths were 75.4, 51.0, and 43.1 mg N kg$^{-1}$ h$^{-1}$, respectively. On average, drained SINM rates were 1.99 (S.E. = 0.22) times greater than SINM rates under flooded conditions. The best-fit linear regression between drained and flooded SINM rates yielded a slope of 2.14 (Fig. 7).

The difference in rates between flooded and drained samples was likely due to the introduction of O$_2$ into the drained samples. The change in aeration status allowed facultative anaerobes to utilize O$_2$ as an electron acceptor leading to higher microbial rates. A similar difference in aerobic and anaerobic mineralization rates of alanine was reported for an organic soil from central Florida (McLatchey and Reddy, 1998). A previous study in WCA-2A found that aerobic rates of C mineralization were approximately three time greater than anaerobic rates in Everglades soil (DeBusk and Reddy, 1998).

There was found a significant (P < 0.05) weak correlation between SINM and distance from inflow for both drained and flooded samples (r = −0.32; −0.38 respectively). A highly significant (P < 0.001) seasonal effect was found with higher ammonification rates in the summer (August 1996) when compared with the winter (March, 1997). A portion of the difference in rates can be attributed to differences of in situ field incubation temperatures (−6°C) between sampling dates. Total P was not significantly correlated with either drained or flooded SINM.

Extractable NH$_4^+$ exhibited a significant weak corre-
tion to SINM \((P < 0.01; r = 0.53, 0.59)\) for both drained and flooded samples. This result is expected, as extractable \(\text{NH}_4^+\) concentrations in the soil are a direct result of net N mineralization processes. The microbial biomass C and N pools were significantly correlated to SINM for the flooded samples. The size of the microbial pool, represented by MBN for the drained samples, was also significantly correlated with SINM. The release or de-amination of \(\text{NH}_4^+\) from amino acids has been seen by others in lake systems where N was not limiting (Gardner et al., 1987; Hollibaugh, 1978). The SINM rates were not strongly correlated with measured soil properties and therefore, does not appear to be a useful assay for determining relative rates of potential organic N mineralization in these soils.

There was a significant difference \((P < 0.001)\) in N mineralization rates of native organic matter and L-alanine, with the average PMN rate ~23 times slower than the SINM rates, after a temperature correction for potential N mineralization rates \((Q_{\text{10}}^0 = 2)\). Similar results have been observed for amino acid utilization in lake water (Gardner et al., 1986, 1989) and in soil samples (Alef and Kleiner, 1986). The large difference in ammonification rates between the two parameters (PMN and SINM), lends additional support to the theory that organic N mineralization is limited by the breakdown of larger, more complex compounds while simple amino acids compounds are quickly attacked and utilized by the microbial populations.

**SUMMARY AND CONCLUSIONS**

Total P was significantly correlated with PMN and extractable \(\text{NH}_4^+\) for both the transect and P enrichment mesocosm studies and consequently provides an easily measurable biogeochemical indicator in investigating the impacts of eutrophication on organic N mineralization rates. Total C and N proved to be ineffective biogeochemical indicators for prediction of microbial biomass pool size or potential organic N mineralization rates. Total P was a useful indicator for the relative pool size of microbial biomass, as P-loading appeared to significantly increase the microbial biomass of soil and detritus in WCA-2A. The size of the microbial pool of detritus and soil was related to PMN. Results from the P-dosing field study (mesocosm) also suggest that P limitation controlled the release of inorganic N from the microbial pool with significantly higher microbial biomass found in the higher P-dosing treatments. Also, results from P dosing study in both the mesocosm (field) and the bottle incubation (laboratory) lend support to the assertion that differences in potential N mineralization rates along the transect in WCA-2a are related to the differences in soil total P instead of other characteristics such as vegetation type, hydrology, or peat composition.

In summary, eutrophication due to P-loading has appeared to increase the turnover rates of inorganic N from soil and detritus, linked to an increased activity and size of the microbial pool. The microbially mediated mobilization of nutrients, through increased decomposi-

**REFERENCES**


