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Biogeochemical Relationships of a Subtropical Dry Forest on Karst

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Abstract - Tropical dry forests on calcareous substrate constitute the main vegetation cover in many islands of the Caribbean. Dry climate and nutrient scarcity in those environments are ideal to investigate the potential role of high levels of soil calcium (Ca) in regulating plant selection and productivity. We analyzed the elemental composition of soil, loose litter, and leaf samples of the woody vegetation on the plateau of Mona Island, an emergent block of carbonate rock in the Caribbean located between Puerto Rico and the Dominican Republic, to explore the nutrient relationships of plants growing on calcareous substrates. The mineral soil has an elemental composition characterized by high levels of aluminum (Al) and iron (Fe) in agreement with the hypothesis that it derives in part from sediments transported by rivers eroding plutonic rocks, and deposited before the massive lifting of biological limestone. Calcium concentration varied within sites, and Ca–Al and Ca–Fe correlations were negative in soils and positive in plant material, implying that element uptake from these soils depends on acidification of the rhizosphere. This acidification should be high enough to extract carbonate-bound elements and solubilize Al, Fe, and probably phosphate (P) compounds. The most abundant cation in leaves was Ca, followed by potassium (K) and magnesium (Mg); Ca/K and Ca/Mg molar ratios averaged 2 and 3, respectively, indicating that most species maintain K and Mg uptake in the presence of high Ca levels. Both N and P concentrations and N/P ratios in soils, loose litter, and woody plant leaves indicate comparatively high P availability. Based on elemental composition, 3 species groups were identified: one associated with higher values of N, P, and K; a second characterized by the lowest concentrations of Mg, Ca, and Al; and a third containing species with greater relative amounts of Ca, Mg, and Al, and higher Ca:K ratios. Differences are probably the result of species-specific requirements, but ecological implications are not clear yet. Phylogenetic arrangement of species showed that Ca concentration varies smoothly between botanical orders, revealing a phylogenetic signature. Brassicales, Ericales, and Lamiales had the highest Ca values, whereas Fabales and Arecales were at the lower end of the concentration scale. Aluminum showed a tight correlation with Ca within the plant orders.

Introduction

The Caribbean region contains numerous islands located within 10º to 28º N and 59º to 86º W. About 130,000 km^2 of those islands are covered by thick layers of limestone deposits of marine origin, up to several hundred meters deep, on top

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of volcanic substrates (Frank et al. 1998). On these deposits, a range of plant communities develop according to rainfall, from open thorn thickets to predominantly deciduous forests with a similar tree flora throughout the lowlands of the Caribbean (Asprey and Loveless 1958, Asprey and Robbins 1953, Borhidi 1993, Kelly et al. 1988, Lötschert 1958, Loveless and Asprey 1957). Limestone is homogeneous in chemical composition, mainly containing calcium carbonate, with varying amounts of magnesium (dolomites) and iron (clay) (Lugo et al. 2001, Marschner 1995, Monroe 1980). On limestone bedrock, a variety of shallow, calcium- and organic matter-rich soils develop. These soils derive from the weathering of the calcareous rock and the accumulation of plant organic matter. In addition, Caribbean karst formations frequently host bauxitic sediments resulting from the ferralitization of aluminum silicate residues derived from sediments of volcanic origin (Day 2010, Gow and Lozej 1993). In karst areas of Puerto Rico, a sand–silt–clay mixture overlies the limestone formations (Briggs 1966). This material is not residual from the underlying limestone, but derives from plutonic rocks in the mountainous interior of Puerto Rico. Weathered material was transported by superficial water run-off and deposited on limestone formations that were still under water, and remained there after retreat of the sea.

Calcium carbonate-rich soils generally have lower availability of phosphorous (P) and iron (Fe) because they are immobilized as insoluble salts. These conditions may also create physiological stress in plants due to the accumulation of large amounts of calcium (Ca) ions (Rorison and Robinson 1984). Plant nutrient stress in calcareous soils may be caused by (a) inhibition of potassium (K) uptake by high concentrations of Ca ions in the soil solution; (b) low P availability because the high pH and high concentration of Ca leads to the formation of only slightly soluble calcium-phosphate; or (c) reduction of Fe availability, which may cause a yellowish discoloration of leaves (lime-induced chlorosis) (Marschner 1995; White and Broadley 2003; Zohlen and Tyler 2000, 2004).

Biogeochemical characterizations of vegetation on calcareous soil in tropical environments are scarce, and little is known about the stoichiometry of plant nutrients. Herein we document the elemental composition of mineral soil, loose litter, and adult leaves of woody species collected from dry forests on the plateau of the Mona Island, PR (Brandeis et al. 2012, Medina et al. 2014). We determined inter-elemental relationships and group species using multivariate statistical techniques to explore the following aspects:

(a) stoichiometric changes of nitrogen (N), P, K, and Ca in soil, loose litter, and leaves of woody plants as indicator of biogeochemical processes;
(b) variations in the N/P and Ca/K ratios in leaf tissues as potential indicators of P deficiency and presence of Ca-accumulating species;
(c) Ca/Al and Ca/Fe correlations in both soils and leaves as indicators of the contribution of allochtonous sediments to the composition of plant tissues;
(d) identification of woody plant species groups characterized by similar elemental composition; and
(e) potential phylogenetic determination of Ca concentration in leaf tissues.

Field-site Description

The subject of this study is the vegetation of Mona Island described previously by Woodbury et al. (1977) and Cintrón and Rogers (1991). This island is a natural reserve and a protected area. It has been subjected to several human-caused disturbances since the 16th century, first by the introduction of *Sus scrofa* L. (Pig) and *Capra hircus* L. (Goat), and the accidental establishment of feral *Felis catus* L. (Domestic Cat) and rat populations, and later by the exploitation of bat-guano and military uses in the eastern side of the island. However, the vegetation is well preserved (Brandeis et al. 2012) and therefore provides a unique opportunity to investigate soil–plant nutrient relationships in a relatively undisturbed vegetation and to explore to which extent the calcareous substrate constitutes an environmental constraint for vegetation development.

Methods

Soil and loose-litter sampling

Soil and loose-litter samples were collected from 27 plots by a joint team of the Forest Inventory Analysis (FIA) program and the International Institute of Tropical Forestry (IITF) of the US Forest Service (USFS) during the period 17–22 November 2008, in conjunction with a study of the vegetation cover of upland terraces of Mona Island (Brandeis et al. 2012).

Soils were consistently similar at the plots sampled. Most sampling sites provided adequate forest-floor samples for lab analysis, with some sites having large amounts of duff in the cracks in the limestone. It was possible to reach mineral soil before hitting rock at about 1/3 of the sampling sites, but only rarely did mineral soil extend beyond 10 cm deep. Mineral soil was defined as where there was an abrupt and highly noticeable color change to red-brown and consistent texture with no recognizable plant parts. This soil was given a field texture of loamy, but was also considered as organic. The FIA-IITF field team was instructed to locate the soils-sampling frame in places representative of what was present in the general area being sampled. There was often a thick layer of roots right at the transition from forest floor (O horizon) to mineral soil (A horizon). Sixty-two mineral soil samples were collected from 21 sites. Soil samples were oven dried at 40 ºC and ground to pass through a 20-mesh sieve. The soil pH was determined in a soil-water paste (1:1).

Loose litter and detritus on top of the mineral soil (27 sites, 3 replicates per plot) was collected and divided in the laboratory into leaf, wood, miscellaneous (consisting of fragments of leaves and wood too small to be separated), and detritus (partly decomposed organic matter probably containing small amounts of mineral soil).

Leaf sampling

Leaves of tree species identified in the field as present on a set of plots on Mona Island were sampled by field teams of the Center for Applied Tropical Ecology and Conservation (CATEC) of the University of Puerto Rico Rio Piedras, the IITF, the

USFS FIA program, and the Department of Natural Resources and the Environment (DNER) of the Commonwealth of Puerto Rico, during the period 17–22 November 2008. Sampling was conducted in conjunction with a survey of vegetation cover data. Thirty-seven plots were sampled following a modified version of the FIA plot design (USDA FS 2007). Adult, sun-exposed leaves with petioles, of woody species (shrubs and trees) occurring at each plot were collected and placed in paper bags that were left open to the air until brought to the IITF lab for oven drying within 4 days or less of collection. Diurnal ambient temperature (>25 ºC) and humidity (<70%) prevented decomposition of leaf samples during the storage period. In the laboratory, samples were dried in a ventilated oven at 65 ºC. The samples included fully expanded leaves from similar developmental stages representing the range of upper canopy leaves of each species at each plot. The number of samples per species varied according to their occurrence in the plots. A total of 285 leaf samples were analyzed belonging to 47 species.

Elemental analyses

We digested leaves, loose litter, and soil samples using a modification of the procedure of Huang and Schulte (1985). Concentrated HNO_3 (5 ml) was added to the sample (0.5 g) and left to stand overnight at room temperature. The next day, the samples were submitted to the following procedure: heating at 100 °C for 15 minutes; cooling; addition of 10 mL of a mixture (1:1) of concentrated HNO_3 and deionized H_2O; heating at 95 °C for 2 hours; cooling; addition of 4 ml of H_2O_2; heating to 95 ºC for 10 minutes; cooling; addition of 2 ml of concentrated HCl; heating at 95 ºC for 30 minutes. Finally, the mixture was diluted to 50 mL with deionized H_2O and filtered into acid washed 60-mL low-density polypropylene bottles (Sánchez et al. 2015).

We analyzed the concentration of Na, K, Mg, Al, Ca, Fe, Mn, and P in the resulting digests simultaneously in a Spectro ICP emission spectrometer model Ciros CCD. We determined total carbon, nitrogen, and sulfur by the macro dry combustion method using the LECO CNS- 2000 Analyzer (LECO Corp. 2005). Moisture (100º C) and ash content estimated from the weight loss on ignition at 490º C (% LOI), were determined in leaves subsamples using a thermogravimeter (LECO TGA701).

Statistical analysis

For each data set (soils, loose litter, plant leaves) of mass and nutrient concentrations, we fitted the best distribution according to their Akaike information criteria (AIC), and tested for homoscedasticity (Bartlett test). Few parameters were optimally fitted by normal (Shapiro-Wilk W test) or log-normal (Kolmogorov's D test) distributions. Normalization procedures were too diverse, therefore we decided to use medians and median absolute deviation (MAD), instead of averages and standard deviations, to characterize each variable. When median comparisons were necessary, we used non-parametric tests (Wilcoxon/Kruskall-Wallis, and Wilcoxon multiple pair comparison). We also conducted correlation analyses among variables to examine the inter-element associations, and used a non-parametric method

(Spearman ρ) to estimate significance. The set of species samples with complete elemental analyses was evaluated using a clustering procedure (Ward method) and principal component analysis (PCA) grouping species according to their leaf element composition. All statistical analyses were conducted using JMP v.12 program (SAS Institute, Inc. 2015).

Results

Elemental composition of soil samples

The median soil pH was 7.6, varying from 7.1 to 8.1 in correspondence to the high concentration of Ca (Table 1). The mineral soils contained large amounts of total carbon (C) and N, resulting in a C/N molar ratio of 13. Those elements showed a significant positive linear correlation (Table 2), suggesting that the measured C originated mostly from the vegetation and not from the carbonaceous substrate. This assumption is based on the observed regression between % LOI and total C (% C) (Fig. 1). The regression is linear after eliminating 3 outliers probably influenced by the presence of carbonate residues, and 1 outlier with lower % C than expected. It runs parallel to and nearly overlaps the diagonal line representing a 50% C fraction in the % LOI. Another indication of the strong influence of organic matter in the soil samples is that % LOI is positively correlated with Ca and negatively correlated with Al, Fe, and Mn (Table 2). Concentrations of P and N were relatively high compared to karst soils (Lugo and Murphy 1986), and N to P ratios do not suggest P deficiency. Relative variation (MAD/Median x 100) was lower than 25% for all elements except Ca and Na. The fraction of metallic elements was dominated

Table 1. Composition of mineral soils collected in Mona Island plots (*n* = 21). Concentrations in mmol/kg air dry soil. MAD = median absolute deviation.

Element	Median	MAD
pH	7.6	7.1–8.1
LOI % $_{(490°C)}$	41.6	7.7
C	13,942	3959
N	1043	250
P	62	15
Na	13	3
K	48	9
Mg	171	32
Ca	745	284
Al	1076	107
Fe	741	175
Mn	29	6
C/N	13	1
N/P	16	3
Ca/K	16	9
Ca/Mg	5	2
Ca/Al	1	0.4
Ca/Fe	1	0.6
Ca/Mn	25	14

by Al, Ca, Fe, and Mg, with smaller concentrations of K, Mn, and Na (Table 1). The influence of limestone is shown by high ratios of Ca/Mg (5) and Ca/K (16). Ratios of Ca/Al (1) and Ca/Fe (1) indicate the strong contribution of allochthonous sediments. Inter-elemental correlations were positive and significant for the pairs of Al–Fe, Al–Mn, Fe–Mn, Al–K, K–Fe, and Mn–K, but negative and significant for the pairs Ca–Fe, Ca–Al, Ca–Mn (Table 2). Notably, Mg concentration was not correlated with any of the elements measured.

Table 2. Inter-elemental non-parametric correlations (Spearman ρ) in Mona Island soil samples (n = 21). Concentrations other than in % are in mmol/kg.

Variables	ρ	*P*
C–N	0.922	<0.0001
Al–Fe	0.810	<0.0001
Al–Mn	0.590	0.005
Fe–Mn	0.787	<0.0001
Al–K	0.708	0.0003
K–Fe	0.561	0.0081
Mn–K	0.453	0.0386
Ca–Fe	-0.827	<0.0001
Ca–Al	-0.600	0.0040
Ca–Mn	-0.664	0.0010
%LOI–%C	0.565	0.0076
%LOI–%N	0.673	0.0008
%LOI–%S	0.683	<0.0001
%LOI–Al	-0.643	0.0017
%LOI–Ca	0.843	<0.0001
%LOI–Fe	-0.595	0.0044
%LOI–Mn	-0.610	0.0033

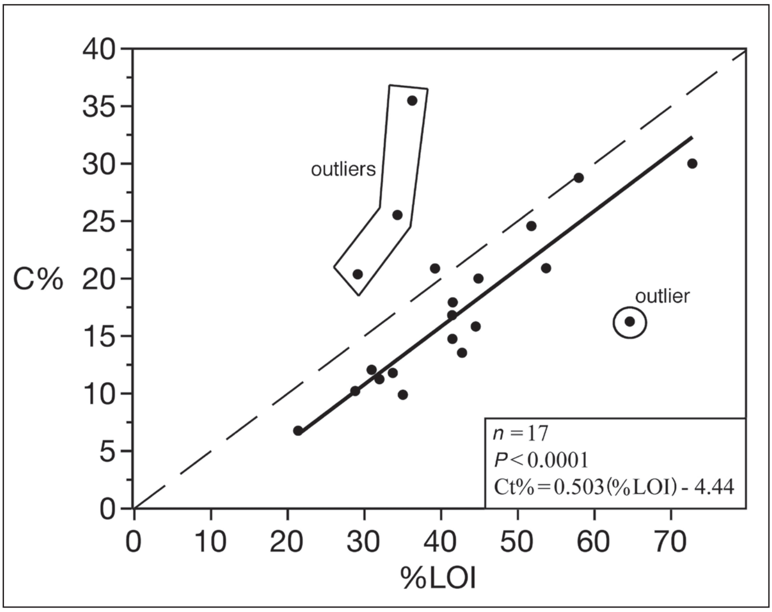

Figure 1. Relationship between % LOI and % total C as measured by combustion. The dashed diagonal indicates the theoretical regression line if the C content of the weight loss due to ignition were exactly 50%.

The biomass and elemental composition of loose litter

A considerable amount of loose litter was accumulated on top of the mineral soils, but it varied substantially from plot to plot and within plots. The total median value amounted to 2.6 kg/m^2 of loose litter (Table 3), consisting of 14% leaves, 6% wood, 19% miscellaneous material, and 49% detritus. The large variability of the loose-litter data set is revealed by the relative variation reaching 45% for the whole sample. There is a significant increase in N and P concentrations in the miscellaneous and detritus fraction compared to the leaf and wood fractions that runs in the opposite direction of C values (Table 4). Concentrations of Al, Mn, and Fe were all higher in the detritus fractions. Concentrations of C and N were not correlated in any of the fractions analyzed, and the C/N ratio was similar in the leaves and wood fractions, decreased markedly in the miscellaneous and still more in the detritus.

Table 3. Median and median absolute deviation (MAD) of loose litter mass and detritus (kg/m^2) in Mona Island (averages of 27 plots). Material on top of mineral soil collected within a 30.48 cm diameter circular frame. Proportion calculated from medians.

Fraction	Median	(MAD)	Relative variation	Proportion (%)
Leaves	0.361	(0.193)	53	14
Wood	0.149	(0.093)	10	6
Miscellaneous	0.501	(0.283)	56	19
Detritus	1.259	(0.816)	65	49
Total	2.580	(1.168)	45	

Table 4. Median and median absolute deviation (MAD; in parentheses) of the concentration of ash (%) and minerals (mmol/kg) in loose litter fractions collected from 27 FIA plots in Mona Island (3 samples per plot). Differences between means tested with non-parametric analysis of variance (Wilcoxon/Kruskall-Wallis: ** <0.0001, *0.0001) and multiple comparisons (Wilcoxon pairwise) as distributions were not normal (Shapiro-Wilk W test), and variances were not homogeneous (Bartlett test). *P* for mean Wilcoxon pair-wise comparison was 0.001, *n* = 27. In-rows, numbers followed by the same letter are statistically similar.

	Leaves	Wood	Miscellaneous	Detritus	χ^2
Ash %	10.0 (1.0)c	11.0 (2.0)c	21.0 (3.0)b	30.0 (3.0)a	86**
C	42,371 (449)a	41,257 (778)b	35,997 (1592)c	32,074 (1910)d	86**
N	814 (79)c	827 (87)c	1421 (89)b	1800 (128)a	88**
P	18 (3)c	13 (3)d	31 (6)b	49 (12)a	80**
Na	31 (7)a	20 (5)b	19 (4)b	15 (3)b	38**
K	37 (8)a	25 (6)b	37 (6)a	42 (8)a	37**
Mg	145 (25)a	89 (22)b	161 (35)a	174 (30)a	46**
Ca	939 (101)b	1103 (124)ab	1445 (196)a	1323 (250)a	51**
Al	32 (7)c	28 (5)c	305 (101)b	587 (115)a	85**
Fe	9 (3)c	5 (2)c	97 (26)b	229 (74)a	87**
Mn	1.2 (0.3)c	1.2 (0.4)c	5.6 (1.2)b	12.0 (3.1)a	76*
C/N	52 (4)	50 (5)	26 (1)	18 (1)	
N/P	44 (9)	60 (10)	43 (9)	34 (10)	

The N to P ratio was similar in the dead leaves and miscellaneous fractions, markedly higher in the wood and lower in the detritus fractions.

The element concentration sequence varied qualitatively from detritus (Ca > Al > Fe > Mg) to leaf litter (Ca > Mg > K = Al) revealing the admixture of soil into the detritus fraction (Al and Fe), and the biological effect of concentrating K and Mg in the leaf fraction.

The dominant non-alkaline metals in soil (Al, Fe, Mn) were well correlated in all fractions of the loose litter and showed a sequence that appeared correlated with the degree of organic matter decomposition. Concentrations of Ca and Al showed weak positive correlations in the leaf litter ($r = 0.25$, ns) and wood ($r = 0.44$, $P = 0.02$) fractions, and negative correlations in detritus (r = -0.44, $P = 0.02$) and miscellaneous ($r = -0.36$, $P = 0.07$) fractions.

Element concentrations and statistical distributions in plant leaves

The set of species analyzed included 47 woody species (trees and shrubs) within 26 families (Table 5). The most common families were Rubiaceae and Euphorbiaceae (5 species each), Myrtaceae (4 species), and Polygonaceae and Rhamnaceae (3 species each). The orders best represented were the Malpighiales (9 species), Gentianales and Sapindales (6 species each), and Caryophyllales, Myrtales, and Rosales (4 species each).

The more frequent species were those from which a larger number of individuals were sampled. We calculated a constancy factor ([(number of plots in which the species was sampled) / (total number of plots) x 100]) as an indicator of the species abundance. The highest constancy values were found for *Coccoloba microstachya* (59.5), *Bursera simaruba* (56.8), *Stenostomum acutatum* (48.6), *Reynosia uncinata* (43.2), *Croton glabellus* (40.5) and *Tabebuia heterophylla* (35.1). These 6 species (13% of the species sampled) contributed 105 individuals, composing 44% of the total number of individuals sampled. Nineteen species were represented by only 1 individual. In this paper, only median element concentration values were used for each species.

The most abundant cation was Ca followed by K and Mg (Table 6). The median of Ca/K and Ca/Mg molar ratios were 2 and 3, repectively, decreasing from 16 and 5, respectively, in the soils. Nitrogen and P median values did not indicate deficiency of these elements, and the median molar N/P ratio was 32. The relative variation of the medians was high (≥40%) for Mn and Na, intermediate (27 to 34%) for P, Ca, Al, Mg, and K, and low (≤22%) for C, Fe, and N. The median for the C/N ratio was 44, well above that estimated for soils (Table 1).

There were several significant correlations among elements (Table 7). Carbon was negatively correlated with % ash, Ca, Al, and Mg, whereas % ash was significantly and positively correlated with the major cations except Na. The highly significant and positive correlation observed between Ca and Al was unexpected, with only 2 species, *Malpighia setosa* and *Randia aculeata*, departing from the regression trend (Fig. 2).

Table 5. Families and species included in the present study (Angiosperm Phylogeny Group; www.mobot.org/MOBOT/research/APweb/). Plants names updated using Axelrod (2011).

Order	Family	Species
Arecales	Arecaceae	*Thrinax morrisii* H. Wendl (Key Thatch Palm)
Boraginales	Boraginaceae	*Bourreria succulenta* Jacq. (Pigeon Berry)
		Varronia bullata L. (Curaçao Bush)
Brassicales	Capparaceae	*Quadrella cynophallophora* (L.) Hutch. (Jamaica caper-tree)
Canellales	Canellaceae	*Canella winterana* (L.) Gaertn. (Canella)
Caryophyllales	Nyctaginaceae	*Pisonia albida* (Heimerl) Britton ex. Standl. (Corcho Bobo)
	Polygonaceae	*Coccoloba diversifolia* Jacq. (Pigeon Plum)
		Coccoloba microstachya Willd. (Pockhout)
		Coccoloba uvifera (L.) L. (Seagrape)
Celastrales	Celastraceae	*Crossopetalum rhacoma* Cranz (Maidenberry)
Ericales	Sapotaceae	*Sideroxylon obovatum* Lam. (Breakbill)
		Sideroxylon salicifolium (L.) Lam. (Willow-bustic)
Fabales	Fabaceae	*Chamaecrista nictitans* (L.) Moench (Sensisitive Pea)
Gentianales	Apocynaceae	*Plumeria obtusa* L. (Singapore Graveyrad Flower)
	Rubiaceae	*Stenostomum acutatum* DC.
		Erithalis fruticosa L. (Black Torch)
		Exostema caribaeum (Jacq.) Roem. & Schult (Albarillo)
		Guettarda elliptica Sw. (Hammock Velvetseed)
		Randia aculeata L. (White Indigoberry)
Lamiales	Bignoniaceae	*Tabebuia heterophylla* (DC.) Britton (Pink Trumpet-tree)
	Verbenaceae	*Lantana involucrata* L. (Buttonsage)
Malpighiales	Erythroxylaceae	*Erythroxylum areolatum* L. (Swamp-redwood
	Euphorbiaceae	*Croton betulinus* Vahl (Beechleaf Croton)
		Croton discolor Willd.
		Croton glabellus L.
		Euphorbia petiolaris Sims (Manchineel Berry)
		Gymnanthes lucida Sw. (Oysterwood)
	Malpighiaceae	*Byrsonima lucida* (Mill.) DC. (Long Key Locustberry)
		Malpighia setosa Spreng. (Brisly Stingingbush)
	Phyllanthaceae	*Phyllanthus epiphyllanthus* L. (Swordbush)
Malvales	Malvaceae	*Melochia tomentosa* L. (Broomwood)
		Corchorus hirsutus L. (Jackswitch)
		Helicteres jamaicensis Jacq. (Scrwtree)
Myrtales	Myrtaceae	*Calyptranthes pallens* Griseb. (Pale Lidflower)
		Eugenia foetida Pers. (Spanish Stopper)
		Eugenia monticola (Sw.) DC.
		Myrcianthes fragrans (Sw.) McVaugh (Twinberry)
Rosales	Moraceae	*Ficus citrifolia* Mill. (Shortleaf Fig)
	Rhamnaceae	*Krugiodendron ferreum* (Vahl) Urb. (Lewadwood)
		Reynosia uncinata Urb. (Sloe)
		Ziziphus taylori (Britton) M.C. Johnst. (Taylor's Jujube)
Sapindales	Anacardiaceae	*Comocladia dodonaea* (L.) Urb. (Posion Ash)
		Metopium toxiferum (L.) Krug & Urb. (Poisonwood)
	Burseraceae	*Bursera simaruba* (L.) Sarg. (Gumbo-limbo)
	Meliaceae	*Swietenia mahagoni* (L.) Jacq. (West Indian Mahogany)
	Rutaceae	*Amyris elemifera* L. (Sea Torchwood)
	Sapindaceae	*Hypelate trifoliata* Sw. (White Ironwood)

Ranking of element concentrations and ratios per species

The species ranked according to their element concentrations followed a quasi-linear pattern with less-steep slopes at both the lower and upper ends of the concentration range (Fig. 3). The species characteristic of the extreme sections for

Table 6. Median and median absolute deviation (MAD) of element concentrations (ash in % and elements in mmol/kg dry mass) in leaves of woody plant species ($n = 47$) from Mona Island. Relative variation in % = MAD/median x 100.

Element	Median	MAD	Relative variation
% ash	8.5	(2.3)	27
C	44138	(1705)	4
N	1039	(220)	21
P	31	(8)	27
Na	52	(26)	50
K	258	(89)	34
Mg	157	(50)	32
Ca	472	(136)	29
Al	3.6	(1.1)	32
Fe	0.6	(0.1)	17
Mn	0.5	(0.2)	41
C/N	44	(11)	24
N/P	32	(7)	22
Ca/K	2	(1)	39
Ca/Mg	3	(1)	28
Ca/Al	140	(11)	8
Ca/Fe	795	(295)	37
Ca/Mn	1050	(477)	45

Table 7. Significant correlations ($\rho \geq 0.40$) between total elements in leaves of woody species from Mona Island in decreasing order of correlation. ($n = 47$)

Element pair	Spearman ρ	*P*
Ca vs Al	0.95	<0.0001
Ca vs ash %	0.89	<0.0001
Al vs ash %	0.86	<0.0001
N vs P	0.69	<0.0001
Mg vs ash %	0.59	<0.0001
K vs ash %	0.56	<0.0001
P vs Na	0.55	<0.0001
N vs Mn	0.54	<0.0001
Mg vs Al	0.57	<0.0001
Ca vs Mg	0.52	0.0002
Mn vs Na	0.46	0.0010
N vs Na	0.41	0.0045
Mn vs P	0.40	0.0053
C vs ash %	-0.76	<0.0001
C vs Ca	-0.75	<0.0001
C vs Al	-0.73	<0.0001
C vs Mg	-0.48	0.0006

each element may be considered excluders or accumulators and differed in concentrations by factors ranging from 4 to 15 (Table 8).

The nutritional constraints of the Mona vegetation may be represented by the availability of N and P, integrated in the leaf N/ P ratios, whereas the relative uptake

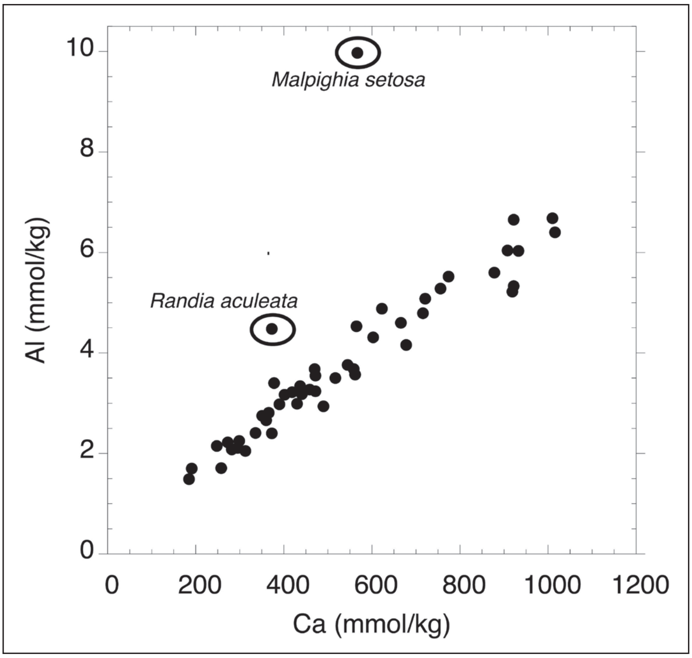

Figure 2. Correlation of Ca and Al concentrations in leaves of woody plants from the vegetation on the Mona Island plateau. Circles indicate outliers corresponding to relative Al accumulators with the species set.

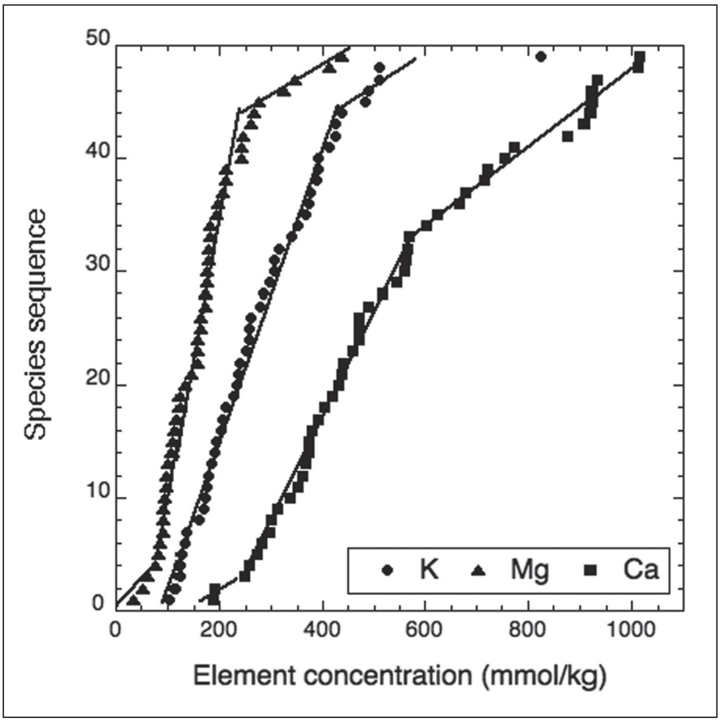

Figure 3. Sequence of increasing cation accumulation of the species analyzed.

of Ca and K under natural conditions is integrated in the leaf Ca/K ratios. Ranked N/P and Ca/K ratios followed a triphasic pattern similar to that observed by ranking of leaf elements (Fig. 4). There was a gradual variation of the N/P ratio from about 24 to nearly 40, with about the same number of species showing ratios above and below 33, the assumed molar threshold for relative P deficiency or excess. Two species had very low ratios, *Erythroxylum areolatum* and *Helicteres jamaicensis*, suggesting potential limitation by N availability. At the other extreme, *Chamaecrista nictitans* and *Quadrella cynophallophora* appeared to be severely limited by P. The Ca/K ratio increased steadily from 1 to 3.5, indicating the regular distribution of Ca uptake capacity among the species assemblage. Three species, *Hypelate trifoliata*, *Eugenia monticola*, and *Tabebuia heterophylla*, had values above 5, whereas *Canella winterana* and *Thrinax morrisii*, with Ca/K ratios well below 1, appeared to exclude Ca from the soil solution.

Species grouping according to elemental composition

The cluster analysis included all elements measured except Na. Concentrations of this element varied strongly probably not because of plant uptake, but due to erratic deposition of marine spray. This analysis rendered 3 well-separated groups of species based on their higher values of N, P, and K (Cluster 1), or the lowest values of Al, Ca, and Mg (Cluster 2), or higher values of Ca, Mg, Al, and Ca/K ratios (Cluster 3) (Table 9). The Ca/K ratio in Cluster 3 was 1.8 times higher than

Table 8. Species with the highest and lowest values of element concentrations (medians, given in mmol/kg dry mass) measured in this study.

	Low		High	
Element	Species	Concentration	Species	Concentration
N	*Sideroxylon obovatum*	419	*Croton betulinus*	1519
	Sideroxylon salicifolum	480	*Chamaecrista nictitans*	1597
P	*Eugenia monticola*	14	*Erythroxylum areolatum*	212
	Sideroxylon obovatum	15	*Helicteres jamaicensis*	212
K	*Chamaecrista nictitans*	104	*Canella winterana*	825
	Erythroxylum areolatum	114	*Sideroxilon obovatum*	510
Mg	*Swietenia mahogani*	34	*Varronia bullata*	436
	Thrinax morrisii	53	*Malphigia setosa*	413
Ca	*Thrinax morrisii*	185	*Sideroxylon obovatum*	1016
	Calyptranthes pallens	191	*Quadrella cynophallophora*	1010
Al	*Thrinax morrissii*	1.5	*Malphigia setosa*	10.0
	Calyptranthes pallens	1.7	*Quadrella cynophallophora*	6.7
Mn	*Byrsonima lucida*	0.1	*Euphorbia petiolaris*	1.3
	Thrinax morrisii	0.1	*Varronia bullata*	1.2
Fe	*Coccoloba uvifera*	0.3	*Thrinax morrisii*	1.8
	Ziziphus taylori	0.3	*Randia aculeata*	1.7

in Clusters 1 and 2. The N/P ratios increased from 29 in Cluster 1, to 35 in Cluster 2, suggesting differences in P availability.

Phylogeny and Ca concentrations

Grouping the species within their corresponding botanical orders and arranging them according to their average Ca concentration showed a systematic variation between orders (Fig. 5). The concentration range of the orders was rather large,

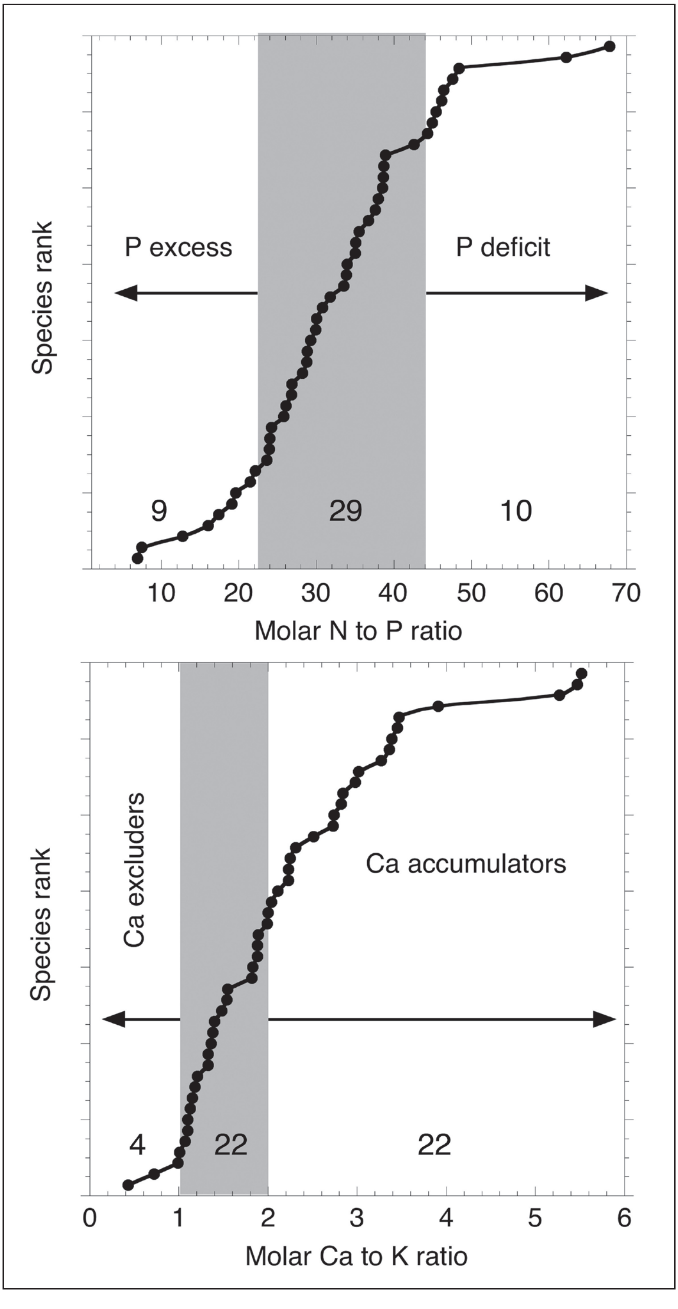

Figure 4. Ranking species according to their N/P ratios (upper panel) and Ca/K ratios (lower panel). The gray rectangles indicate the *P* sufficiency values of N/P in plants in natural environments (Güsewell 2004), and the proposed range of Ca/K values for balanced Ca uptake by plants on karst soils. The numbers at the base of the curves indicate the number of species in each group.

varying from 185 to 1010 mmol Ca/kg. The Brassicales, Ericales, and Lamiales occupied the top, whereas the Fabales and Arecales were located at the bottom of the Ca concentration scale. The other groups, from Caryophyllales to Malpighiales, containing most species, occupied intermediate positions. The strong correlation between Ca and Al concentrations observed in the species set was also revealed in this phylogenetic arrangement (Fig. 5).

Table 9. Species clusters based on correlations (Ward method) obtained from multivariate analyses of leaf mineral composition, excluding Na and % ash, of woody species from Mona Island. Below are the medians of each element in mmol/kg dry mass. Non-parametric analysis of variance (Wilcoxon/Kruskall-Wallis χ^2) and multiple pair-wise median comparisons (Wilcoxon multiple test: $P \leq 0.01$). In rows, numbers followed by the same letter are statistically similar (HSD Tukey-Cramer test: $P = 0.05$).

Cluster 1 (*n* = 16)	Cluster 2 (*n* = 21)	Cluster 3 (*n* = 10)
Amyris elemifera	*Bursera simaruba*	*Coccoloba diversifolia*
Bourreria succulenta	*Byrsonima lucida*	*Coccoloba uvifera*
Canella winterana	*Calyptranthes pallens*	*Crossopetalum rhacoma*
Corchorus hirsutus	*Chamaecrista nictitans*	*Eugenia monticola*
Croton betulinus	*Coccoloba microstachya*	*Hypelate trifoliata*
Croton discolor	*Comocladia dodonaea*	*Malpighia setosa*
Croton glabellus	*Erithalis fruticosa*	*Pisonia albida*
Erythroxylum areolatum	*Eugenia foetida*	*Sideroxylon obovatum*
Ficus citrifolia	*Euphorbia petiolaris*	*Sideroxylon salicifolium*
Gymnanthes lucida	*Exostema caribaeum*	*Tabebuia heterophylla*
Helicteres jamaicensis	*Guettarda elliptica*	
Krugiodendron ferreum	*Metopium toxiferum*	
Lantana involucrata	*Myrcianthes fragrans*	
Melochia tomentosa	*Phyllanthus epiphyllanthus*	
Quadrella cynophallophora	*Plumeria obtusa*	
Varronia bullata	*Randia aculeata*	
	Reynosia uncinata	
	Stenostomum acutatum	
	Swietenia mahagoni	
	Thrinax morrisii	
	Ziziphus taylori	

	Cluster 1	Cluster 2	Cluster 3	Wilcoxon/Kruskall-Wallis χ^2
C	43,085 b	46,031 a	42,301 b	26 ***
N	1356 a	972 b	778 b	26***
P	69 a	29 b	27 b	16**
K	385 a	215 ab	276 ab	13*
Mg	194 a	121 a	219 ab	14*
Ca	618 b	358 c	795 a	28***
Al	4.2 b	2.8 c	5.9a	21***
Fe	0.72	0.75	0.52	ns
Mn	0.70 a	0.49 b	0.32 b	12 *
N/P	29	35	32	ns
Ca/K	1.9 b	1.9 b	3.4 a	8*

***<0.0001; **<0.001; * <0.01.

Discussion and Conclusions

Soils and loose litter

The soils sampled in Mona island were very shallow, usually 10 cm deep or less, forming on highly calcareous materials. These soils, known as leptosols, are the dominant soils in tropical karstic areas, characterized by very fragmented soil-relief patterns (Bautista et al. 2005). Their high content of Al and Fe suggests that they cannot simply have formed through weathering of the underlying calcareous rock constituted mainly by Ca and Mg carbonates. Studies on this type of lateritic sediments in several Caribbean sites indicate the contribution of allochtonous materials derived from erosion of volcaniclastic rocks or eolian dust (Briggs 1966, Goldich and Bergquist 1947, Merino and Banerjee 2008). The heterogeneity of this soil type is caused mainly by topography of the underlying rock (Zhang et al. 2014).

Anchoring roots of trees and shrubs grow through cracks and crevices in limestone rock, thereby reaching mineral soil accumulated below the surface. We assumed that the nature of soil accumulated in rock cracks has a similar composition to that of the samples obtained from the plots below the layer of loose litter. The mineral soil samples had relatively large concentrations of C and N, revealing the contribution of organic matter from the aboveground vegetation. The molar C/N (13) and N/P (16) ratios indicate high potential availability of N, a fact probably related to the accumulation of loose litter and detritus on top of the mineral soil. The elements Al, Mn, and Fe are strongly positively correlated, as would be expected from sediments derived from volcanic rocks, while the negative and significant

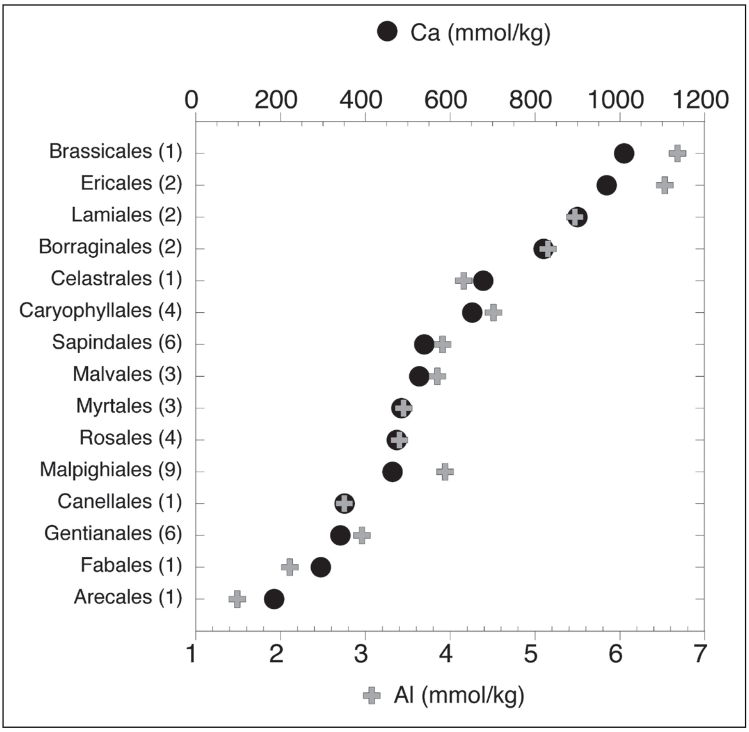

Figure 5. Average concentrations of Ca and Al by botanical order arranged from high to low. The number of species included is given in parenthesis after the order name.

correlations of Ca with these elements reveal the contribution of the underlying carbonaceous rock. The comparison of elemental composition of karst rocks from Puerto Rico (calculated from Monroe 1980) and the averages of the soil samples reported here, shows that concentration of Ca is much higher, whereas that of Al, Fe, and P are much lower than those measured in Mona Island mineral soils (Fig. 6). This pattern suggests the contribution of allochthonous sediments.

The nutrient storage in Mona Island mineral soils is difficult to calculate because there is only a shallow layer on top of the limestone, and certainly most of this material is irregularly distributed within cracks and crevices. Only in the so-called "bajuras", depressions characteristic of the karst landscape, do thicker layers of this mineral soil accumulate more homogenously (Brandeis et al. 2012, Woodbury et al. 1976). Recently, Meléndez-Ackerman et al. (2016) published a comparative study of mineral soils from sites on the plateau and on the depressions in Mona Island. Their analysis of variance showed significant differences between plots for P, Mg, and K, although not associated with site position (plateau vs depression). The average values for plateau sites, except for K, are almost identical to the median values reported here. In the Meléndez-Ackerman et al. (2016) data set, Al and Fe are positively correlated, and both elements are negatively correlated with Ca, a clear indication of the variable proportions of the mixture of 2 substrates of different origin. In addition, as confirmed by results of this study, C and N concentrations showed a highly significant positive correlation. Data from a dry forest in Guánica, PR (Lugo and Murphy 1986), showed much lower concentrations of N, P, K, and Ca than Mona Island soils, probably because those samples were richer in organic carbon. Organic matter (% LOI) and N contents of Mona soils were also markedly

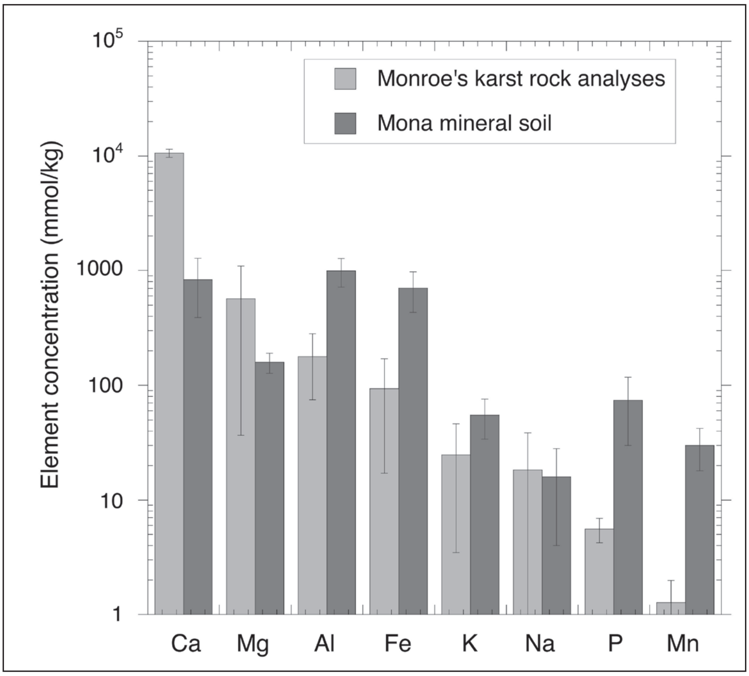

Figure 6. Comparison of average concentrations of elements in rocks from 5 karstic formations in northern Puerto Rico (calculated from Monroe 1980), and 21 mineral soil samples from Mona island.

higher than superficial soils in moist karst from Puerto Rico (Viera Martínez et al. 2008), and a subtropical karst forest in southwest China (Zhangh et al. 2014). Similarly, concentrations of Mg, Ca, and Al, are considerably higher, while those of Fe and Mn are similar, in Mona soils compared to those of the moist karst site in Puerto Rico (Viera Martínez et al. 2008). Causes of these variations are probably related to sediment age, local climate, and vegetation development, but the subject requires a detailed geochemical study for a definitive assessment.

Erickson et al. (2014) analyzed a comprehensive data set of loose-litter biomass and element concentrations in Puerto Rico, which included forests in different climatic zones (humid vs dry), on different geology (karst vs non-karst), and varying ages of secondary succession. They concluded that forests on karst geology, both humid and dry, were separated from non-karst forests by their lower concentrations of P, Al, Mn, and Fe, but higher concentrations of C and Ca, in fallen leaves. In addition, deciduous karst forests differed from evergreen only by their lower C and higher Ca concentrations. The mass of the leaf fraction of loose-litter data from Mona is slightly higher than the values reported by Erickson et al. (2014), but its significance is doubtful considering the variability of the Mona values. However, the total mass of loose litter measured in the Mona sites is about 4 times higher than the values of "forest floor" reported by Erickson et al. (2014). The difference is due mainly to the detritus fraction in Mona samples, which was more than twice as high as the total figures from Puerto Rico karst forests.

In the forest plots of Mona Island, the loose-litter layer amounted to 2.6 kg/m^2. Using the medians of mass (Table 3) and element concentrations of loose litter (Table 4) we estimate that this layer stores per m^2, 964 g of C, 48 g of N, 2.7 g of P, 3.5 g of K, 8.9 g of Mg, and 116 g of Ca. These nutrients are surely mobilized through weathering and organic matter decomposition during humid periods, explaining the proliferation of superficial fine roots observed in several plots by the FIA-IITF team. The fractions of loose litter showed a gradient in C/N ratios from 18 in the detritus, like that calculated for the soil samples, up to 52 in the leaf samples. Similarly, the N/P ratios increased from 34 in the detritus, much larger than the corresponding values for soil samples, up to 44 in the leaf samples. The low values of both ratios in the detritus reveal that this fraction was admixed with mineral soil, richer in P and poorer in C. The fallen leaves fraction from evergreen karst forest plots in Puerto Rico (Erickson et al. 2014), showed higher N, but lower C and P concentrations than those from Mona plots, whereas the deciduous karst forest plots where similar in C and P, and higher in N. As a result, the C to N ratios of the karst plots in Puerto Rico varied from 32 to 35, much lower than the value for Mona plots (52), while the N to P ratios vary from 68 to 78, much higher than the value calculated for Mona plots (44). The latter figures suggest stronger potential P limitation in the Puerto Rico karst forests compared to the woody plant assemblages in Mona Island. In the Mona samples, median concentrations of K, Al, and Fe were lower but Mg was higher than the average of the samples from Puerto Rico. This finding suggests that the influence of volcanic sediments is larger in the Puerto Rico karst, whereas the presence of dolomite influences Mg concentration of leaf litter in Mona Island.

The elemental profile of loose-litter fractions showed the changes occurring during the decomposition process, separating the substrate-dominated (detritus) from biotic-dominated (dead leaves and wood) components. Concentrations of Al, Fe, and Mn reveal the contribution of bauxitic sediments, whereas that of Ca results from weathering and dissolution of the carbonaceous substrate. Depiction of the Al–Fe concentrations of the loose-litter fraction plus soil showed that those elements are linearly correlated on a log-log scale in all compartments (Fig. 7A). The Al–Ca relationship tells a different story; it is negative when the inorganic component predominates, but positive in the organic component (Fig. 7B). Variations in concentrations of Al between inorganic and organic components are between 1 and 2 orders of magnitude. Variations in Ca concentration in the inorganic component vary uniformly, indicating different contributions to the mineral components from the carbonaceous rock and the bauxitic sediments as discussed in the soil section.

Canopy leaves

The overall spectrum of elemental composition of Mona tree leaves shows the predominance of Ca, followed by K and Mg, and comparatively high concentrations

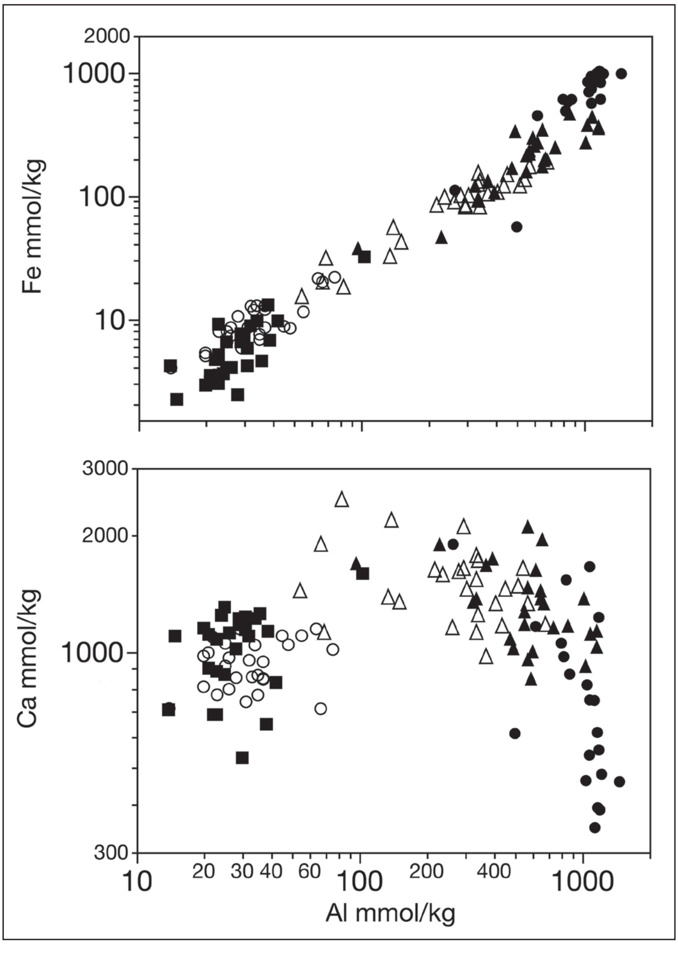

Figure 7. Biogeochemistry of loose litter: (A) Al–Fe relationship, (B) Al–Ca relationship. Concentrations of elements are depicted in log scales to account for the large variations of Fe and Al. Filled circles = soil, filled triangles = detritus, empty triangles = miscellaneous, empty circles = dead leaves, and filled squares = wood.

of C and N. Phosphorus and N are positively correlated, although not as strongly as expected given the role of P in regulating the efficiency of N use for growth and photosynthesis (Marschner 1995, Medina 1984).

Comparison of the Mona leaf data set to the reference values for nutrient concentrations in leaves required for normal functioning (Table 10; Marschner 1995) confirms the expected deficiencies in P, Fe, and Mn, but not in K, whereas their levels of Ca and Mg are well above the reference values. Leaf P concentration reported for a forest on karst in Guánica, PR (Table 10), is much lower than that of Mona island, resulting in N/P ratios indicative of stronger P limitation in the Guánica forest. A recent study of woody plants from karst forests in southwestern China provides a wider nutrient basis for comparison with Mona plants (Liu et al. 2014). In that study, average N and P concentrations were slightly higher than what we found for the average of Mona leaves, but N/P ratios are almost identical (Table 10). The concentrations of alkaline elements were also similar, but the plants from China karst showed lower Ca to K and Ca to Mg ratios, and were not deficient in Mn and Fe (Table 10).

Compared with a set of 43 tree species from a lower montane forest on volcanic substrate in Puerto Rico (Ovington and Olson 1970), the plants from Mona Island have similar concentrations of N, K, and Mg, but higher concentrations of P and Ca (Table 10). Therefore, the Ca/K and Ca/Mg ratios are much higher, whereas the N/P ratio is much lower in Mona plants. In addition, concentrations of Al and Fe are well below those of plants from volcanic substrates in Puerto Rico (Medina 2015).

A general conclusion is that Mona vegetation appears to be less restricted by P availability, contrary to the expectation of P deficiency associated with the abundance of carbonate and the high pH of the Mona soils. However, the N/P ratios distribution revealed that about half of the species were potentially restricted by

Table 10. Average element concentration in leaves (mmol/kg) found in different studies.

	Reference Values[A]	Lower montane rain forest (43 spp.)[B]	China karst (121 spp.)[C]	Jamaica karst dry forest[D]	Guánica, PR composite[E]	Mona Island (47 spp.)
N	1000	1154	1224	1158	1171	1061
P	60	26	47	19	21	42
K	250	283	210	261	199	286
Ca	125	269	517		-	540
Mg	80	157	125		-	167
Fe	2.0	-	2.5		-	0.7
Mn	1.0	-	1.6		0.5	
N/P	17	44	26	62	57	25
Ca/K	0.5	1.0	2.5		-	1.9
Ca/Mg	1.6	1.7	4.2		-	3.2

[A] Marschner 1995; [B] Ovington and Olson 1970; [C] Liu et al. 2014; [D] Kapos 1982; [E] Lugo and Murphy 1986.

P availability, as indicated by values above 33 (Güsewell 2004, McGroddy et al. 2004, Medina 2015). Possible explanations for this result are related to the accumulation of loose litter that could act as a slow, but reliable source of P for fine roots.

The Ca/K ratios based on total element content do not allow a characterization of calciotrophic behavior in the sense of Kinzel (1983). However, despite the apparent large differences in availability of Ca and K, the Mona species seemed to obtain enough K from the substrate, as 27 of 49 species showed Ca/K ratios below 2, and 3 species had ratios below 1. The proportion of soluble and insoluble fractions of Ca in plant leaves remains to be analyzed, which would enable the occurrence of true calciotrophs species to be established.

The linear Ca–Al relationship was unexpected and requires a mechanistic explanation. In Mona soils, the amount of total P (62 mmol/kg) was higher than that reported for a karst forest in Puerto Rico (18 mmol/kg; Lugo and Murphy 1986) and a subtropical karst forest in China (51 mmol/kg; Zhang et al. 2014). Plants extract P from those soils by secreting organic acids into the rhizosphere, or through symbiotic associations with micorrhizal fungi (Marschner 1995). Rhizosphere acidification mobilizes otherwise insoluble Ca ions, and possibly the P associated with Ca salts. By the same process, insoluble Al from the bauxitic sediments may be solubilized (Tyler 1994). At high Ca concentrations, uptake of this cation increases in proportion to the rate of water transport from the soil to the transpiring leaves. A similar situation develops with Al, because higher plants cannot hinder efficiently the uptake of this cation through growing roots. This process could generate the observed positive correlation between Ca and Al. The cases of P and K are completely different as the roots can take them up efficiently even from highly diluted soil solutions. Phosphorus and K are transported by specific mechanisms, whereas Fe uptake depends on siderophores (Morrissey and Guerinot 2009); therefore, the correlations with Ca and these elements are not so strong. Other processes may also be involved, such as the presence of P solubilizing bacteria, that will also mobilize Al if present in the phosphate rock, and mycorrhizal associations that facilitate P uptake from low-P soils (Read et al. 1976). Literature on both subjects in calcareous soils in the tropics is scarce (Robles-Torre 1999). However, those mechanisms may provide explanations for the tolerance of plants to high-Ca environments.

In a previous analysis, vegetation of the Mona Island plateau was separated into 4 clusters based on concentrations of C, N, lignin, and ash (Medina et al. 2014). The classification in the present paper using 7 mineral nutrients and Al is more informative and identifies 3 well-defined clusters. Cluster 1 is constituted by species with higher concentrations of N, P and K, without apparent P restrictions (N/P ratio = 28) and Ca/K ratios approaching one. On the opposite side, Cluster 2 has a N/P ratio of 37, suggesting potential restrictions in P availability and a Ca/K ratio approaching 3. The true calciotrophic species may be found in this latter cluster, which deserves further experimental analysis. Cluster 3 contained the larger number of species and was characterized by lower concentrations of K, Mg, Ca, and Al. This group of species probably is less capable of dissolving the carbonate substrate, contrasting physiologically with species from Cluster 2.

To fully understand the ecological significance of this species clustering, it would be necessary to establish if the species groups segregate in the field. That is, if they occur as differentiated plant assemblages. The data for that analysis is lacking. However, the sequence of species with highest constancy numbers, showed a characteristic distribution. The 4 most abundant species (*C. microstachya, B. simaruba, S. acutatum* and *R. uncinata*) all belong to Cluster 3, the fifth (*C. glabellus*) and the sixth species (*T. heterophylla*) belong to the Clusters 1 and 2, respectively. The implications of this distribution cannot be assessed with available data.

Broadley et al. (2004) carried out a greenhouse experiment growing 117 angiosperm species (mostly herbaceous), selected to represent a balanced proportion of species within 24 orders of eudicots and 5 orders of monocots. The authors concluded that "the calcium concentration of plant shoot tissues varies systematically between angiosperm orders" (Broadley et al. 2004:321). The highest Ca concentrations were found in the orders Cucurbitales, Brassicales, and Malvales (>600 mmol Ca/kg dry mass), whereas the lowest values were measured in commelinoid monocots (Poales and Arecales). Our data set contains woody species from 14 angiosperm orders in common with the study of Broadley et al. (2003) (Table 5). The arrangement of orders according to their average Ca concentration in our study showed that Ca concentrations indeed "varies systematically between orders", but the sequence overlaps only partially with the greenhouse study. In the latter, the concentrations of the orders in common vary from 260 to 699 mmol Ca/kg, whereas in our field study the variation is 185 to 1010. In agreement with Broadley et al. (2003) results, the Brassicales and Lamiales are at the top, whereas the Arecales are at the bottom of the Ca concentration scale. The Rosales, Malpighiales, and Sapindales occupy nearly the same position in both data sets. In contrast, the Fabales are at the upper end in the greenhouse study, and at the lower end in the our field data, while the contrary is the case of the Ericales. Thus, it appears that Ca concentrations in leaf tissues are in part related to the phylogenetic group. However, by some groups, Ca concentration is well regulated, while in others it appears to depend on the level of Ca availability (Ericales, Malvales). The strong correlation between Ca and Al concentrations is revealed also in this phylogenetic arrangement of our data set (Fig. 5).

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