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Multivariate analysis of chemical and microbial properties in histosols as influenced by land-use types

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ABSTRACT

The histosols of the Everglades agricultural area in South Florida, USA, were drained in early 1900s and converted from wetlands to agricultural use, which subsequently increased soil oxidation and altered soil properties. The objectives of this study were to determine land-use effects on integrated soil chemical properties and how their discriminations regulate microbial community composition and function using multivariate analytical methods. Soil was collected from sugarcane, cypress, and uncultivated sites. Cluster analysis (CA) and discriminant analysis (DA) were applied to determine differences in soil chemistry and microbial community structure and function, while principal components analysis (PCA) was used to reduce variables. Canonical correlation analysis (CCA) evaluated dependent relationships between soil chemical and microbial parameters. Soils under different land-uses were perfectly clustered into their own groups, which was distinguished by labile inorganic P and total P. Discriminations on integrated soil microbial characteristics were significant. Microbial biomass C and N, community-level physiological profile components, and potentially mineralizable N contributed most to such differentiations. Canonical correlations between soil chemical and microbial indexes were significant on both canonical variates ($R_1 = 0.91$, $p = 0.0006$; $R_2 = 0.65$, $p = 0.03$). Cumulatively, 63% of the variances in microbial indices were explained by chemical canonical variates. Agricultural management, especially historic P fertilization, altered soil nutrient availability and consequently modified the microbial community composition and function. Future land-use changes and management should consider the role of labile P on the functioning of microbial communities and their control of nutrient cycling since this parameter had the most influence on changing soil properties.

1. Introduction

Univariate data analysis is essential in many experiments, but it is considered appropriate when just one variable is measured for several samples (Sena et al., 2002). To better understand whole soil ecosystem processes, various properties are systematically collected and multivariate analytical methods are employed, which allows analysis of multiple variables simultaneously while interpreting results with better summarized information (Sena et al., 2002). Although considered underutilized (Sena et al., 2000), multivariate methods have been well recognized and commonly applied in soil research. For instance, principal component analysis (PCA) has been performed to investigate management impacts on soil quality (Wander and Bollero, 1999) and microbial community structure and function (Grayston et al., 2004; Bossio et al., 2005; Allison et al., 2007; Cookson et al., 2007). Recently, application of canonical correlation analysis (CCA) and discriminant analysis (DA) has also been reported for soils research (Zhang et al., 2006; Banning and Murphy, 2008; Sanchez-Moreno et al., 2008).

Canonical correlation analysis is a method used to assess the dependent relationships between two data sets. The method is designed to find linear combinations of variables in one data set that account for the most variation in a linear combination of variables for the other data set (Lattin et al., 2003). In this way, much of the relationship between two data sets is detected and visualized. A potential application for CCA in soil research is the identification of the relationship between soil chemical properties and microbial community structure and function. Soil supports diverse microbial populations and, in natural systems, microbial activity is essentially related to the efficiency of nutrient cycling and organic matter turnover (Wright et al., 2009). Yet, the richness, abundance, and activity of microbial communities are influenced by chemical properties such as organic matter content and nutrient availability (Rutigliano et al., 2004; Ye et al., 2009). Alterations in the chemical conditions of soils may lead to shifts in microbial community composition and changes in microbial function, which

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is frequently observed upon change in land-uses (Nogueira et al., 2006; Cookson et al., 2007; Castillo and Wright, 2008a).

The Everglades agricultural area (EAA) in South Florida was drained in the early 1900s and converted from wetlands to sugarcane and vegetable cropping. Soils of the EAA are primarily histosols with high organic matter content, and contain high N yet low P and micronutrient concentrations that require supplemental fertilization (Snyder, 2005; Ye et al., 2010). These soils have undergone subsidence since they were drained at rates currently at 1.5 cm/year (Shih et al., 1998). This has decreased the soil depth to bedrock to the point where major changes in soil chemical properties, such as pH, are becoming problematic and increasingly restrictive for agricultural use. Sugarcane is the major land-use in the EAA and requires approximately 30 kg P ha^{-1} $year^{-1}$ and extensive tillage (Rice et al., 2006). Its long-term cultivation typically changes soil chemical properties and microbial community structure and function (Grayston et al., 2004; Cookson et al., 2007; Castillo and Wright, 2008a,b). Due to economic factors, soil subsidence, and water management concerns, current land-use patterns indicate a shift from sugarcane cropping back to the historic seasonally-flooded wetland prairie ecosystem, tree islands, or pastures (Snyder, 2005).

The purpose of this study was to evaluate land-use effects on soil biochemical processes using multivariate analytical methods. Land-uses under sugarcane cropping and cypress were compared with uncultivated land. Application of multivariate methods was performed to answer the following questions: (1) whether land-uses distinguished by integrated soil chemical properties and microbial parameters? (2) which variables contributed most for those differentiations? (3) and whether discriminations in microbial parameters were dependent on soil chemical properties?

2. Materials and methods

2.1. Site description

The study sites are located in the northern EAA near Belle Glade, FL (26°9′N, 80°38′W). All soils are dania muck (euic, hyperthermic, shallow lithic medisaprists) with depths to the bedrock of approximately 45 cm. Three land-uses with different management history were selected for this study: soils under forest for 21 years, soils under sugarcane production for approximately 50 years, and reference soils that have never been cultivated since drainage. The forest soils were previously cropped to sugarcane but planted to bald cypress (*Taxodium distichum*) and pond cypress (*Taxodium ascendens*) in 1988. These did not receive any fertilization after land-use change, but were tilled prior to seedling establishment with no further management applied. The sugarcane soils were managed for vegetable production from the early 1900s to the 1950s, but mainly for sugarcane since the 1960s. Phosphorus fertilization is commonly applied at 30 kg ha^{-1} $year^{-1}$ prior to planting (Rice et al., 2006). Tillage operations included several diskings (to 15 cm depth) after crop harvest, subsoil chiseling (to 30 cm depth) to improve drainage, and frequent in-season tine cultivations (to 4 cm depth) for weed control (Morris et al., 2004). The uncultivated soils were primarily occupied by paragrass [*Panicum purpurascens* (L.) Raddi] and bermudagrass [*Cynodon dactylon* (L.) Pers] and mowed periodically with residues returned to soil, and received no fertilization and tillage.

2.2. Soil chemical properties

Surface soil (0–15 cm) samples were collected from four replicate sites of each land-use in March 2007. The soils were homogenized after the removal of visible plant particles and stored at 4 °C. Soil organic matter content was measured by the loss-on-ignition method after ashing at 550 °C for 4 h (Wright et al., 2008). Total C, total N, and total P were determined using the oven dried (70 °C) soil. Total C and N were measured with a Carlo-Erba NA 1500 CNS Analyzer (Haak-Buchler Instruments, Saddlebrook, NJ) while total P was measured after ashing (Bremner, 1996) using the ascorbic acid–molybdenum blue method (Kuo, 1996) with an AQ2+ discrete analyzer (Seal Analytical Inc., Mequon, WI). Dissolved organic C (DOC) and labile inorganic N (LIN) was determined by extraction with 0.5 M K_2SO_4 followed by total C determination (Shimadzu TOC-5050A total organic carbon analyzer) and colorimetric analysis of NH_4 (Castillo and Wright, 2008b). After digestion, the K_2SO_4 extracts were measured for total Kjeldahl N (TKN) using an AQ2+ discrete analyzer (Seal Analytical Inc., Mequon, WI). Labile organic N (LON) was calculated from the difference between TKN and LIN (Castillo and Wright, 2008b). Labile inorganic P (LIP) was analyzed as described previously after $NaHCO_3$ extraction (Kuo, 1996). The $NaHCO_3$ extracts were measured for total P after Kjeldahl digestion (Castillo and Wright, 2008b), with labile organic P (LOP) being difference between total extractable P and LIP. Soil chemical properties are listed in Table 1.

Table 1
Chemical properties of cypress, sugarcane, and uncultivated soils (0–15 cm) with standard error values, $n = 4$.

	Unit	Cypress	Sugarcane	Uncultivated
Loss-on-ignition	%	83 (3)	81 (2)	85 (1)
Total C	g kg^{-1}	449 (3)	445 (2)	461 (2)
Total N	g kg^{-1}	30 (0.5)	29 (0.3)	32 (0.3)
Total P	g kg^{-1}	1.3 (0.07)	1.0 (0.01)	0.8 (0.01)
Dissolved organic C	g kg^{-1}	1.1 (0.1)	2.3 (0.1)	1.1 (0.2)
Labile organic N	mg kg^{-1}	141 (11)	197 (8)	132 (16)
Labile inorganic N	mg kg^{-1}	12 (1)	10 (1)	42 (5)
Labile organic P	mg kg^{-1}	79 (5)	69 (4)	80 (4)
Labile inorganic P	mg kg^{-1}	43 (9)	96 (4)	17 (3)

2.3. Soil microbial parameters

2.3.1. Microbial biomass

Microbial biomass C (MBC), biomass N (MBN) and biomass P (MBP) were measured by the fumigation-extraction method (White and Reddy, 2001). The amount of K_2SO_4-extracted C was determined with a Shimadzu TOC-5050A total organic carbon analyzer. The MBC was calculated as the difference in extractable C between fumigated and unfumigated samples using a conversion factor of 0.37. The MBN was calculated as the difference in total N between fumigated and unfumigated samples using $k = 0.54$. The MBP was determined as the difference in total P of $NaHCO_3$ extracts between fumigated and unfumigated soil. The P content of the $NaHCO_3$ extracts was measured as previously described (Kuo, 1996).

2.3.2. Mineralization potentials

Potentially mineralizable N (PMN) was determined according to methods of White and Reddy (2000) based on a 10 d incubation followed by extraction with 2 M KCl and analysis of NH_4. Potentially mineralizable P (PMP) was measured using the method of Corstanje et al. (2007) with slight modifications. Soil (0.5 g) was placed in 30 mL serum bottles and mixed with 5 mL double distilled water. The bottles were then capped and incubated in the dark at 40 °C for 10 d. At 10 d, 20 mL of 1 M HCl was injected and samples were shaken for 3 h. Extracts were then filtered through 0.45 μm membrane filters. Another set of equivalent weight samples, without incubation, were directly extracted with 25 mL of 1 M HCl and extracts analyzed for P. The PMP was determined as the difference in P concentration between extracts from incubated and non-incubated soil.

2.3.3. Enzyme activity assay

Four enzyme activities were measured in this study, including glucosidase in the C cycle, leucine aminopeptidase in the N cycle, alkaline phosphatase in the P cycle and arylsulfatase in the S cycle. Approximately 1 g moist soil was placed in polypropylene centrifuge tubes, mixed with 30 mL of water, and shaken for 25 min. Homogenized samples were further diluted 5 times for enzyme assays, which were conducted in triplicate with controls to assess non-enzymatic production. Leucine aminopeptidase assay was conducted in 96-well microtiter plates (Prenger and Reddy, 2004) with 200 μL samples incubated with 50 μL of 5 mM L-leucine 7-amino-4-methylcoumarin (Biosynth, Naperville, IL) at 20 °C for 8 h. The fluorescence readings were collected at 1 h intervals using a Bio-TEK FL600 fluorescence plate reader (Bio-TEK Instruments Inc., Winooski, VT) at a setting of 365 μm excitation and 450 μm emission. Enzyme activity was determined by calculating the mean fluorescent reading changes over time with a standard curve and expressed as mg 7-amino-4-methylcoumarin released g^{-1} soil h^{-1}. Alkaline phosphatase and sulfatase assays were conducted according to Wright and Reddy (2001).

2.3.4. Community-level physiological profile by BIOLOG assay

Community-level physiological profiles (CLPPs) were determined by direct incubation of fresh soil extracts in BIOLOG Eco-Plates (31 substrates) (BIOLOG Inc.). Approximately 1 g moist soil was mixed with 20 mL of water and gently shaken for 20 min. The homogenized samples were then diluted 400 times and soil particles allowed settling for 15 min at 4 °C. 150 μL of the supernatant was subsequently dispensed into each well of Eco-Plates and incubated at 20 °C for 7 d. Optical densities were measured every 6 h or 12 h using Bio-TEK FL600 (Bio-TEK Instruments Inc., Winooski, VT) at 590 μm. Absorbance values of each well with C sources were blanked against control wells before analysis. Negative values were considered as zero. Average well color development (AWCD) was determined as described by Garland (1996,1997). To overcome possible interference by inoculum density on color development, absorbance values for various C sources were standardized by dividing the blanked value of each well by the AWCD of the plate and were subsequently used for PCA.

2.4. Data analysis

To classify soils by integrated chemical properties, data were standardized to zero mean and unit variance and subject to cluster analysis (CA) according to K-means partitioning method, which involves classifying objects into groups so that objects within each group are relatively similar, while objects in different groups are relatively dissimilar (Lattin et al., 2003). The importance of a given variable X in defining the differences among clusters was justified by the ratio of between-cluster sum of squares of X to the total sum of squares of X (R^2) and the ratio of between-cluster sum of squares of X to within-cluster sum of squares of X [$R^2/(1-R^2)$]. High ratios indicate high importance. Microbial data were analyzed with DA to determine differences among land-uses, and stepwise variable selection was conducted to identify the important variables in discriminating land-uses. A p value of 0.1 and 0.05 was used as the entry and staying values, respectively, in the stepwise selection method. Due to the existence of missing data, MBP and PMP were not eligible for DA and hence excluded from the analysis. Principal components analysis was used to reduce the numbers of chemical and microbial variables by extracting the most important principal components separately. Canonical correlation analysis was then carried out to investigate the dependent relationship between extracted chemical and microbial principal components. The significant level was set at $\alpha = 0.05$. Application of PCA and DA was conducted with JMP 7 (SAS Institute Inc., NC), while CA and CCA were performed with SAS 9.1 (SAS Institute Inc., NC).

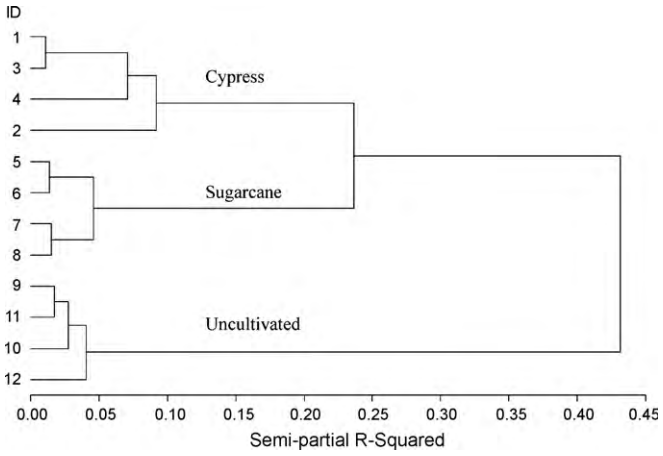

Fig. 1. Dendrogram from K-means cluster method applied to soil chemical data.

3. Results

3.1. Land-use effects on integrated soil chemical properties

The K-means procedure suggested a three group clustering as the best clustering scheme for these land-uses (Fig. 1). The dendrogram clearly displayed that cluster 1 contained only soils from cypress, cluster 2 included soils solely under sugarcane production, and cluster 3 exclusively was comprised of uncultivated soils. The cluster of sugarcane soil was closer to cypress than to uncultivated soil. Analysis of the variances revealed that LIP, LIN, DOC, and total P were the most important parameters defining the differences among clusters (Table 2). Additionally, the plot of cluster means across all chemical variables demonstrated that sugarcane, cypress, and uncultivated soils were highly distinguished by total P and LIP (Fig. 2).

Variable reduction was made by PCA while retaining as much as possible the original variances. The analysis extracted two principal components, C-Prin1 and C-Prin2, representing 54% and 19% of the original variances, respectively. Score plots showed that sugarcane, cypress, and uncultivated soils were distributed separately along ordination axis Prin1, while on axis Prin2 cypress was separated from sugarcane and uncultivated (Fig. 3). Variables with significant loadings on Prin1 were total C ($R^2 = 0.80$), total N ($R^2 = 0.84$), DOC ($R^2 = -0.81$), LIN ($R^2 = 0.81$), LON ($R^2 = -0.75$), and LIP ($R^2 = -0.95$), while total P had high negative loadings on Prin2 ($R^2 = -0.74$) (Fig. 4).

Table 2
The ratio of between-cluster sum of square to total sum of square (R^2) and the ratio of between-cluster sum of square to within-cluster sum of square ($R^2/(1-R^2)$) for chemical variables in defining differences among clusters, $n = 12$.

	R^2	$R^2/(1-R^2)$
Loss-on-ignition	0.21	0.26
Total C	0.72	2.60
Total N	0.63	1.72
Total P	0.86	6.10
Dissolved organic C	0.86	6.30
Labile organic N	0.65	1.83
Labile inorganic N	0.87	6.49
Labile organic P	0.30	0.43
Labile inorganic P	0.91	10.43

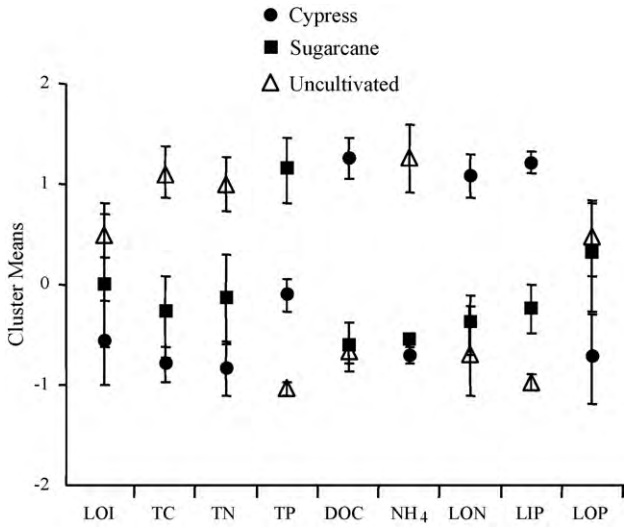

Fig. 2. Cluster means across chemical variables. LOI, loss-on-ignition; TC, total C; TN, total N; TP, total P; DOC, dissolved organic C; NH_4, labile inorganic N; LON, labile organic N; LIP, labile inorganic P; LOP, labile organic P.

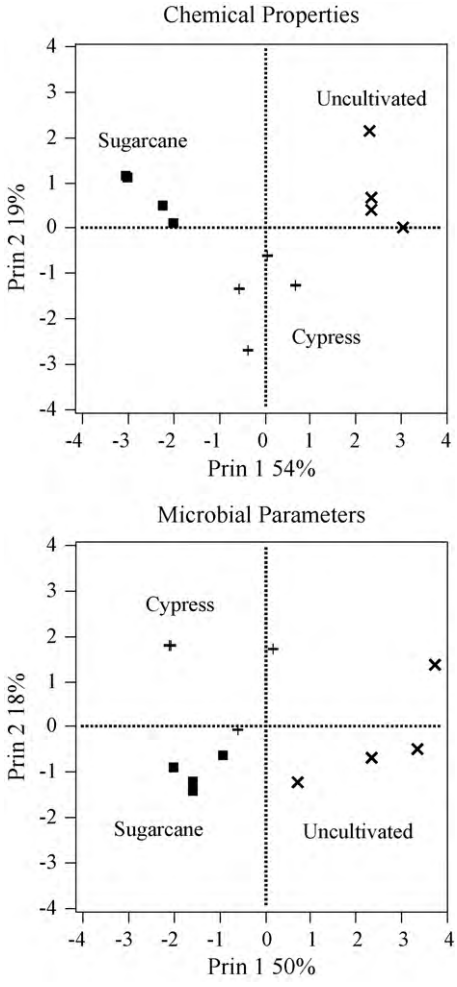

Fig. 3. Score plots of principal components analysis on soil chemical properties and microbial parameters.

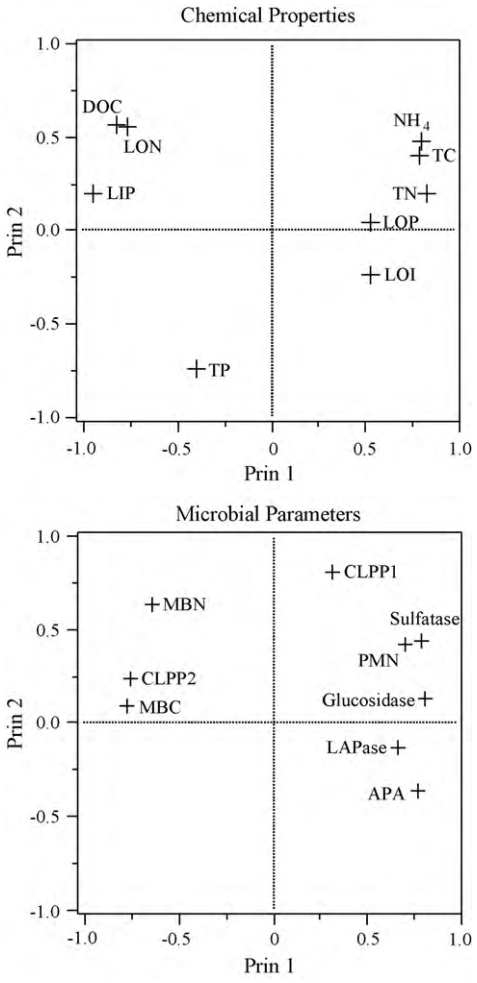

Fig. 4. Loading plots of principal components analysis on soil chemical properties and microbial parameters. LOI, loss-on-ignition; TC, total C; TN, total N; TP, total P; DOC, dissolved organic C; NH_4, labile inorganic N; LON, labile organic N; LIP, labile inorganic P; LOP, labile organic P; MBC, microbial biomass C; MBN, microbial biomass N; MBP, microbial biomass P; LAPase, leucine aminopeptidase; APA, alkaline phosphatase; CLPP1, 2, first and second principal components extracted from datasets of community-level physiology profiles.

3.2. Land-use effects on integrated microbial properties

Considering the high numbers of variables (31) for BIOLOG data, PCA was first conducted to create new variables representing the carbon utilization patterns. Two variables were extracted (CLPP1 and CLPP2), and each explained 20% of the total variance in the BIOLOG data set. The new variables were then combined with other microbial parameters and used for DA (Table 3). Canonical plots showed that cypress, sugarcane, and uncultivated soils were significantly different on the first discriminant function (Canonical 1), and the second discriminant function (Canonical 2) demonstrated the differences between uncultivated soils and those under sugarcane and cypress (Fig. 5). To identify variables that significantly contributed to the discriminations, stepwise variable selection was conducted. Variables selected into the final discriminant model were CLPP2, MBC, MBN, and PMN with a partial R^2 of 0.78, 0.79, 0.60, and 0.84, respectively (Table 4).

Application of PCA extracted two principal components, M-Prin1 and M-Prin2, from the original microbial data, which together explained 68% of the total variance (Fig. 3). Score plots indicated that uncultivated soils were distinct from sugarcane and cypress soils on the ordination axis M-Prin1, while sugarcane and

Table 3
Microbial parameters in cypress, sugarcane, and uncultivated soils (0–15 cm) with standard error values, $n = 4$.

	Unit	Cypress	Sugarcane	Uncultivated
Microbial biomass C	$g\,kg^{-1}$	13 (1.0)	14 (0.5)	9 (0.6)
Microbial biomass N	$g\,kg^{-1}$	0.2 (0.04)	0.2 (0.01)	0.1 (0.01)
Potentially mineralizable N	$mg\,kg^{-1}\,d^{-1}$	10 (2.5)	3 (0.3)	13 (1.6)
Leucine aminopeptidase	$mg\,kg^{-1}\,d^{-1}$	1.4 (0.4)	2.0 (0.2)	2.6 (0.7)
Phosphatase	$mg\,g^{-1}\,h^{-1}$	0.7 (0.12)	1 (0.08)	1 (0.08)
Sulfatase	$mg\,g^{-1}\,h^{-1}$	0.3 (0.04)	0.2 (0.04)	0.4 (0.05)
Glucosidase	$mg\,g^{-1}\,h^{-1}$	0.2 (0.01)	0.1 (0.02)	0.3 (0.04)
CLPP1	None	1.2 (1.0)	−1.6 (0.4)	0.4 (1.8)
CLPP2	None	2 (0.7)	1 (0.2)	−3 (0.9)

CLPP1 and 2, first and second principal components extracted from datasets of community-level physiology profiles.

cypress were separated on axis M-Prin2. Glucosidase had the highest loading ($R^2 = 0.81$) on M-Prin1 followed by sulfatase ($R^2 = 0.80$), phosphatase ($R^2 = 0.77$), MBC ($R^2 = -0.77$), PMN ($R^2 = 0.70$), leucine aminopeptidase ($R^2 = 0.67$) and MBN ($R^2 = -0.65$), while CLPP1 had the highest loading on M-Prin2 ($R^2 = 0.81$) (Fig. 4).

3.3. Dependent relationship between chemical properties and microbial parameters

Canonical correlation analysis was performed with chemical (C-Prin1, 2) and microbial (M-Prin1, 2) principal components, and two pairs of canonical variates (CVs) were extracted. Canonical correlation between the first chemical canonical variate (C-CV1) and the first microbial canonical variate (M-CV1) was significant ($R = 0.91$), with a goodness of fit of $p = 0.0006$. Approximately 83% of the variance in M-CV1 was explained by C-CV1. The importance of a given principal component for obtaining the maximum correlation between C-CVs and M-CVs was expressed as a standardized canonical coefficient. The coefficient of M-Prin1 for M-CV1 was 0.99 and of M-Prin2 was −0.17. Therefore, M-Prin1 gave a greater contribution increasing the M-CV1, while M-Prin2 contributed less in an opposite way. The coefficients of C-Prin1 and C-Prin2 for C-CV1 were 0.81 and 0.58, respectively. The second pair of canonical variates explained 43% of the variance shared by M-CV2 and C-CV2 ($R = 0.65$, $p = 0.03$). The canonical coefficients of M-Prin1 and M-Prin2 for M-CV2 were 0.17 and 0.99, respectively, while those of C-Prin1 and C-Prin2 for C-CV2 were 0.58 and −0.81, respectively. Further redundancy analysis revealed that 42% of total variance in M-Prin1 and M-Prin2 was explained by C-CV1 and another 21% by C-CV2.

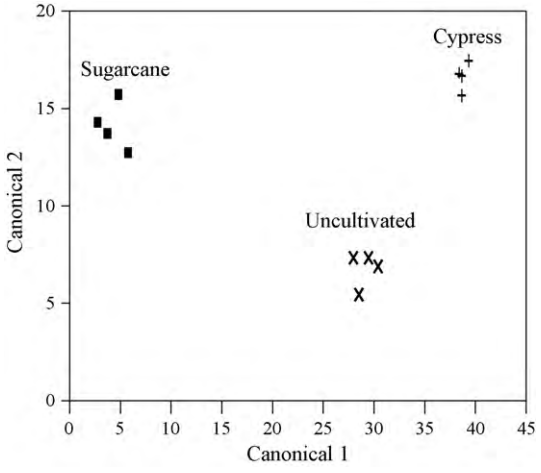

Fig. 5. Canonical plots of discriminant analysis on soil microbial parameters.

Table 4
Stepwise discriminant model for differentiating cypress, sugarcane, and uncultivated soils based on microbial parameters, $n = 12$.

Variable	Partial R^2	F value	P value
CLPP2	0.78	15.9	0.001
Microbial biomass C	0.79	15.3	0.002
Microbial biomass N	0.60	5.3	0.039
Potentially mineralizable N	0.84	16.0	0.004

CLPP2, second principal component extracted from datasets of community-level physiology profiles.

4. Discussion

4.1. Land-use effects on soil chemical properties

Cluster analysis has been used to identify discriminations of management effects on soil properties (Sena et al., 2002; Gila et al., 2008; Micó et al., 2008). In the present study, a K-means partitioning method was utilized to describe the heterogeneity of EAA soils under different land-uses by integrated analysis using chemical parameters. Soils were clustered into three groups in corresponding to the three land-uses (Fig. 1), indicating that cypress, sugarcane, and uncultivated soils were highly different in the sense of soil chemistry. Apparently, long-term cultivation of the EAA soils resulted in such discrimination. It has been reported that agricultural management poses remarkable impacts on the distribution of C (Wu et al., 2004; Zhang et al., 2006), N (Cookson et al., 2007) and P (Castillo and Wright, 2008b; Wright, 2009). Soils of the EAA are primarily organic and contain high N and low P and micronutrient concentrations. Therefore, sugarcane production requires supplemental fertilization and as well as extensive tillage (Rice et al., 2006). Fertilization is likely to promote nutrient accumulation, especially P, in the soil profile (Wright, 2009), while tillage disrupts organic matter dynamics and leads to changes in the depth profile of nutrients in soil (Vu et al., 2009). Analysis of cluster means across all variables further revealed that cypress, sugarcane, and uncultivated soils were highly distinguished by total P and LIP (Table 1, Fig. 2), indicating a significant difference in P availability between land-uses. It was reasonable since sugarcane soils received long-term P fertilization of approximately 30 $kg\,ha^{-1}\,year^{-1}$, and those of uncultivated did not, whereas the cypress soils received P application up until their establishment. This may also explain why LIP ($R^2 = 0.91$; $R^2/(1 - R^2) = 10.4$) was the most important variable in defining clusters (Table 2), and why the cluster of cypress was closer to sugarcane rather than to uncultivated soil (Fig. 1).

4.2. Land-use effects on microbial parameters

In the present study, DA was applied to differentiate soils under multiple land-uses as well as to determine whether the difference was actually significant. Analysis of discriminant functions clearly showed that sugarcane, cypress, and uncultivated soils had distinctive microbial characteristics (Fig. 5). Similar results have also been observed for other ecosystems, in which nutrient availability caused shifts in microbial community structure and function (Allison et al., 2007; Cookson et al., 2007; Matsushita et al., 2007). The stepwise variable selection procedure initially suggested that phosphatase ($R^2 = 0.63$), sulfatase ($R^2 = 0.51$), glucosidase ($R^2 = 0.62$), PMN ($R^2 = 0.67$), MBC ($R^2 = 0.68$), MBN ($R^2 = 0.57$), and CLPP2 ($R^2 = 0.78$) were all significant and individually eligible to enter the discriminant model. However, only CLPP2, MBC, MBN, and PMN were finally included, which indicated their great significance to the discrimination (Table 4). In other words, cypress, sugarcane, and uncultivated soils were

highly distinguished by those four variables. Considering the fact that CLPP2, MBC, and MBN characterized the microbial community composition and population size, it may be true that microbial community structure, rather than function, was more sensitive to agricultural management. This concept was also supported by other studies (Bossio et al., 2005; Zhang et al., 2006).

4.3. Variable reduction

The objective of variable reduction is to make data analysis more manageable and straightforward. Application of PCA in the present study successfully reduced nine chemical variables to two principal components and was able to capture 73% of the original variance, while nine microbial parameters were reduced to two principal components explaining 68% of the total variance. The reduction increased the sample size to variable ratio and made subsequent CCA analysis and interpretation easier. In addition, score plots showed that cypress, sugarcane, and uncultivated soils were dispersed differently along ordination axes (Fig. 3), indicating their significant difference in both soil chemistry and microbial community structure and function, which further supports the aforementioned results of CA and DA. Principal component loadings determine the amount of variance of a given variable accounted by principal components (Lattin et al., 2003). Our results demonstrated that LIP had the highest loadings on C-Prin1 ($R^2 = -0.95$), while total P was the only variable that had significant loadings on C-Prin2 ($R^2 = -0.74$), which is in agreement with the previous statement that LIP and total P significantly contributed to the discrimination in soil chemistry (Figs. 2 and 4).

4.4. Dependent relationship between chemical properties and microbial parameters

Microbial communities are in close contact with soil micro-environments, and therefore are easily subjected to change following alteration of soil chemical properties (Corstanje et al., 2007). Thus, land-use patterns are likely to affect the microbial community structure and function, which can be described by the changes in microbial parameters such as respiratory capacities, microbial biomass and extracellular enzymatic activities (Wright and Reddy, 2001; Castillo and Wright, 2008a). In the present study, multivariate data analysis demonstrated the contrasting soil chemistry and distinctive microbial community composition and function across soils under different land-uses (Figs. 1 and 5). It was likely that discriminations in microbial parameters were directly connected to variations in soil chemical properties, and CCA suggested that the dependent relationship was indeed significant. Changes in soil chemical properties resulted in alterations in microbial community composition and function. Nutrient availability often plays a critical role in regulating the microbial community structure and function (Cookson et al., 2007; Corstanje et al., 2007; Wright and Reddy, 2008). Cluster analysis suggested that LIP and total P were the most important variables discriminating the integrated chemistry of cypress, sugarcane, and uncultivated soils (Table 2, Fig. 2), which was further supported by the results of PCA (Fig. 4). In consideration of the fact that Everglades soils are historically P limited (Wright and Reddy, 2008), it was likely that P availability was the major factor that controlled the microbial community of these land-uses, which indeed are P limited (Table 1).

Soil chemical properties and the microbial community are two key components in ecosystem function (Finlay et al., 1997). Net results of chemical–microbial interactions define the way ecosystems function. Factors capable of interrupting or modifying the interactions are likely to cause significant ecological impacts. Applications of multivariate analytical methods in the present study suggested that agricultural cultivation has drastically altered soil chemical properties within the EAA, especially P availability, which further modified microbial community composition and activity. Intensive application of P fertilizers in the EAA is likely to stimulate microbial communities, which in turn would enhance organic matter decomposition rates and contribute to greater rates of soil subsidence. Thus, future P fertilization should be evaluated well for its potential long-term impact on microbial activity, which in turn may affect the sustainability of different land-uses of these subsiding soils.

5. Conclusions

Long-term cultivation in the EAA significantly altered nutrient distribution and availability for different land-uses, especially for P cycling. Correspondingly, soil microbial community composition and function was modified, and applications of CA and DA successfully described the alterations. Canonical correlation analysis clearly demonstrated a significant dependence relationship between chemical properties and microbial community composition and function. Phosphorus availability was one of the major factors regulating the soil microbial activity and function for the land-uses. Intensive application of P fertilizer is likely to stimulate microbial community and subsequently increase soil oxidation and subsidence. Future land-use changes in the EAA should consider effects of P on the functioning of microbial communities and their control of nutrient cycling.

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