Microbial Response of a Calcareous Histosol to Sulfur Amendment

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Abstract: The objective of this study was to assess the functional response of microbial communities to sulfur amendment in calcareous organic soils of the Everglades Agricultural Area in south Florida. Soils under sugarcane cultivation were amended with elemental S at four rates up to 448 kg S ha^{-1} to decrease pH and enhance nutrient availability. Soil samples were collected 2, 6, 9, and 13 months after S application and subjected to microbial, enzyme, and nutrient analysis. Application of S at rates up to 448 kg S ha^{-1} enhanced labile P availability by 103% compared with unamended soils. Nonetheless, stimulatory effects were limited and temporary because of the high buffering capacity of this calcareous organic soil against acidification. Activities of leucine aminopeptidase and sulfatase were independent of S application. Phosphatase activities were 115% higher and glucosidase activities 573% higher for soils receiving the highest S rates than unamended soils at 2 months. Microbial biomass C and N were not affected by S amendment, but biomass P was 314% higher for soils amended with the highest S rates at 2 months than soils receiving the lowest rates, primarily as a result of increased P availability. The C, N, and P mineralization rates were not affected by S, although all rates varied seasonally, suggesting that S application did not stimulate soil oxidation. Overall, application of S generally did not result in pulse or flux of nutrients from soil, suggesting that S application had minimal benefits for increasing nutrient availability to sugarcane.

Key words: Sulfur, Everglades, Histosols, microbial activity, enzymes.

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Microbial communities play important roles in organic matter degradation and nutrient cycling in soils. Likewise, soil physical and chemical properties and environmental factors greatly influence microbial activities and community composition (Allison et al., 2007). Microbial functional activities, such as extracellular enzyme activities (Allison et al., 2007), microbial respiration (Castillo and Wright, 2008; Iovieno et al., 2009), and nutrient mineralization rates (Corstanje et al., 2007; Wright et al., 2009), have been widely used as indicators to assess soil disturbance. Monitoring the microbial response to soil disturbance provides insights in understanding their effects and extent on nutrient cycling and organic matter turnover (Corstanje et al., 2007).

The Everglades Agricultural Area (EAA) is located south of Lake Okeechobee and north of the Water Conservation Areas in south Florida. It consists of an area of approximately 283,300 ha, which was artificially drained for agricultural production in the early 1900s. Long-term drainage has resulted in oxidation of these Histosols, resulting in a decreased depth to bedrock. The current estimate of soil loss is 1.5 cm y^{-1}, and many soils are less than 51 cm in depth, such as those classified as the Dania series (Shih et al., 1998; Snyder, 2005). Land use conversion and soil oxidation have contributed to nutrient export from agricultural fields into adjacent wetlands, resulting in sulfate (SO_4^{2-}) and phosphorus (P) enrichment. Sulfate export from the EAA into Everglades wetlands has been implicated in causing stimulation of Hg methylation (Gabriel et al., 2008). Long-term cultivation of these drained Histosols, specifically the use of tillage, coupled with soil oxidation, has also resulted in incorporation of bedrock calcium carbonate ($CaCO_3$) into surface soil and has gradually increased the pH from the historic 5.0 to 5.5 to approximately 7.0 to 7.5 today (Snyder, 2005; Gabriel et al., 2008). As a result, P and micronutrient availability to crops have decreased and necessitated new fertilizer management practices. Elemental S is occasionally applied as soil amendment for the purpose of reducing pH and therefore increasing P and micronutrient availability (Gabriel et al., 2008). The microbial oxidation of elemental S to SO_4^{2-} produces acidity, which in turn releases P bound to Ca minerals, which may result in a pulsed flux of P to soil solution. Thus, there is concern that widespread S application may stimulate nutrient export from these soils, which can harm downgradient wetlands. However, the buffering capacity of these calcareous Histosols is strong and may counteract the acidifying effects of S oxidation; thus, the effects of amendments may be only temporary and minimally effective (Beverly and Anderson, 1986; Ye et al., 2010).

Increased nutrient availability resulting from S amendment is likely to stimulate microbial activity and subsequently alter nutrient cycling and organic mater turnover (Wright and Reddy, 2001; Castillo and Wright, 2008). Because of the increases in pH and the decreasing depth to bedrock of soils in the EAA, the need for S amendments may increase in the future. Investigations of the responses of microbial functional activities to variable S application rates are necessary and would provide insight in understanding the response of this system to S application. These results can then be used to help formulate fertilizer and nutrient management solutions for better soil management in the EAA.

MATERIALS AND METHODS

Site Description

The experimental site is located in the central EAA on Dania muck (euic, hyperthermic, shallow Lithic Haplosaprist) with a depth to bedrock of approximately 50 cm. Mean annual temperature is 23°C, and precipitation is 131 cm. The experimental design was a randomized complete block with four S application rates and four field replications. Each field plot measured 9 × 13 m and consisted of six rows of sugarcane (*Saccharum officinarum* L.). Sugarcane cultivar CP 89-2143 was planted in November 2007 and harvested in February 2009. Elemental granular S (90%) was applied at rates of 0, 112, 224,

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and 448 kg S ha^{-1} to furrows and covered after planting. Other fertilization was provided using typical recommendations and guidelines for this region and soil type (Rice et al., 2006). All fertilizers were soil-applied before planting and included 17 kg N ha^{-1} and 37 kg P ha^{-1} as monoammonium phosphate, 228 kg K ha^{-1} as KCl, 8.5 kg Mn ha^{-1}, 4.5 kg Cu ha^{-1}, 5.6 kg Fe ha^{-1}, 2.8 kg Zn ha^{-1}, and 1.1 kg B ha^{-1}. All plots received common cultural practices including tillage and herbicide application. Water was applied as needed via seepage irrigation in field ditches approximately 182 m apart.

Soil Sampling and Analysis

Soil samples were collected from furrows before planting and fertilizer application and again in January 2008, May 2008, August 2008, and December 2008, corresponding to approximately 0, 2, 6, 9, and 13 months after planting, respectively. Twelve soil (0–15 cm) cores (2.54-cm diameter) were randomly collected within each field plot and composited, and samples were homogenized after the removal of visible plant residues and stored at 4°C.

Soil pH was measured using a soil-to-water ratio of 1:3 after equilibration for 30 min. Total organic C was measured by loss-on-ignition at 550°C for 4 h after conversion to organic C with a coefficient factor of 0.51 (Wright et al., 2008). Dissolved organic C was extracted with 0.5 *M* K_2SO_4 and analyzed with a TOC-5050A total organic C analyzer (Shimadzu, Norcross, GA). Total N was measured by Kjeldahl digestion followed by NH_4^+ analysis (Wright and Inglett, 2009). Extractable NH_4-N and NO_3-N were determined by extraction with 2 *M* KCl followed by colorimetric analysis (Castillo and Wright, 2008). Total P was determined using the ascorbic acid–molybdenum blue method after Kjeldahl digestion, and labile inorganic P measured after Mehlich−1 extraction (Wright, 2009).

Microbial biomass C and N were measured by the fumigation-extraction method using a conversion factor of 0.37 and 0.54, respectively (Wright et al., 2009). The microbial biomass P was determined as the difference in total P of sodium bicarbonate ($NaHCO_3$) extracts between fumigated and unfumigated samples (Ye et al., 2009). Potentially mineralizable N and P were determined based on a 10-day incubation followed by extraction with 2 *M* KCl (for N) and 1 *M* HCl (for P) and subtraction of initial N and P concentrations (Ye and Wright, 2010). Mineralized S was calculated as the difference in water-extractable SO_4^{2-} between residual soil and after a 10-day incubation, with SO_4^{2-} being analyzed by ion chromatography (Gharmakher et al., 2009). Microbial respiratory activities were measured as CO_2 production after 5- and 10-day incubation (Corstanje et al., 2007) and expressed as the slope of the regression of cumulative CO_2 production over the 10-day incubation period (Wright and Reddy, 2008).

In consideration of the potential acidification effect induced by S oxidation and to resemble real soil pH variations between treatments, all enzyme activities were assayed with nonbuffered solutions. Approximately, 1 g of moist soil was placed in polypropylene centrifuge tubes, mixed with 30 mL of sterile water, and shaken for 25 min. Homogenized samples were further diluted five times for enzyme assays. Enzyme assays were conducted using six replicates with controls to offset nonenzymatic production. Phosphatase, glucosidase, and leucine aminopeptidase assays were conducted in 96-well microtiter plates. The enzyme substrates were 1 m*M* 4-MUF-phosphate (Sigma, St Louis, MO), 1 m*M* 4-MUF-β-D-glucopyranoside (Sigma), and 2.5 m*M* L-leucine 7-amino-4-methylcoumarin (Biosynth, Naperville, IL), respectively. Two hundred microliters of sample was incubated with 50-μL substrates at 20°C for 3 h. The florescence readings were collected at 0.5-h intervals using a Bio-TEK FL600 fluorescence plate reader (Bio-TEK Instruments Inc., Winooski, VT) at a setting of 365-ηm excitation and 450-ηm emission. Enzyme activity was determined by calculating the mean florescent reading changes over time. Arylsulfatase activity was determined colorimetrically as described by Tabatabai and Bremner (1970).

Statistical Analysis

A mixed model was fit using restricted maximum likelihood in the MIXED procedure of SAS (Littell et al., 2006). The fixed effects were S application rate, time, and their interaction, with block as a random effect. Degrees of freedom were adjusted using the Kenward-Roger adjustment. An exponential covariance structure was used to model the correlation among observations taken from the same plot over time. Significant differences among individual treatments and time intervals were analyzed with Tukey test at $\alpha = 0.05$. Pearson correlation was used to evaluate relationships between variables. All statistical analyses were carried out with SAS 9.1 (SAS Institute, Cary, NC).

RESULTS

Soil Physical and Chemical Properties

Application of S at a range from 0 to 448 kg S ha^{-1} did not affect soil pH; extractable NH_4-N, NO_3-N, and SO_4^{2-}; and dissolved organic C (Tables 1 and 2). Averaged across treatment effect, dissolved organic C increased significantly from 2 (1,346 mg kg^{-1}) to 6 months (1,572 mg kg^{-1}) and then decreased toward the end of the season (Table 2). Inversely, extractable NO_3-N decreased from 383 mg kg^{-1} at 2 months to 16 mg kg^{-1} at 6 months, but then increased to 49 mg kg^{-1} at 13 months. Extractable NH_4-N and SO_4^{2-} both decreased gradually throughout the growing season. Sulfur application significantly affected the concentration of labile P, which, however, greatly depended on time (Table 1). At 2 months after

TABLE 1. Two-Way Analysis of Variance on Selected Chemical and Microbial Variables in Soils Amended With Various S Application Rates

	P		
Variable	**Treatment**	**Time**	**Interaction**
pH	0.14	<0.0001	0.58
Dissolved organic C	0.43	<0.0001	0.76
Extractable NH_4-N	0.61	<0.0001	0.49
Extractable NO_3-N	0.88	<0.0001	0.99
Extractable SO_4^{2-}	0.09	<0.0001	0.94
Labile P	0.19	0.16	0.03
Microbial biomass C	0.45	<0.0001	0.36
Microbial biomass N	0.92	0.10	0.99
Microbial biomass P	<0.0001	<0.0001	<0.0001
Potentially mineralizable C	0.41	<0.0001	0.47
Potentially mineralizable N	0.62	<0.0001	0.36
Potentially mineralizable P	0.79	0.39	0.80
Potentially mineralizable S	<0.0001	<0.0001	0.48
Phosphatase	0.04	0.0002	0.21
Glucosidase	0.02	<0.0001	0.16
Leucine aminopeptidase	0.19	0.03	0.99
Sulfatase	0.70	<0.001	0.76

TABLE 2. Extractable Nutrients (mg kg^{-1}) and pH in Soil Amended With Elemental S During the Sugarcane Growing Season

	Dissolved Organic C	Extractable NH_4-N	Extractable NO_3-N	Extractable SO_4^{2-}	pH
Treatment, kg S ha^{-1}					
0	1,315 (58)[a]*	12 (2)[a]	110 (41)[a]	107 (40)[b]	6.2 (0.1)[a]
112	1,583 (93)[a]	10 (1)[a]	115 (39)[a]	145 (40)[a]	6.5 (0.1)[a]
224	1,419 (56)[a]	10 (1)[a]	120 (42)[a]	141 (37)[a]	6.3 (0.1)[a]
448	1,381 (45)[a]	12 (3)[a]	121 (42)[a]	179 (42)[a]	6.1 (0.1)[a]
Time, mo					
2	1,346 (47)[c]	19 (3)[a]	383 (19)[a]	376 (32)[a]	6.0 (0.1)[c]
6	1,572 (67)[a]	10 (0)[a]	16 (1)[c]	129 (13)[b]	6.3 (0.0)[b]
9	1,436 (80)[b]	9 (1)[a]	19 (1)[c]	21 (3)[c]	6.4 (0.1)[ab]
13	1,344 (66)[bc]	6 (0)[b]	49 (2)[b]	47 (7)[c]	6.4 (0.1)[a]

Values denote means and the S.E. is in parentheses.

*Superscript letters represent significant differences ($\alpha = 0.05$).

application, labile P was substantially higher in soils amended with 448 kg S ha^{-1} (118 mg kg^{-1}) than soils receiving lower S rates (49 mg kg^{-1}) (Fig. 1). However, the higher P concentrations were not observed at later months.

Extracellular Enzyme Activities

The S application effect on phosphatase activity was significant (Table 1; Fig. 2A). Phosphatase activity at 2 months was considerably higher for soils receiving 448 kg S ha^{-1} (131 mg MUF kg^{-1} h^{-1}) than soils receiving 0, 112, and 224 kg S ha^{-1}, which averaged 61, 76, and 81 mg MUF kg^{-1} h^{-1}, respectively. However, no sulfur application effects were observed thereafter. Phosphatase activity fluctuated seasonally, with the lowest activity observed at 6 months, and the highest activity at 9 months within each treatment level. Glucosidase activity significantly increased at 2 months, averaging 15, 56, 58, and 99 mg MUF kg^{-1} h^{-1} for the increasing S application rates (Fig. 2B). Such difference did not appear at the other months. Leucine aminopeptidase activity was not affected by any S rate, averaging 68 mg MUF kg^{-1} h^{-1} (Table 1; Fig. 3A). Nonetheless, it decreased significantly toward the end of the sugarcane growing season. Sulfatase activity did not respond to S application at any rate (Table 1; Fig. 3B) and was largely unaffected by seasonality.

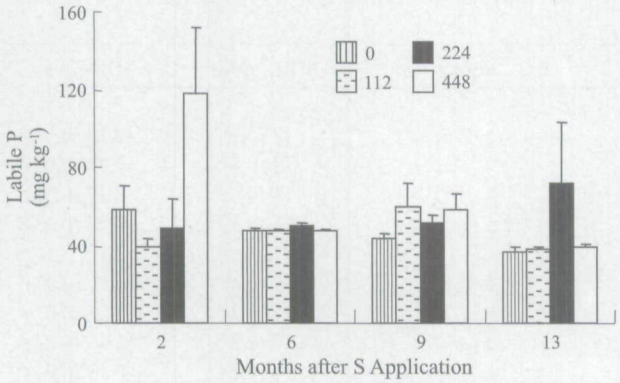

FIG. 1. Concentrations of labile P in soils at 2, 6, 9, and 13 months after elemental S application at different rates (0, 112, 224, and 448 S kg ha^{-1}). Error bars represent the S.E.M.

Microbial Biomass

Microbial biomass C was not altered as a result of S amendment, but did fluctuate during the growing season (Tables 1 and 3). The highest concentration occurred at 9 months (17 g kg^{-1}), followed by 2 months (13 g kg^{-1}) and 6 and 13 months (11 g kg^{-1}). Similarly, the size of the microbial biomass N pool did not change after S amendment and was stable throughout the growing season. Microbial biomass P increased significantly at 2 months at the highest S rate (177 mg kg^{-1}), which was about three times higher than for lower S application rates (Fig. 4). However, the stimulating effect did not extend beyond 2 months.

Microbial-Mediated Mineralization

Aerobic CO_2 production rates did not differ between soils receiving variable S application rates (Tables 1 and 3). The

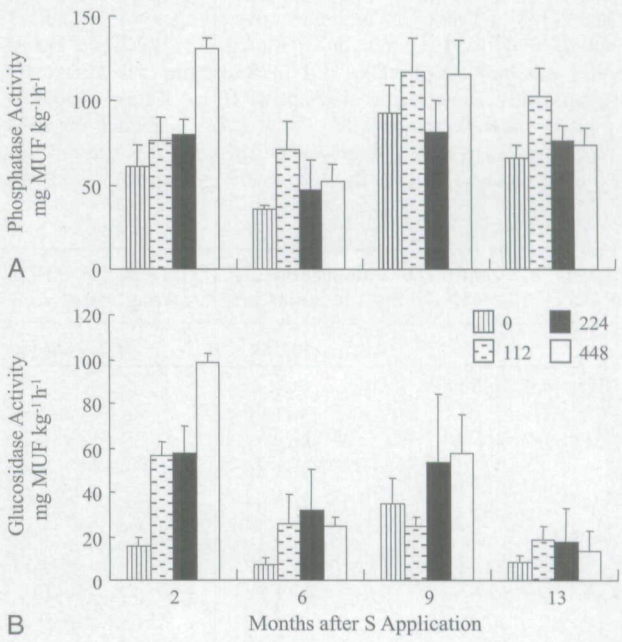

FIG. 2. Activities of phosphatase (A) and glucosidase (B) in response to different elemental S application (0, 112, 224, and 448 kg S ha^{-1}) throughout the sugarcane growing season. Error bars represent the S.E.M.

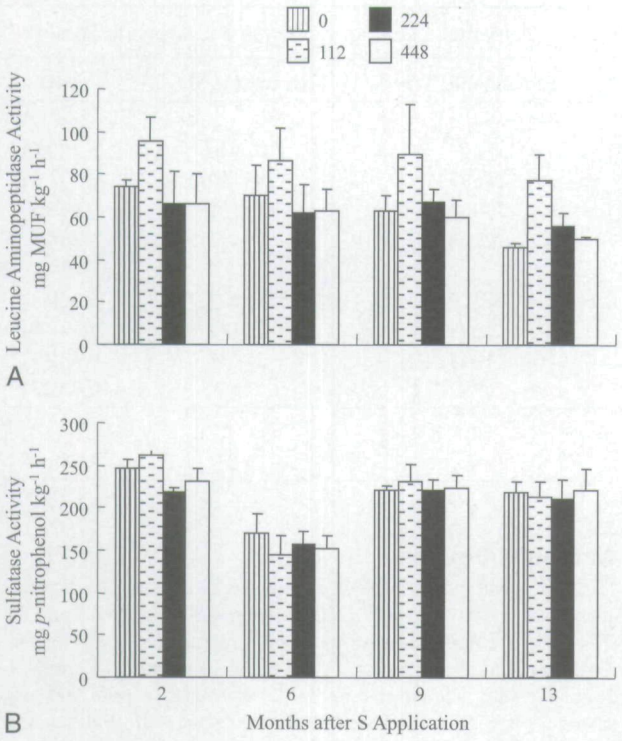

FIG. 3. Activities of leucine aminopeptidase (A) and sulfatase (B) in response to different elemental S application rates (0, 112, 224, and 448 kg S ha^{-1}) throughout the sugarcane growing season. Error bars represent the S.E.M.

production was the highest at 9 months after S application (44 mg CO_2-C kg^{-1} d^{-1}) and lowest at 2 months (26 mg CO_2-C kg^{-1} d^{-1}), corresponding to temperature patterns. Similarly, N and P mineralization rates were not influenced by S amendment (Tables 1 and 3). The highest overall rates of N mineralization were found at 9 months (10 mg kg^{-1} d^{-1}), and the lowest rates at 13 months (2 mg kg^{-1} d^{-1}). Sulfur mineralization rates significantly increased as a result of S application (Table 1; Fig. 5). Overall, mineralized S was 1,421% greater for soils receiving 448 kg S ha^{-1} than unamended soil, 375% greater than soils receiving 112 kg S ha^{-1}, and 219% greater for soils receiving 224 kg S ha^{-1}. Nonetheless, mineralized S rates significantly decreased throughout the growing season.

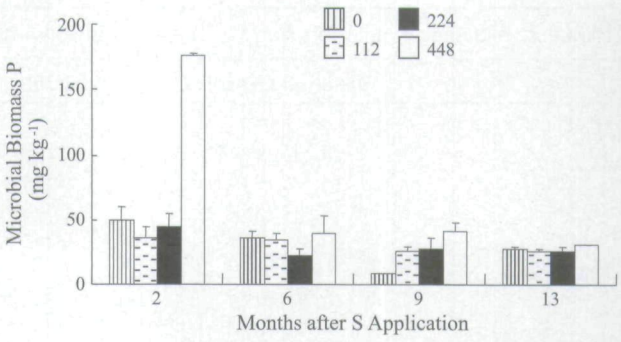

FIG. 4. Microbial biomass P in soils at 2, 6, 9, and 13 months after elemental S application at different rates (0, 112, 224, and 448 S kg ha^{-1}). Error bars represent the S.E.M.

DISCUSSION

Labile P is considered the most bioavailable form of P in soils. Sulfur application at 448 kg S ha^{-1} significantly increased concentrations of labile P at 2 months (Fig. 1), suggesting increased P availability to sugarcane as well as soil microorganisms (Codling, 2008). There are two primary mechanisms by which S influences P availability: lowering of soil pH (Gabriel et al., 2008) and replacement of PO_4 with SO_4^{2-} and the release of P from association with Ca (Jaggi et al., 2005). These results are supported by the fact that labile P was significantly correlated with pH ($R = -0.35$, $P = 0.005$) and extractable SO_4^{2-} ($R = 0.29$, $P = 0.019$). However, increased P availability was not observed at later months, indicating limited long-term effects of S on the reduction in soil pH due to the high buffering capacity of this calcareous organic soil (Jaggi et al., 2005; Snyder, 2005).

Enzymatic Activities

Extracellular enzymes are excreted by the microorganisms to the soil for the purpose of sequestering nutrients. The enzyme activities, especially hydrolases, are known to be involved in

TABLE 3. Potentially Mineralizable C (C_{min}), N (N_{min}), P (P_{min}), and Microbial Biomass C (MBC) and N (MBN) in Soil Amended With Elemental S During the Sugarcane Growing Season

	C_{min}, mg kg^{-1} d^{-1}	N_{min}, mg kg^{-1} d^{-1}	P_{min}, mg kg^{-1} d^{-1}	MBC, g kg^{-1}	MBN, g kg^{-1}
Treatment, kg S ha^{-1}					
0	34 (1.9)[a]*	6 (0.8)[a]	6 (3.6)[a]	12 (0.9)[a]	0.28 (0.03)[a]
112	39 (2.7)[a]	8 (1.1)[a]	4 (1.5)[a]	13 (0.8)[a]	0.27 (0.03)[a]
224	38 (3.4)[a]	7 (1.0)[a]	5 (2.3)[a]	13 (0.6)[a]	0.27 (0.03)[a]
448	32 (2.0)[a]	6 (0.9)[a]	2 (1.2)[a]	13 (0.8)[a]	0.29 (0.03)[a]
Time, mo					
2	26 (1.3)[c]	8 (1.1)[b]	6 (2.8)[a]	13 (0.4)[b]	0.27 (0.01)[a]
6	36 (1.8)[b]	6 (0.3)[b]	6 (2.7)[a]	11 (0.9)[c]	0.27 (0.02)[a]
9	44 (3.3)[a]	10 (0.3)[a]	3 (2.3)[a]	17 (0.2)[a]	0.35 (0.04)[a]
13	37 (1.2)[b]	2 (0.2)[c]	1 (0.6)[a]	11 (0.2)[c]	0.23 (0.01)[a]

Values denote means and the S.E. is in parentheses.

*Superscript letters represent significant differences ($\alpha = 0.05$).

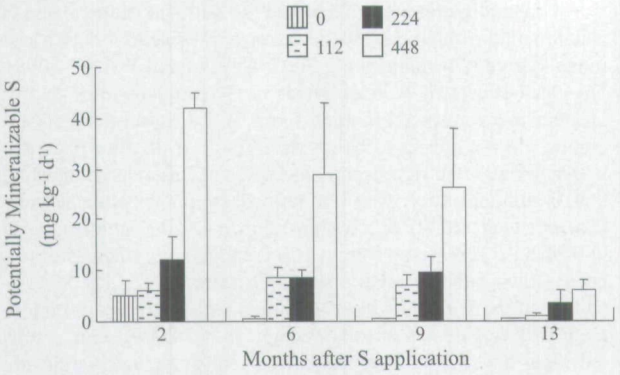

FIG. 5. Mineralized S in soils at 2, 6, 9, and 13 months after S application at different rates (0, 112, 224, and 448 S kg ha^{-1}). Error bars represent the S.E.M.

organic matter turnover and nutrient cycling in terrestrial systems (Wright and Reddy, 2001; Corstanje et al., 2007). Phosphatase catalyzes the hydrolysis of organic P ester, resulting in the release of P, and thus plays an important role in P regeneration from soils (Wright and Reddy, 2001). Glucosidase catalyzes the hydrolysis of glycosides, and its activity reflects the state of organic matter and processes occurring therein (Tejada et al., 2006). Our results showed that phosphatase activities were 115% higher for soils receiving the highest S rates than unamended soils at 2 months after S application, whereas glucosidase activities were 573% higher (Fig. 2). High enzyme activity may indicate nutrient limitation (Sinsabaugh et al., 1993; Allison et al., 2007), and the highest activity occurred during winter sampling when soil oxidation rates are typically the lowest (Snyder, 2005). Thus, the nutrient-supplying capacity of this soil was low at this time. In fact, soil oxidation typically provides a major portion of the sugarcane nutrient requirements for this soil (Rice et al., 2006). Negative correlations between nutrient availability and related enzyme activities have been demonstrated (Wright and Reddy, 2001; Allison and Vitousek, 2005). However, no such correlations were observed in the present study, but instead, both phosphatase and glucosidase activities were positively correlated to the concentrations of labile P (Table 4), which together may suggest the P limitation for organic matter turnover in these high C soils (Allison et al., 2007). The EAA soils are primarily Histosols with high organic matter content, approximately 85% by weight, which contain high N yet low P and micronutrient concentrations that require supplemental fertilization (Snyder, 2005; Castillo and Wright, 2008). The background total C–total N–total P molar ratio at this site was 1,274:98:1, indicating this soil was indeed P limited, which may constrain the activities and growth rates of microorganisms (Fontaine et al., 2003). As demonstrated previously, application of S increased the concentration of labile P at 2 months (Fig. 1), and both activities of phosphatase and glucosidase simultaneously increased (Fig. 2). It is plausible that application of S had a transitory effect on soil pH, which stimulated P release to the soil environment and hence enhanced the microbial enzymatic activities. Thus, application of S at the current recommended rate had a short-lived effect on microbial activity. The combination of soil buffering capacity and sugarcane nutrient uptake likely minimized P release and accumulation in soil that was initiated by S application. These results indicate that higher S rates may be needed to prolong the response of the soil microbial community and maintain nutrient availability throughout the growing season.

Leucine aminopeptidase and sulfatase were also assayed in this study to represent N and S cycles. Leucine aminopeptidase is involved in the degradation of proteins (Larson et al., 2002), whereas sulfatase hydrolyzes aromatic ester sulfates (Knauff

TABLE 4. Significant Correlation Coefficients ($P < 0.05$) Between Selected Chemical Properties and Microbial Functional Activities (n = 64)

	pH	DOC	NH_4^+	NO_3	P*i*	SO_4^{2-}	LAP	PHO	GLU	SUL	C_{min}	N_{min}	P_{min}	S_{min}	MBC	MBN	MBP
pH	1																
DOC	0.63*	1															
NH_4	−0.42	NS	1														
NO_3	−0.51	NS	0.61	1													
P*i*	−0.35	NS	0.60	0.28	1												
SO_4	−0.46	NS	0.60	0.88	0.29	1											
LAP	0.42	0.57	NS	NS	NS	NS	1										
PHO	NS	NS	NS	NS	0.28	NS	NS	1									
GLU	−0.28	NS	0.30	0.41	0.35	0.35	NS	0.64	1								
SUL	NS	−0.29	NS	0.41	NS	NS	NS	NS	NS	1							
C_{min}	0.52	0.50	−0.33	−0.51	NS	−0.45	NS	NS	NS	NS	1						
N_{min}	NS	NS	NS	NS	NS	NS	0.44	NS	NS	0.29	NS	1					
P_{min}	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	1				
S_{min}	−0.38	NS	0.31	NS	0.49	0.39	NS	0.28	0.41	NS	NS	NS	NS	1			
MBC	NS	NS	NS	NS	NS	NS	NS	0.34	NS	NS	NS	0.41	NS	NS	1		
MBN	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.36	NS	NS	NS	1	
MBP	−0.38	NS	0.57	0.49	0.63	0.47	NS	NS	0.35	NS	−0.37	NS	NS	0.61	NS	NS	1

*Significant at $P = 0.05$.

NS: not significant; DOC: dissolved organic C; NH_4: extractable NH_4-N; NO_3: extractable NO_3-N; P*i*: extractable labile P; SO_4^{2-}: extractable SO_4-S; MBC: microbial biomass C; MBN: microbial biomass N; MBP: microbial biomass P; LAP: leucine aminopeptidase; PHO: phosphatase; GLU: glucosidase; SUL: sulfatase; C_{min}: potentially mineralizable C; N_{min}: potentially mineralizable N; P_{min}: potentially mineralizable P; S_{min}: potentially mineralizable S.

et al., 2003). Both enzyme activities were not influenced by any rate of S application (Fig. 3). Because enzymes are biological catalysts capable of catalyzing specific chemical reactions, their responses may vary based on substrate quality and real microbial community composition (Corstanje et al., 2007). Soil enzyme activities may also depend on other parameters, such as seasonal variation in soil moisture, pH, and temperature (Knauff et al., 2003; Wallenstein et al., 2009), which may explain the lack of response to S amendment in this soil.

Microbial Biomass

Microbial biomass P was more sensitive to S addition than biomass C and N at 2 months, as biomass P for soils receiving 448 kg S ha^{-1} was 314% higher than soils receiving the lower rates (Fig. 4). Correlation analysis revealed that microbial biomass P was significantly correlated to labile P, total P, extractable SO_4, pH, NH_4-N, and NO_3-N (Table 4). Considering the fact that this Histosol is P limited, it was likely that increased labile P at 2 months after S application caused P immobilization into microbial biomass, which may explain the lower labile P concentrations at subsequent sampling times during the growing season. Increasing biomass P as a result of increased P availability in Everglades soils has been well documented (Corstanje et al., 2007; Castillo and Wright, 2008; Wright et al., 2009).

Microbial-Mediated Organic Matter Mineralization

Agricultural practices in EAA soils, such as tillage and P fertilization, showed variable effects on organic C mineralization (Morris et al., 2004). The present study indicates that S amendment did not appear to influence microbial aerobic respiration rates, indicating that S application will not further stimulate soil oxidation. Correspondingly, the metabolic coefficient (qCO_2), the proportion of aerobic respiration to microbial biomass C, did not change across S rates (Table 5), suggesting that application at rates up to 448 kg S ha^{-1} did not alter organic matter turnover. Glucosidase activity is known to reflect the state of organic matter cycling (Tejada et al., 2006). However, glucosidase activity was not correlated with C mineralization rates (Table 4). Glucosidase is one of several soil enzymes involved in C mineralization process, and hence its activities alone may not be able to reflect the overall real C mineralization, which may explain why no increased CO_2 production was observed at 2 months at 448 kg S ha^{-1} when glucosidase activity was significantly enhanced.

Potentially mineralizable N and the quotient (mineralized N as a function of biomass N) characterize the potential N turnover in the system (Corstanje et al., 2007; Castillo and Wright, 2008). No clear effects of S amendment on N turnover rates in this Histosol were observed (Tables 3 and 5). Likewise, net P regeneration did not appear to be impacted by S, even after phosphatase activities were significantly increased at 2 months, suggesting that P mineralization was not limited by phosphatase activity (Carreira et al., 2000). Studies have shown no correlation between phosphatase activity and gross P mineralization, whereas others have shown positive relationships (Carreira et al., 2000). Agriculture in the EAA has been identified as a source of P enrichment in Everglades wetlands (Wright et al., 2009). Our results indicated that S application under current guidelines would not enhance the microbial-mediated P regeneration in EAA soils and thus minimize the risk of P export from agricultural fields.

Potential S mineralization was the only mineralization process showing a clear response to S amendment. It has been proposed that S mineralization involved two processes, biological and biochemical mineralization, in which SO_4^{2-} is released as a by-product of C oxidation and as a product of enzymatic hydrolysis (McGill and Cole, 1981; Chen et al., 2001). Nonetheless, in the present study, mineralized S was not correlated with CO_2 production or sulfatase activity. Instead, S mineralization was highly correlated with glucosidase activity (Table 4), which may suggest that net mineralization of S was not simply dependent on either biological or biochemical processes (Eriksen et al., 1998). Oxidation of elemental S also contributed to the observed increased mineralized S rates with increasing S application rate. Elemental S is oxidized by soil microorganisms producing SO_4^{2-}, which can accumulate in soil solution. The elemental S oxidation rate may be slow (DeLuca et al., 1989), and SO_4^{2-} accumulation in this study is likely a result of both organic S mineralization and elemental S oxidation to SO_4^{2-}. Our results demonstrated that mineralized S increased concurrently with higher S application rates, and the effects continued throughout the growing season, although stimulatory effects diminished with subsequent sampling times (Fig. 5).

Seasonal Fluctuations in Microbial Indices

Seasonal variations in soil microbial activities are common (Wallenstein et al., 2009) and dependent on environmental

TABLE 5. Microbial Metabolic Coefficient (qCO_2), Microbial Biomass C to Organic Matter Content Ratio (MBC/OM), and Potential N and P Mineralization Quotient (qPMN and qPMP) in Soils Amended With Elemental S During the Sugarcane Growing Season

	qCO_2, ×100	MBC/OM, %	qPMN, mg N g^{-1} MBN	qPMP, mg P g^{-1} MBP
Treatment, kg S ha^{-1}				
0	0.31 (0.04)[a]*	1.5 (0.1)[a]	22 (3.3)[a]	192 (130)[a]
112	0.31 (0.04)[a]	1.7 (0.1)[a]	28 (4.1)[a]	136 (54)[a]
224	0.29 (0.02)[a]	1.6 (0.1)[a]	26 (4.4)[a]	153 (71)[a]
448	0.28 (0.04)[a]	1.6 (0.1)[a]	23 (5.0)[a]	42 (22)[a]
Time, mo				
2	0.20 (0.01)[c]	1.6 (0.1)[b]	31 (4.0)[a]	160 (59)[a]
6	0.39 (0.05)[a]	1.3 (0.1)[c]	24 (2.3)[a]	212 (121)[a]
9	0.27 (0.02)[b]	2.1 (0.0)[a]	36 (4.8)[a]	109 (72)[a]
13	0.33 (0.01)[a]	1.4 (0.0)[c]	8 (0.9)[b]	45 (24)[a]

Values denote means and the S.E. is in parentheses.

*Superscript letters represent significant differences ($\alpha = 0.05$).

factors such as rainfall and temperature. Soil disturbance resulting from agricultural practices is also known to pose impacts on microbial community composition and activity (Knauff et al., 2003; Morris et al., 2004; Wright and Reddy, 2008). During the growing season, tillage was applied to improve drainage and weed control, which has been found to affect the size of the microbial biomass pool and organic matter mineralization rates (Morris et al., 2004; Castillo and Wright, 2008). Our results showed clear seasonal fluctuations for most of the microbial parameters, suggesting the net results of interactions between environmental factors, soil management, and the sugarcane growing season. Most effects occurred within 2 months of S application, suggesting a strong effect of fertilization for this site. Nutrient concentrations decreased during the growing season because of sugarcane uptake; thus, nutrient limitations may have minimized microbial responses to S application at subsequent sampling times. In fact, these soils are often low in plant-available nutrients with the exception of N (Rice et al., 2006).

CONCLUSIONS

Application of elemental S at 448 kg S ha^{-1} increased P availability at 2 months, which subsequently stimulated some enzyme activities and simultaneously promoted labile P to be immobilized in microbial biomass. However, these effects were temporary and not observed beyond 2 months. There was limited effect of S application on increasing the P availability due to the high buffering capacity of this organic soil against pH reduction. Overall, S amendment at rates up to 448 kg S ha^{-1} did not appear to pose significant impacts on organic matter turnover and N and P regeneration rates, suggesting that S application will not stimulate soil oxidation and result in large-scale nutrient flux from soil. Using the current recommended S application guidelines and rates, impacts on microbial activities and functions should be minimal. However, because of the increasing pH trend for these soils, there may be a need for higher S application rates in the future. These higher S rates may overcome the soil's buffering capacity and release more P and micronutrients and increase their availability to crops, potentially stimulating microbial functional activities and altering organic matter dynamics.

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