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Fungal inhibition by *Bromelia pinguin* (Bromeliaceae) and its effect on nutrient cycle dynamics

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Abstract: Few studies have been done on the ecology of *Bromelia pinguin* (Bromeliaceae), a bromeliad with unique characteristics found throughout Central America and the Caribbean. In particular, the fruit pulp has antifungal properties against many fungal strains. We looked at the differences in belowground nutrient processes and the microbial community within bromeliad soil and in adjacent ecotonal and primary forest soils. PCR-based analysis of universal fungal 18S rRNA and universal bacterial 16S rRNA were used as indicators of inhibition, while analysis of basidiomycete internal transcribed space (ITS) was used to see if inhibition was preferential towards basidiomycota. Rhizobial 16S rRNA PCR-based abundance methods were used to determine if the plant affected nitrogen-fixing bacteria. The data suggest that this bromeliad does inhibit fungi and Basidiomycetes within the soil. Moreover, the increased soil dissolved organic carbon and decreased microbial biomass, and the lack of fungal and basidiomycete DNA, in the bromeliad soils suggests a reduced biomass of fungi and basidiomycetes in these soils may decrease the capacity to breakdown the more complex forms of organic carbon. There was decreased abundance and diversity, but greater dominance (i.e., decreased evenness) of Rhizobium 16S rRNA in the bromeliad soil. This, along with the similar amount of ammonium in all soils, and the greater amount of nitrate in the bromeliad soils, suggests that the properties of bromeliad soil select for fewer, more dominant rhizobial species, and that there may be more ammonium oxidizing activity occurring in these soils.

Resumen: Se han hecho pocos estudios sobre la ecología de Bromelia pinguin (Bromeliaceae), una bromeliácea con características únicas que se encuentra en toda América Central y el Caribe. En particular, la pulpa de los frutos tiene propiedades antifúngicas contra muchas cepas de hongos. Examinamos las diferencias en los procesos subterráneos de los nutrientes y la comunidad microbiana en el suelo de la bromelia y en suelos contiguos ecotonales y de bosque primario. Se utilizaron análisis basados en PCR de ARNr 18S fúngico universal y de ARNr 16S bacteriano universal como indicadores de la inhibición, mientras que el análisis del espacio transcrito interno (ITS, siglas en inglés) de los basidiomicetos fue usado para observar si la inhibición era preferencial hacia los Basidiomycota. Se usaron métodos basados en PCR de abundancia de ARNr 16S de Rhizobium para determinar si la planta afectaba a las bacterias fijadoras de nitrógeno. Los datos sugieren que esta bromelia sí inhibe a los hongos y los basidiomicetos en el suelo. Además, la cantidad mayor de carbono orgánico disuelto en el suelo y la cantidad menor de biomasa microbiana, así como la ausencia de ADN fúngico y de basidiomicetos en los suelos de la bromelia, sugieren que la biomasa de los hongos y los basidiomicetos está reducida y que en estos suelos puede disminuir la capacidad de

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descomponer las formas más complejas de carbono orgánico. En el suelo de la bromelia la abundancia y la diversidad de ARNr 16S de Rhizobium fueron menores, pero la dominancia fue mayor (i.e., una equidad menor). Todo esto, aunado a que la cantidad de amonio fue similar en todos los suelos, y a que la cantidad de nitrato fue mayor en los suelos de la bromelia, sugiere que las propiedades del suelo de la bromelia ejercen una selección hacia un número menor de especies de Rhizobium, aunque éstas son más dominantes, y que en estos suelos puede haber una mayor actividad oxidativa del amonio.

Resumo: Poucos trabalhos foram realizados sobre a ecologia da Bromelia pinguin (Bromeliaceae), uma bromelidia com características únicas encontrada através da América Central e das Caraíbas. Em particular, a polpa do fruto tem propriedades antifúngicas contra muitas estripes de fungos. Analisaram-se as diferenças nos processos dos nutrientes abaixo da superfície e a comunidade microbiana com solo de bromelidia, num ecótono adjacente, e em solos florestais primários. A análise com base no PCR do 18S rRNA universal para fungos e o 16S rRNA universal para as bactérias foi usada como indicadores de inibição, enquanto se usou o espaço interno transcrito (ITS) dos basidiomicetas para ver se a inibição era preferencial em relação àqueles. A análise de aplicação de PCR com base nos métodos da abundância de 16S rRNA rizobial foi usada para determinar se a planta afectou as bactérias fixadoras de azoto. Os dados sugerem que esta bromelidia inibe fungos e Basidiomicetas no solo. Além disso, o aumento do carbono orgânico dissolvido e o decréscimo da biomassa microbiana, assim como a falta de DNA de fungos e basidiomicetas em solos de bromelidia, sugerem uma biomassa fúngica e de basidiomicetas reduzida nestes solos, o que pode diminuir a capacidade de reduzir as formas mais complexas do carbono orgânico. Houve um decréscimo de abundância e diversidade, mas maior dominância (i. é decréscimo de uniformidade) de 16rRNA de Rhizobium no solo de bromelidia. Isto, juntamente com um teor semelhante de amónio em todos os solos, e uma maior quantidade de nitrato nos solos de bromelidias, sugerem que as propriedades destes solos de selecionarem, para menos, espécies de rizóbios mais dominantes o que poderá estar a induzir, nestes solos, mais atividade oxidante do amónio.

Key words: Antifungal properties, *Bromelia pinguin*, basidiomycete fungi ITS, carbon cycle, Costa Rica, Maquenque Reserve, microbial ecology, nitrogen cycle, rhizobial 16s rRNA, tropical ecology, universal fungi 18s rRNA, universal bacterial 16s rRNA.

Introduction

Bromelia pinguin (Bromeliaceae), also known as maya, is commonly found near forest edges throughout Central America and the Caribbean. Known for its spiny fronds, this bromeliad deters grazers and large mammals, and provides important habitats for many small insects and invertebrates. Maya were planted as hedges and living fences around Indian villages serving as barriers to potential intruders (Hallwachs 1983).

There have been few studies done on the ecology of this bromeliad (Woodley & Janzen 1995). It is known that the fruit of *B. pinguin* has many unique characteristics. The fruit pulp is very acidic and can cause ulcers on the fingertips when handled without gloves (Payrol *et al.* 2005). In traditional medicine, the fruit pulp of *B. pinguin* is used as an antihelminthic and to treat whooping cough and scurvy (Camacho-Hernández *et al.* 2002). It has also been found to be a great source of natural proteases, especially pinguinain (Toro Goyco *et al.* 1968). Camacho-Hernández *et al.* (2002) observed that the methanol extract from the maya fruit pulp possessed antifungal activity against most of the fungal strains tested.

The ecological role of this bromeliad in tropical forests is unclear. We conducted a study of this bromeliad in the Maquenque Reserve located in the lowland forest of Northern Zone of Costa Rica (approximately N 10° 41' 11", W 84° 12' 22"). This region of Costa Rica has experienced a variety of extraction-based land management activities, both legal and illegal, over the past 30-plus years, resul-

ting in the loss of approximately 70 % of its forests - the highest deforestation rate in the country (Monge *et al.* 2002; Schelhas *et al.* 2006). The Maquenque Reserve was established by the San Juan-La Selva Biological Corridor (SJLSBC) Commission to protect this fragmented area, and to encourage the establishment of ecosystem monitoring activities following initial characterization of the different habitats.

The goals of this study were to investigate the belowground nutrient processes and the microbial community structure occurring in bromeliad soils in comparison to adjacent primary forest and connecting ecotonal area soils, and to determine if antifungal activity similar to that found in the fruit by Camacho-Hernández *et al.* (2002) occurs within bromeliad soils. The PCR-based presence/absence and abundance analysis of universal fungal 18s rRNA and bacterial 16s rRNA genes, and the basidiomycete fungi internal transcribed space region (BAS ITS) between of the 18s rRNA gene, respectively, were used as indicators of microbial inhibition and to assess whether inhibition was preferential towards basidiomycota (Gardes & Brun 1993). This could have an impact on soil dissolved organic carbon (DOC) development, as basidiomycota are principal decomposers of lignin (Lynch & Thorn 2006). Lastly, the PCR-based presence/absence and abundance analysis of the rhizobial 16s rRNA gene was used to determine if the nitrogen (N) fixation community was affected by these bromeliads.

Materials and methods

Sampling

Sampling occurred over a 3-day period in July, 2009, during the rainy season. Four 30 x 40 m plots were chosen in a primary forest in the Maquenque Reserve that has previously been described (McNulty & Barry 2009; Quirós-Brenes 2002). Three 5 x 20 m subplots were chosen within each of these plots, representing an area of primary forest, an area composed of very high densities of the terrestrial bromeliad *Bromelia pinguin* (from 32 to > 100 plants per subplot), and an ecotonal area between the forest and the bromeliad patches. The primary forest region was defined by its lack of *B. pinguin* and is typical of the area, whereas the ecotone included a few bromeliads (from 9 to 16 plants per subplot). Each of the subplots were chosen so that they were adjacent to each other to assure similar topography and inherent soil characteristics and to better ensure that differences found between soil ecosystems were due to the effects of the bromeliads.

Percent saturation of the soil and pH were determined at 7 randomly located sites within each subplot using a Kelway HB-2 Soil and pH meter (Wyckoff, NJ, USA). Sixteen 2 cm wide x 15 cm deep soil cores were collected at eight randomly chosen locations within each subplot, and composited for analysis. Four other 2 cm x 15 cm soil cores were collected from each subplot for bulk density analysis. The composited samples were sieved at 5 mm prior to nutrient analysis and DNA extraction. The nutrient values from the study were adjusted for dry weight and bulk density.

Microbial biomass, respiration and nutrient analysis

The amount of ammonium (NH_4-N), nitrate (NO_3-N), and total mineral N (TMN) were determined using 50 mL of 2M KCl extraction of 10 g soil samples followed by the ammonium salicylate and cadmium reduction spectrophotometric methods using the HACH DR 2700 system (Hach Company, Loveland, Colorado, 80539-0389; HACH methods 8155 and 8192 respectively). The TMN was calculated as the amounts of NH_4-N and NO_3-N. Phosphate (PO_4) content was measured following Bray 1 extraction from 2 g of soil using the molybdenate reduction method (HACH method # 8048) and the HACH DR 2700 system.

Microbial biomass C (C_{mic}) was determined by the fumigation-extraction method (Jenkinson 1988) as the difference between 0.5 M K_2SO_4 - extracted soil dissolved organic carbon (DOC) levels in ethanol-free chloroform-fumigated and unfumigated 10 g soil subsamples. The DOC levels were determined by dry combustion analysis at the CATIE labs in Turrialba, Costa Rica, using the methods of Anderson & Ingram (1993) and an autoanalyzer (Alliance Instruments). The rate of respiration was determined as CO_2 released using a Qubit SR1LP Respiration system (Kingston, ON, Canada). The efficiency of utilization of organic C was estimated by determining the microbial metabolic quotients (qCO_2, as a ratio of CO_2 from respiration/C_{mic}) and the ratio of C_{mic} to SOC (Anderson 2003).

DNA analysis

DNA was extracted from the subplot composited soil samples using the Power Soil DNA Isolation kit (MO BIO Carlsbad, CA). Three replicates

Table 1. Conditions used for target DNA analysis using the primers universal fungal 18S rRNA, BAS ITS, universal bacterial 16S rRNA, and *Rhizobium* 16S rRNA.

	Primers (reference)	Annealing Temperature (°C)	Restriction Enzymes
Fungal 18S rRNA	EF4/EF3 (Smit *et al.* 1999)	48	HinfI, TaqI
BAS ITS	ITS1f/ITS4 (Gardes & Bruns 1993)	55	Not Done
Bacterial 16S rRNA	27f/1492r 16s rRNA (Martin-Laurent *et al.* 2001)	55	Hhal
Rhizobium 16S rRNA	63f/Rhiz-1244r (Singh *et al.* 2006)	55	HhaI, MspI, RsaI

were used for each subplot to increase the likelihood of finding less common groups of microbes. After DNA extraction, the replicates were pooled. DNA was stored at 4 °C until use, and the purity of the DNA samples were checked for appropriate purity assuring that all A_{230} values were < 0.1.

Endpoint PCR was used to determine whether target DNA was present in the soil samples using the primers for universal fungal 18S rDNA, universal bacterial 16S rDNA, Basidiomycete (BAS) ITS, and *Rhizobium* 16S rDNA (primers and conditions listed in Table 1). Quantitative PCR was performed on all endpoint PCR-positive samples to estimate the abundance of the target gene in the soil DNA. The semi-quantitative PCRs were performed using the same methods as above, except that aliquots were collected at the end of a cycle and the fluorescence values determined. These values were used to compare the threshold cycle (C_t) level and PCR product amount for sample DNA to that of purified positive control DNA of known concentration (6.5 to 28.4 ng μL^{-1} of cloned target gene DNA with sequences confirmed in GenBank) to allow for an estimation of the abundance of the target DNA concentration in the soil DNA.

Microbial diversity analysis

Restriction fragment length polymorphism (RFLP) analysis was performed on approximately 0.2 µg of PCR-amplified DNA products to assess the relative diversity of the microbial communities using Fermentas (Applied Biosystems, Foster City, CA) restriction endonucleases (Table 1) and the conditions suggested by the enzyme manufacturer. The resulting size and quantity (per µg of starting PCR product) of each DNA band was determined for each DNA sample using agarose (2 %) gel electrophoresis and Gene Tools software to analyze the RFLP banding patterns (size and quantity of discrete DNA bands). The RFLP-based richness (S), diversity (H′), and evenness (E) indices were determined for the different subplots and habitat types (Shannon & Weaver 1963).

Data analysis

The t-test, *P* and Cohen's *d* (effect size) statistics were used to suggest biological meaningfulness of the differences in mean values of the different parameters measured between any two plots. The data from all habitats were combined and examined for possible relationships between the different metrics and the amount of C_{mic} and fungal rRNA by calculating correlation coefficient *r* and r^2 values.

The DNA band size and quantity from the RFLP analyses and the qPCR-based abundance of the genes were also examined for relationships with respect to subplots and habitat types via multivariate cluster analysis in which a similarity matrix was developed using Clustal W methods and Ward's minimum variance method of measuring Euclidean distances (Hartmann & Widmer 2006) in NCSS software (NCSS, Kaysville, UT). In particular, cluster analyses were performed to determine the effect that the bromeliads may have had on the microbial community structure. Cluster analysis was performed using all DNA abundances and RFLP assays to show overall genetic relationships when examined in the aggregate. As well, cluster analysis was performed using only the DNA data from the *Rhizobium* and universal bacterial analyses to determine if the bromeliad plants had an effect on components of the microbial community other than the fungi.

Results

Nutrient and carbon analyses

The primary forest, ecotone, and bromeliad soils had bulk densities of 0.69, 0.66 and 0.58 g cm^{-3}, pH values of 6.25, 6.38, and 6.49, and percent saturations of 50 %, 46 %, and 46 %, respectively.

Table 2. A comparison of the physical, nutrient and microbial activity characteristics in soil associated with Bromeliad Plants, Primary Forest, and the Ecotone between them in an old growth forest region in the Northern Zone of Costa Rica. The mean levels are presented (± SD; n = 4).

Soil Characteristics	Bromeliad Soil	Ecotone Forest Soil	Primary Forest Soil
Biomass C (μg cm^{-3})	120 ± 75	170 ± 47	313 ± 41
Respiration (mg cm^{-3} h^{-1})	3.57 ± 0.34	3.43 ± 0.63	4.74 ± 1.42
Organic C (μg cm^{-3})	811 ± 29	740 ± 38	677 ± 75
Biomass/Organic C	0.15 ± 0.10	0.23 ± 0.07	0.47 ± 0.09
qCO_2 (Resp/Cmic)	0.039 ± 0.022	0.021 ± 0.003	0.015 ± 0.003
NO_3-N (μg cm^{-3})	1.87 ± .47	2.12 ± 0.86	1.13 ± 0.58
NH_4-N (μg cm^{-3})	5.13 ± 3.06	6.09 ± 1.95	6.16 ± 2.08
TMN (μg cm^{-3})	7.00 ± 2.91	8.21 ± 2.72	7.29 ± 2.50
PO_4 (μg cm^{-3})	6.85 ± 4.46	5.50 ± 3.86	10.10 ± 5.57

Table 3. Two-way comparisons of mean values of the physical, nutrient and microbial activity characteristics and abundance of microbial group DNA within soil associated with Bromeliad Plants, Primary Forest, and the Ecotone between them within an old growth forest region in the Northern Zone of Costa Rica.

Metric	Comparison	*P* value	*d* value
Nitrate	Bromeliad v. Primary	0.100	1.400
	Bromeliad v. Ecotone	0.600	0.360
	Ecotone v. Primary	0.129	1.350
Ammonium	Bromeliad v. Primary	0.100	0.390
	Bromeliad v. Ecotone	0.657	0.380
	Ecotone v. Primary	0.143	0.030
TMN	Bromeliad v. Primary	0.971	0.110
	Bromeliad v. Ecotone	0.600	0.430
	Ecotone v. Primary	0.586	0.350
Cmic	Bromeliad v. Primary	0.014	2.750
	Bromeliad v. Ecotone	0.414	0.600
	Ecotone v. Primary	0.014	3.460
Respiration	Bromeliad v. Primary	0.157	1.130
	Bromeliad v. Ecotone	0.729	0.280
	Ecotone v. Primary	0.114	1.190
qCO2	Bromeliad v. Primary	0.057	1.290
	Bromeliad v. Ecotone	0.243	0.950
	Ecotone v. Primary	0.229	1.010
SOC	Bromeliad v. Primary	0.014	2.360
	Bromeliad v. Ecotone	0.043	2.110
	Ecotone v. Primary	0.229	1.060
Cmic/SOC	Bromeliad v. Primary	0.029	3.460
	Bromeliad v. Ecotone	0.314	0.820
	Ecotone v. Primary	0.014	3.430
Phosphate	Bromeliad v. Primary	0.185	0.640
	Bromeliad v. Ecotone	0.514	0.330
	Ecotone v. Primary	0.157	0.960

The PO_4 levels were the greatest in the primary forest soils, and the total mineral nitrogen (TMN) levels were similar in all three soil types, although the NO_3-N was greater in the sites with the bromeliad plants (bromeliad and ecotone soils), and the NH_4-N was somewhat greater in the primary forest and ecotone soils (Tables 2 & 3). The microbial respiration was much greater in the primary forest soils (4.74 μg CO_2-C cm^{-3} dry soil h^{-1}) than in either of the other soil types, which exhibited similar respiration rates (3.58 and 3.43 μg cm^{-3} dry soil h^{-1} in the bromeliad soil and the ecotone soil, respectively). However, the dissolved organic C (DOC) was lowest and the C_{mic} was highest in the primary forest soils (677 and 313 μg C cm^{-3} dry soil, respectively), while the bromeliad soils had the highest DOC (811 μg C cm^{-3} dry soil) and the lowest C_{mic} values (120 μg C cm^{-3} dry soil). The qCO_2 was lowest and Cmic to SOC ratios highest greatest in the primary forest soils, and opposite in the bromeliad soils (Tables 2 & 3). Analysis of all the data showed that the C_{mic} values were positively correlated with the amount of mineralized N, respiration, fungal rRNA and BAS ITS in the soils, and negatively correlated with qCO_2 values. The amount of fungal rRNA was positively correlated with the amount of rhizobial rRNA, the Cmic to SOC ratio, and negatively correlated with qCO_2 (Table 4).

DNA analysis

It is important to note that we interpreted the absence of a microbe by endpoint PCR as indicating they were not detectable at the levels of sensitivity of these assays, rather than implying they are completely absent from the soils. There was

Table 4. Pearson's correlation results (r and r^2 values) from comparisons between parameters measured in the soil associated with Bromeliad Plants, Primary Forest, and the Ecotone between them within an old growth forest region in the Northern Zone of Costa Rica within the Northern Zone of Costa Rica.

Correlations	r value	r^2 value	P value
Ammonium & Cmic	0.554	0.307	0.061
TMN & Cmic	0.408	0.167	0.188
Respiration & Cmic	0.705	0.494	0.011
qCO_2 & Cmic	-0.737	0.543	0.006
Fungal rRNA & Cmic	0.583	0.34	0.047
BAS ITS & Cmic	0.626	0.391	0.03
qCO2 & Fungal rRNA	-0.551	0.304	0.064
Cmic/SOC & Fungal rRNA	0.657	0.432	0.02
Rhizobial & Fungal rRNA	0.531	0.282	0.076

Table 5. Presence/absence of fungal 18S rRNA and basidiomycete ITS DNA associated with soil from the various subplots studied within an old growth forest region in the Northern Zone of Costa Rica within the Northern Zone of Costa Rica. The presence of the target DNA was determined by agarose electrophoresis of PCR-amplified DNA from the total soil community DNA, and was evaluated as no signal (-), weak signal (+), moderate signal (++) and strong signal (+++) based on DNA band intensity.

	Endpoint PCR Target Gene Signal Results	
	Fungal DNA	Basidiomycete DNA
Bromeliad subplot 1	-	-
Bromeliad subplot 2	-	-
Bromeliad subplot 3	-	-
Bromeliad subplot 4	-	-
Ecotone subplot 1	+	-
Ecotone subplot 2	+++	-
Ecotone subplot 3	++	-
Ecotone subplot 4	++	+++
Primary subplot 1	+++	+
Primary subplot 2	+++	++
Primary subplot 3	+	-
Primary subplot 4	+++	-

no detectable fungal DNA signal in the bromeliad soil using either the universal fungal 18S rRNA or BAS ITS primers in the PCR analysis (Table 5). By contrast, moderate and strong signals were found for universal fungal target DNA in the ecotone and primary forest soils, respectively. In addition, one subplot in the ecotone soils and two in the primary forest soils had positive signals for BAS ITS target DNA. Considerable amounts of universal bacterial 16S rRNA and rhizobial 16S rRNA target DNA were found in all three habitats, with the strongest signals found in the primary forest soil samples (Table 5).

The average abundance of the universal fungal 18S rRNA and BAS ITS target DNA were greater in the soil from the primary forest than the ecotonal area. Universal bacterial 16S rRNA target DNA was most abundant in the bromeliad forest, followed by the primary forest and ecotone. However, the ecotone and the primary forest soils both had greater abundance of rhizobial 16S rRNA than the bromeliad soils (Table 5).

There were minimal differences in the RFLP-based diversity (H′), richness (S), and evenness (E) of universal fungi 18S rRNA target DNA between the primary forest and ecotone soils. There was slightly greater diversity and richness in the bromeliad soil than in the ecotone and primary forest soils for universal bacteria16S rRNA target DNA, but the evenness was about the same for all three soil types. The diversity of the rhizobial 16S rRNA was greater in the primary forest, followed by the ecotone and bromeliad soil. However, the bromeliad soil demonstrated greater dominance (lower E value) in the rhizobial 16s rRNA than the primary forest and ecotone soils (Table 6).

Cluster analysis

The qPCR-based DNA abundance values and DNA band groups were used to look for degrees of relatedness between the three habitat types, and possible effects of the bromeliad plants on the soil microbial community structure. Two cluster analyses of the DNA data using (i) all abundance and RFLP assay data and (ii) using only the data from the *Rhizobium* and universal bacterial 16S rRNA assays, both showed the primary forest and ecotone soils were closely related, and the bromeliad soils clearly distinct (Fig. 1).

Discussion

Vegetation influences the makeup of soil microbial communities (Kageyma *et al.* 2008; Westover *et al.* 1997). In the current study, the soil microbial community and nutrient composition

Table 6. A comparison of the quantitative PCR-based abundance of target DNA (as fg target DNA / ng soil DNA),and community level restriction fragment length polymorphism (RFLP) analysis of the diversity of PCR-amplified soil community DNA from three different soil types within an old growth forest in the Northern Zone of Costa Rica. The RFLP-based Shannon-Weaver diversity (H′), richness (S), and evenness (E) indices are presented.

Target DNA	Soil Type	Abundance (fg/ng soil DNA)	H′	S	E
	Bromeliad	0	0	0	0
Universal Fungi 18S rRNA	Ecotone	139 ±105	2.37	17	0.84
	Primary	155 ± 67	2.45	18	0.85
	Bromeliad	0	N/A	N/A	N/A
BAS ITS	Ecotone	19 ± 0	N/A	N/A	N/A
	Primary	1375 ± 10	N/A	N/A	N/A
	Bromeliad	50490 ±18470	2.28	14	0.87
Universal Bacterial 16S rRNA	Ecotone	10240 ± 0.0	2.12	12	0.83
	Primary	37240 ± 24130	2.11	13	0.82
	Bromeliad	4 ± 3	1.33	8	0.64
Rhizobial 16S rRNA	Ecotone	8 ± 12	1.64	7	0.85
	Primary	7 ± 5	1.74	10	0.76

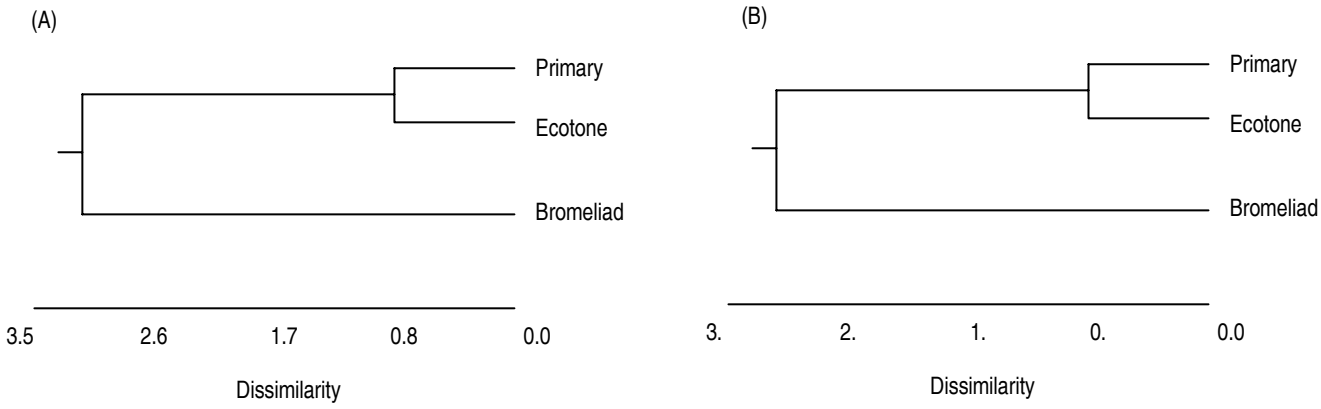

Fig. 1. Results of a multivariate cluster analysis (using Clustal W methods and Ward's minimum variance method) to determine the effect that the bromeliad plants had on the microbial community structure. Cluster analysis was performed on (A) all DNA abundances and RFLP to show overall genetic relationships; and (B) using only the DNA data from the Rhizobium and universal bacterial analyses to determine if the bromeliad plants had an effect on microbes other than the fungi.

beneath the bromeliad plant, *Bromelia pinguin* (Bromeliaceae), were clearly different from the adjacent forest soils. The universal fungal 18S rRNA and BAS ITS target DNA were not found in the bromeliad soil, but were found in the adjacent ecotonal and primary forests. The primary forest soils had more P and NH_4-N and less NO_3-N than the bromeliad soils. As well, the primary forest soils had greater microbial biomass and respiratory activity, and more fungal, basidiomycete, and *Rhizobium* DNA, but less bacterial DNA overall. These soils also appeared to be more efficient at the use of organic C than the bromeliad soils as they had lower qCO_2 and greater C_{mic} to DOC ratios. These data suggest that the bromeliads within these forests are playing important roles in the composition of the microbial community and associated nutrients within these soils by inhibiting the fungal and rhizobial microbes, resulting in the primary forests having a more nutrient-

rich and fungal-dominant soil. It is plausible that there could also be a seasonal affect occurring within these soils that warrants investigation.

Higher levels of PO_4, especially with respect to inorganic N, help stimulate fungal growth. However, when PO_4 is limiting in the presence of normal levels of inorganic N, fungal development in soils is often suppressed (Bittman *et al.* 2005). The data from this study support this relationship in that the primary forest soils had the most PO_4 and similar mineral N as the other soils, and had the greatest abundance of fungal and basidiomycete DNA. Conversely, the bromeliad soils had much lower PO_4 levels and similar N levels, with no fungal or basidiomycete DNA detected. It is certainly possible that nutrient conditions play a role in the lack of fungal DNA in these bromeliad soils. However, it is interesting that the ecotone N levels were very similar to those found in the bromeliad soils, and the PO_4 lower than in the Bromeliad soils, yet the ecotone soils had both fungal and basidiomycete DNA present. This suggests that the absence of fungi in the bromeliad soils could be a function of the antifungal properties of the bromeliad plant, similar to those reported for its fruit in an *in vitro* assay by Camacho-Hernández *et al.* (2002).

This study provided some preliminary evidence that the C cycle dynamics may be affected within the bromeliad soil, due to possible impacts on fungal populations. The absence of detectable fungal and basidiomycete DNA in the bromeliad forest may explain why there were lower qCO_2 values and greater ratios of C_{mic} to DOC as indicators of decreased efficiency in the use of organic C in these soils relative to the primary forest and ecotone soils, which is indicative of soils that are less fungal-dominant (Anderson 2003). As the basidiomycetes are significant decomposers of complex organic compounds such as lignin (de Boer *et al.* 2005), the lower levels of these fungi, along with the greater density of bromeliads as ground cover vegetation could result in a build-up of complex organic compounds like lignin, accounting for the greater levels of DOC, the lower efficiency of organic C use, and the lower levels of microbial biomass found in the bromeliad soils.

The levels of NH_4-N were somewhat less in the bromeliad soils than in the primary forest and ecotone soils. This, along with the decreased abundance and diversity, but greater dominance (lower E value) of the *Rhizobium* DNA in the bromeliad soils, suggests that the bromeliad plants could be selecting for a smaller, more efficient *Rhizobium* population than in the primary forest, that perform N-fixation in these soils. There was also less NO_3-N in the primary forest than the other soils. Sahrawat (2008) has identified this as a common observation in the more complex soils from older temperate forests due to smaller population sizes of ammonia oxidizing bacteria (mostly associated with lower pH values), and more rapid uptake of NO_3 into the biomass. However, it is not clear if these patterns are found in tropical soils.

The indicators of microbial activity (respiration and the ratio of C_{mic} to DOC) were positively correlated with the microbial biomass, which was greatest in the primary forest soils, and lowest in the bromeliad soils. These and other indicators suggestive of a fungal-dominated soil (higher PO_4 in relation to N levels, lower qCO_2, and greater C_{mic} to DOC ratios) were all correlated with abundance of fungal and basidiomycete DNA, and suggesting a more fungal-dominated soil habitat in the primary forest. The results of the cluster analysis suggest that the bromeliad plants may be influencing both the bacterial and the fungal components of the microbial community composition in these soils in that the microbial community within the bromeliad soils was the most unique among the three areas tested. Thus, the data from this study demonstrated differences exist in the nutrient levels, C cycle dynamics, and fungal populations between the bromeliad and primary forest soils.

Currently, the use of synthetic pesticides are the primary method used for treatment of pathogenic diseases in plants. Even though fungi are the principal cause of these diseases (Chang *et al.* 2008), fungicides only constitute 6 % of the total amount of pesticides used worldwide (Barnard *et al.* 1997). Thus, there is a need to develop more fungicidal treatments for plants, especially those that are less harmful to humans and the environment (Barnard *et al.* 1997). The bromeliad's antifungal activities suggested in this study and by Camacho-Hernández *et al.* (2002) could represent a source of a natural fungicide for many valued crops.

Conclusions

The presence of *Bromelia pinguin* seems to inhibit fungi in general, as well as the specific basidiomycete group of fungi, affect the soil microbial diversity, and alter the nutrient cycle components in soil in comparison to adjacent forest soils. This impacts the microbial biomass and organic C dynamics within the soils, resulting in a poten-

tially reduced capacity to decompose organic C. The impact on the fungal community in the soils diminishes moving from the bromeliad plants towards the primary forest, coincident with increases in microbial activity and biomass, efficiency of organic C use, and increases in levels of P-all of which are indicators of a more fungal-dominated soil community. It is also interesting that there seems to be a decrease in complexity, but not in functionality of the rhizobial 16S rRNA in the bromeliad soil, and that the amount of N fixation does not appear to have changed, but the N fixing community may have. Future studies will focus on identifying the specific groups of bacteria and fungi that are inhibited by the bromeliad plants, the changes in the rates of microbial activity due to the effects of the plant, and whether these plants have antifungal activity against common soil-borne fungal pathogens.

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