

# ECOSPHERE

# Controls on fallen leaf chemistry and forest floor element masses in native and novel forests across a tropical island

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**Abstract.** Litter chemistry varies across landscapes according to factors rarely examined simultaneously. We analyzed 11 elements in forest floor (fallen) leaves and additional litter components from 143 forest inventory plots systematically located across Puerto Rico, a tropical island recovering from large-scale forest clearing. We assessed whether three existing, independently-derived, landscape classifications (Holdridge life zone, remotely sensed forest type (leaf longevity combined with geology generalized to karst vs. non-karst), and plot-based measures of forest assemblage) would separate observed gradients. With principal component and regression analyses, we also tested whether climate-, landscape- (geology, elevation, aspect, percent slope, slope position, distance from coast), and stand-scale (tree species composition, basal area, density, stand age) variables explained variation in fallen leaf chemistry and stoichiometry. For fallen leaves, C, Ca, Mg, Na, and Mn concentrations differed by Holdridge life zone and C, P, Ca, Mn, Al, and Fe concentrations differed by forest type, where leaf longevity distinguished C and Ca concentrations and geology distinguished C, P, Ca, Mn, Al, and Fe concentrations. Fallen leaf C, P, Ca, and Mn concentrations also differed, and N concentrations only differed, by forest assemblage. Across several scales, fallen leaf N concentration was positively related to the basal area of putatively N2-fixing tree legumes, which were concentrated in lower topographic positions, providing for the first time a biological explanation for the high N concentrations of fallen leaves in these locations. Phosphorus concentrations in fallen leaves by forest assemblages also correlated with the basal area of N2-fixing legumes, and P and N concentrations decreased with mean age of assemblage. Fallen leaves from younger (<50 yr, 86% of the plots) and often novel forests had higher P, Fe, and Al and lower C concentrations and lower C/P and N/P ratios than fallen leaves from older forests, the latter due to a decrease in P rather than changes in N. These findings suggest that both N and P availability may currently be greater on the island than pre-deforestation, and substantiate the unique roles that state factors play in contributing to the spatial heterogeneity of fallen leaf chemistry.

**Key words:** C/N ratio; forest floor mass; landscape analyses; leaf litter chemistry; N/P ratio; novel forests; precipitation effects; stand age; stoichiometry; subtropical forests; tropical legumes; variance analysis.

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### INTRODUCTION

Leaf litter is a major input of carbon (C) and mineral nutrients to forest soils. The concentrations of elements in litter and how they vary in the context of one another (i.e., their stoichiometry) influence key ecosystem processes such as primary productivity and decomposition (Kaspari et al. 2008, Wieder et al. 2009, Barantal et al. 2012), trophic interactions (Gessner et al. 2010), soil gas release (Erickson et al. 2002), and soil C storage and turnover (Bréchet et al. 2009, Posada and Schuur 2011). Knowing how and why leaf litter chemistry varies within and across landscapes is crucial for understanding broad-scale patterns of soil fertility and biogeochemical cycling.

In the tropics, agricultural use after deforestation is often temporary, leading to abandonment followed by forest regrowth. Yet a solid understanding of how these dynamics influence litter chemistry is missing. As tropical secondary forests increase, we need to understand their effects on ecosystem processes (Brown and Lugo 1990, Guariguata and Ostertag 2001, Myster 2004).

Forests on the Caribbean island of Puerto Rico are recovering from almost complete clearing for agriculture a century ago; by 2003 forests covered nearly 60% of the island (Brandeis et al. 2007). Some of these secondary forests are novel communities formed from naturalized tree species, including symbiotic dinitrogen (N<sub>2</sub>)-fixing tree legumes (cf. Chinea and Helmer 2003, Lugo and Helmer 2004, Martinuzzi et al. 2013) which have greater litter nitrogen (N) concentrations than non-fixing legumes (Pons et al. 2007, Fyllas et al. 2009). As well, stands with N<sub>2</sub>-fixing tree legumes have elevated leaf litter N (Erickson et al. 2002, Hughes and Denslow 2005, Siddique et al. 2008).

Litter chemistry is also influenced by plant species other than tree legumes (Cuevas and Lugo 1998, Townsend et al. 2007, Cornwell et al. 2008, Hättenschwiler et al. 2008), by green leaf chemistry and the degree of nutrient resorption before leaf drop (Lugo 1992, McGroddy et al. 2004, Hättenschwiler et al. 2008, Richardson et al. 2008, Inagaki et al. 2011), and by soil nutrient availability (Vitousek and Sanford 1986, Richardson et al. 2008). Soil nutrient availability, in turn, is regulated by the combination of climate, substantial biological diversity (in the tropics), wide ranges in topographic relief and parent material, and by the type and timing of disturbance (Jenny 1941, Townsend et al. 2007, 2008, Hättenschwiler et al. 2008).

Despite the importance of leaf litter chemistry, only a few studies (e.g., McGroddy et al. 2004, Yuan and Chen 2009) characterize litter chemistry at landscape or larger scales. At these scales, the diverse and interacting factors that potentially influence litter chemistry, while complex, can be simultaneously considered to learn which of these emerge as prominent regional controls on litter chemistry. For example, some litter elements may be strongly influenced by climate (see H1 below), while others may be influenced by both climate and parent material (see H2 below).

Concentrations of nutrients and their ratios in leaf tissues may also serve as indices of nutrient limitation of plant growth (Vitousek 1984, Medina et al. 1994, Cuevas and Lugo 1998, Tanner et al. 1998, Güsewell 2004, Townsend et al. 2007, Richardson et al. 2008, Vitousek et al. 2010). Thus, characterizing broad-scale gradients in litter chemistry may provide insights into potential nutrient limitation across landscapes. For example, low litter P concentrations or high litter C/P ratios likely reflect low soil P availability (e.g., Silver 1994), which is widely believed to limit productivity on highly weathered and stable soils in lowland tropical forests (Vitousek 1984, Vitousek et al. 2010). The prolonged and elevated rainfall typical of humid tropical forests leads to loss of rock-derived P and base cations (Jenny 1941, Walker and Syers 1976, Porder and Chadwick 2009). However on unstable surfaces where erosion exposes and transports less weathered soils downslope, P availability may be enhanced (Silver et al. 1994, Scatena and Lugo 1995, Vitousek et al. 2003, Porder et al. 2005, Wu et al. 2007, Mage and Porder 2013) and potentially less limiting to growth.

Parent material also contributes to nutrient availability (Barthold et al. 2008, Mage and Porder 2013) by regulating both initial concentrations and supply rates. In the Caribbean for example, karst soils, derived from carbonatebearing minerals, are common on many islands and influence the availability of various elements, including P, via pH-related reactions. As well, N<sub>2</sub>-fixing legumes may enhance P availability under some circumstances (Hughes and Denslow 2005, Houlton et al. 2008, Nardoto et al. 2008). Nitrogen availability is often high in lowland tropical forests (Vitousek 1984, Vitousek and Sanford 1986) and apparently is little affected by rainfall (Townsend et al. 2007, Rentería and Jaramillo 2011). Low N availability however is thought to limit primary productivity in montane environments due to temperaturelimited N mineralization (Vitousek and Sanford 1986, Tanner et al. 1998, Fisher et al. 2013), on young geologic substrates where N has not yet accumulated (Vitousek and Farrington 1997), and in forests previously cleared and burned that experience high N loss (Davidson et al. 2004). While nutrient additions are needed to confirm postulated limitations, exploring relationships across landscapes may lead to new hypotheses.

Taken together then, we might expect leaf litter chemistry to reflect these various drivers, i.e., litter P concentrations would be greater in less weathered environments (see H1 below), in disturbed forests stands (see H4 below), and in lower landscape positions (see H5 below) while litter N concentrations would be greater at lower elevations, in stands with N<sub>2</sub>-fixing tree legumes (see H3 below) and in older stands (see H4 below). Similarly, we might expect increasing litter N/P ratios as stands age and P becomes less available and N cycling recovers (see H4 below; Davidson et al. 2007), and as elevation decreases, broadly reflecting potentially greater P over N limitation in these contexts. We emphasize controls on litter N and P as they are considered the elements most limiting in tropical systems (cf. Tanner et al. 1998). Yet, the geographical extent and identification of limiting nutrients across much of the tropics is largely unknown (Wright et al. 2011).

Puerto Rico presents an exceptional opportunity to examine controls on litter chemistry. Steep topographic and climatic gradients exist which, when combined with various geologic substrates and widespread forest recovery, yield drivers relevant for much of the tropics. Together with factors widely regarded to affect nutrient availability in the tropics, we developed the following five hypotheses regarding the variation in leaf litter chemistry across the island, and present complementary hypotheses in the discussion. We take advantage of forest inventory plots systematically located across Puerto Rico to characterize this large-scale variation in litter chemistry. We analyze chemistry from mixed-species leaves (hereafter 'fallen leaves') and other litter components collected from the forest floor. We also assess the utility of using the systematic samples for characterizing fallen leaf chemistry and forest floor element masses.

H1: Fallen leaf concentrations of the base cation elements (Ca, Mg, K, Na) and P will decrease as rainfall increases across the island. H2: Fallen leaf concentrations of Ca, P, Fe, Mn, and Al in karst will be distinct from non-karst regions due to distinct chemical processes (i.e., adsorption, fixation, precipitation) occurring in their soils.

H3:  $N_2$ -fixing tree legumes, as they occur in secondary forests, will show a positive correlation with fallen leaf N and possibly P concentrations.

H4: Fallen leaf N concentrations will increase and P concentrations decrease as forest stands recover from agricultural abandonment, leading to the highest fallen leaf N/P ratios in older forests.

H5: Fallen leaf P concentrations will be greatest in lower landscape positions.

Classifications of landscapes, based on any number of criteria (e.g., climate, potential or current vegetation, bedrock geology, or combinations, etc.), may be useful for summarizing litter properties if differences exist between categories. We considered three independent, non-hierarchical classification schemes (additional details in methods) to describe the patterns of litter chemistry across Puerto Rico: (1) Holdridge life zones, (2) forest types as combined with coarse-scale geology, and (3) forest tree species assemblages as identified by Brandeis et al. (2009) from plot-scale data. These classifications lend themselves to a ready familiarity and hence may be effective as teaching tools and as a way to scale properties across landscapes.

# **M**ethods

## Island description

Puerto Rico (17°45′ N, 66°15′ W) encompasses

a biologically diverse 8,800 km<sup>2</sup> of land in the Caribbean. Mean annual precipitation (MAP) ranges from <700 mm in the southwest to >4500 mm in the Luquillo Mountains in the northeast. Geology is complex and includes uplifted calcareous marine sediments (karst) and extruded volcanics. Nine soil orders have been identified and tree species diversity is high (Brandeis et al. 2007, 2009).

Moist broadleaf evergreen and seasonal evergreen forests (hereafter 'evergreen') dominate. Other forest types include drought deciduous and semi-deciduous (including semi-evergreen) forests (hereafter 'semi-deciduous' or SD), and cloud forests at the highest elevations above the cloud condensation level. All forests are subtropical sensu Holdridge (1967). Tree species composition varies with climate and geology (Ewel and Whitmore 1973), and with previous land use (Aide et al. 1996, Thompson et al. 2002). At the landscape scale, environmental factors interact with previous land use to determine species composition (Chinea and Helmer 2003, Brandeis et al. 2009).

## Study plots and forest floor data

Given the high tree species diversity in the tropics, a challenge is to understand the effects of multiple species on litter chemistry. Leaves collected from litterfall traps or from the forest floor offer the advantage of reflecting in-situ species abundances. As well, the collection of fallen leaves enables sampling of widely-spaced plots relatively quickly compared with the greater time needed for installing, maintaining, and collecting samples from litterfall traps across a large landscape. A potential caveat is that uneven leaching of nutrient elements (cf. Schreeg et al. 2013) and inconsistent decomposition across environmental gradients may contribute to unaccounted-for variation in fallen leaf chemistry; we address this in the discussion. We focus on the chemistry (concentrations) of fallen leaves in our analyses because (1) litterfall is usually dominated by leaves (except during storms, Silver et al. 2004), (2) fallen leaf chemistry likely reflects nutrient availability better than total forest floor chemistry or element mass, and (3) fallen leaf chemistry is less variable than forest floor element mass (see Results).

We used an extensive and unbiased dataset

from forest inventory plots systematically located across the island, coupled with plot-scale information on climate, topography, geology, tree species composition and forest age, to describe and examine hypothesized controls on litter chemistry. Forest floors (O-horizons) were collected once from 147 forested plots over a 3-year period (2001–2003) largely following FIA (Forest Inventory and Analysis) protocol. Thus the data include an unknown amount of temporal variation that cannot be accounted for in statistical analyses (see discussion). The FIA plots (0.067 ha) are located in the center of 2428 ha hexagons systematically placed across the island (Bechtold and Scott 2005). Nested within the plots are four systematically located 7.32 m radius subplots for sampling forest floors (O'Neill et al. 2005).

Depending on year, O horizons were collected from either 3 or 2 subplots, which were processed separately. Organic materials (excluding wood >5 mm diameter) were collected from a 30.48 cm diameter circular area and placed in paper bags. Sampling occurred during the typical dry season island-wide (January–July) when leaching losses are likely low. There were no hurricanes over the collection period, which may have added nutrient-rich green leaves to the forest floor.

Forest floor samples were air-dried at the IITF-USFS lab in Río Piedras, PR, and then separated into leaves, wood, fruits and flowers, and an "other" category. Fractions were dried at 65°C for at least 48 h, weighed to the nearest 0.1 g and ground using a Tecator grinding mill (18 mesh). Total C, N and S were determined for each fraction by combustion using a LECO-CNS 2000 Analyzer and P, Ca, Mg, K, Na, Mn, Al, and Fe by a plasma emission spectrometer (Spectro Ciros ICP) after digestion in concentrated nitric and hydrochloric acids and 30% hydrogen peroxide (Luh-Huang and Schulte 1985). Results are reported on an oven-dry (105°C) mass basis.

To calculate litter and element masses for each plot, masses of litter fractions and elements were first calculated for the subplots (element mass for each fraction = element concentration  $\times$  mass for that fraction). If full chemical analyses could not be conducted on a subplot due to small amounts of litter, rather than code the element mass as missing, which would result in an undesirable

bias away from plots with low masses, concentrations from an adjacent subplot were used to calculate element masses. (We note this was only done for mass calculations and not in the concentration only dataset). Fractional masses were then summed for each subplot and converted to an area basis. We used plot means for all analyses, except for the nested ANOVA where subplot values were used.

# Landscape, climate, and plot-scale data

FIA plot center coordinates were used to extract the landscape and environmental attributes of each plot from digital maps of climate, topography, geology, historical land-cover, and nearest distance to a coastline, potentially important for base cations (Whipkey et al. 2000). Climate data included 30-yr average annual precipitation and temperatures (Daly et al. 2003). Topographic variables, extracted from 30m digital elevation models (Gesch et al. 2002), included elevation, aspect, percent slope, and slope position (Hatfield 1999). Slope positions, originally coded as numeric, were converted into three classes: 0-25 = valley bottoms, 26-84 =hillslopes, 85-100 = ridgetops, and checked for reasonableness by visually comparing the digital elevation model with aerial photos. Land-cover data included forest type in 2000 and forest age class (Kennaway and Helmer 2007; see Landscape classifications). Generalized geology came from several maps (Garrison et al. 1972, Krushensky 1995). Stand-scale data comprised forest structure information (species identity, density, and basal area) obtained from direct measurements.

#### Landscape classifications

We considered three independently derived, non-hierarchical classification schemes, as well as forest age class. Because the plots were systematically located across the island and categories within the classifications are not equally distributed across the landscape, sample sizes across categories within a single classification scheme are not equal; sample frequency roughly approximates the proportional representation of a category across the island.

*Holdridge life zone.*—Holdridge life zones (Ewel and Whitmore 1973) are climatically derived, based on MAP, bio-temperature, and potential evaporation. The plots represent four subtropical forested life zones: dry, moist, wet, and lower montane wet, indicating increasingly wetter and colder climates (Table 1). Across the life zones a geologic gradient also exists; 77% of the plots in the subtropical dry life zone are located on karst, while only 4% of the plots in the subtropical wet life zone are on karst.

Forest type. – Forest types were assigned from a map of forest type and land cover for the year 2000 that was produced with Landsat image mosaics (Kennaway and Helmer 2007). Based mainly on leaf longevity (evergreen versus semideciduous (SD)) and broad-scale geology, they include lands with  $\geq 25\%$  tree canopy cover. Plots with 10-25% tree cover, designated as forests in FIA but as other land-cover types in the imagederived map, were assigned a forest type based on the forest type of pixels surrounding the plot. Five plots were dropped from analyses: 2 plots with minimal forest cover classified as low density urban or agriculture, and 3 plots on ultramafic substrates. Evergreen on karst (3 plots) was combined with seasonal evergreen on karst. The 5 remaining forest types considered were SD forests on karst (n = 14), SD forests on other substrates (n = 11), everyreen forests on karst (n = 24), evergreen forests on other substrates (n = 90), and cloud forest (n = 4).

Forest assemblage and tree species information.— Because species distributions are rarely random across landscapes (Clark et al. 1999, Condit et al. 2000), we used an aggregation of forest plots into forest assemblages, i.e., forest communities, to simplify testing for the effects of tree species on fallen leaf chemistry. Fourteen forest tree species assemblages were derived from plot-scale forest inventory data (Brandeis et al. 2009), where plots were assigned species assemblages based on the relative dominance (basal area for each species divided by total basal area) and relative density (trees per hectare for each species divided by total trees per hectare) in each plot with hierarchical agglomerative clustering of importance values (average of relative dominance and relative density). From the species lists at each plot, we identified putative N2-fixing legume genera based on a review of the literature (Appendix: Table A1). We then calculated relative dominance, relative density, and importance values for each functional group (N2-fixers [or simply 'legume'] and non-fixers) at each plot.

Landscape classification	n	MAP (mm)	MAT (°C)	Elevation (m)	Mid-point age (years)†	Geology index‡	N2-fixing legumes§
Holdridge life zone							
Subtropical dry	12	995	25.6	97	23	0.23	49
Subtropical moist	73	1749	24.4	229	29	0.63	19
Subtropical wet	56	2050	22.9	472	24	0.96	14
Subtropical lower montane	2	3080	20.8	740	37	1	36
Forest type							
SD Karst	14	1058	25.6	81	23	0	36
Evrg Karst	24	1870	24.4	240	32	0	4
SD Nonkarst	11	1420	24.8	174	11	1	37
Evrg Nonkarst	90	1942	23.4	376	19	1	20
Cloud Forest	4	2609	20.9	803	42	1	25
Forest assemblage							
CasSyl/PsiGua	14	1764	23.8	339	28	0.64	24
CecSch/CitSin	9	1979	22.8	483	15	0.89	27
GuaGui/DenArb	35	1869	23.1	410	23	0.88	18
CitFru/ExoCar	3	1219	25.6	75	14	0.33	28
BurSim/BucBuc	9	1451	24.7	219	33	0.22	4
ThoStr	2	1857	23.6	349	47	0.50	11
SpaCam	22	1945	24.3	240	20	0.77	8
NeoRes/GueSca	2	1800	24.1	296	47	0.50	1
SyzJam	11	2001	23.2	413	21	0.82	4
CasGui/AndIne	7	1937	23.9	322	34	0.71	17
TabHet/CasArb	9	1961	24.1	342	23	0.78	9
LeuLeu	10	1312	25.4	98	3	0.60	61
PreMon/MirGar	2	3198	22.3	531	57	1	0
AcaFar/ProPal	5	1133	25.7	51	2	0.60	75

Table 1. Averages for climate, elevation, age, geology, and potentially N<sub>2</sub>-fixing legume abundance for forest plots according to Holdridge life zone, forest type, and forest assemblage classifications.

*Notes:* Abbreviations are: MAP, mean annual precipitation; MAT, mean annual temperature; SD, semi-deciduous; Evrg, evergreen. Forest assemblage codes are explained in Appendix: Table A2.

 $\dagger$  Ages are biased low for plots in the oldest age class (50–64+ yr); stands >64 yr could not be accurately dated and were assigned an age of 64 yr.

‡ Proportion of plots on non-karst soils.

§ Values are percentages of basal area.

*Forest age class.*—Forest age came from Kennaway and Helmer (2007). That study created maps of forest type and land cover with Landsat image mosaics dated 1991 and 2000, and then combined these with maps of land cover in 1951 and 1978 based on photo interpretation. Four forest age classes (0–9 years, 10–22 years, 23–49 years, and 50–64+ years) were identified, as well as a low forest-cover class mapped as pasture.

### Hypothesis testing and data analyses

*Overall approach.*—Further detail will be provided in upcoming sections but our general approach was the following: We aimed to identify differences in fallen leaf chemistry and forest floor element masses within the three island-wide classifications (Holdridge life zone, 2000 forest type, and forest assemblage) as well as across forest age. We also sought to describe the distribution of variation in the nested dataset across chemical and element mass variables. Because life zones and forest types are partly

defined by differences in climate and geology, this step provides one test of H1 and H2 (rainfall and karst effects, respectively, on Ca, Mg, K, Na, P, Fe, Mn, and Al). H4 (age effects on N and P concentrations and the N/P ratio) is tested across forest age classes. Secondly, we used correlation analyses, multiple regression, and ANOVA to examine in more detail effects on fallen leaf N and P concentrations and their ratios (including with C). For example, H3 (effects of legumes on N and P concentrations) is tested by considering legume abundance and fallen leaf concentrations across forest assemblages. Fallen leaf N concentration is also examined across all plots using a multiple regression of climate, landscape, and stand variables. Additional support is provided for H3 and H4 where legume abundance and stand age, respectively, contribute significantly to models. H5 (slope position and P concentration) is tested by comparing across slope position classes. Third, we wished to examine how variation in aggregate fallen leaf chemistry was related with our suite of variables.

Some analyses are exploratory or not linked to specific hypotheses. For example, we are also interested in knowing whether forest age or landscape position influence fallen leaf concentrations of elements not included in H4 or H5, and so include them in the tests. As well, because of the importance of litter C concentrations on ecosystem C dynamics and element interactions, we conducted tests on C although we did not formulate C-related hypotheses.

Most of the chemical (except for C, N, and S concentrations and C/N ratios) and all of the mass variables were non-normally distributed and positively skewed and hence ln-transformed before analyses.

Island wide classifications.—To quantify the influence of geology, we calculated the proportion of plots not on karst substrates (for individual plots, karst = 0, non-karst = 1), hereafter 'geology index'. We then used this index as a factor in ANOVAs to examine differences in fallen leaf chemistry and forest floor element mass across Holdridge life zones and forest assemblages, followed with the posthoc Tukey-Kramer test (p < 0.05). For forest assemblages, MAP (as a covariate) was also included in models when significant (p < 0.05).

To compare forest types, which already distinguish plots based on geology and rainfall, we used a procedure in SAS (PROC MULTTEST, which cannot accommodate a covariate) that minimizes the chances of a Type I error from performing multiple ANOVAs. Linear contrasts (non-orthogonal) were then used to compare: (1) karst versus non-karst and (2) SD versus evergreen.

While not an island-wide classification per se, we also analyzed the effect of forest age as a class variable (5 age classes) in an ANOVA; MAP and geology were included as factors if significant. A linear contrast was used to compare the oldest forests ( $\geq$ 50 years) with the four younger age classes, assumed to be primarily originating from previous land clearing.

*Variance analysis.*—We performed a variance component analysis (SAS, PROC NESTED) on the data (subplots nested within plots, plots nested within landscape categories) to determine how the variance was proportioned among these strata, representing different scales, for each classification scheme. Variance components were computed for the strata assuming random effects. Knowing at which scale most of the variation lies is important for designing or modifying future sampling strategies (Rawlins et al. 2009) and also informs analyses under the current sampling protocol.

Tests on C, N, and P.–We used correlation and multiple regression analyses to examine effects of and test the hypotheses related to landscape, climate and stand variables on fallen leaf N and P (and C) concentrations and their ratios at several scales. First, at the plot-scale, independent variables with p values < 0.20 in univariate regression were considered for inclusion into multivariate models. Effects of geology (karst vs. non-karst), stand age (oldest age class versus others), and slope position (ridgetop, midslope, toeslope) were modeled as classification (dummy) variables. Because relative density, relative dominance, and importance values were highly correlated, we selected the importance value for the influence of functional group (N2-fixing or not) in these analyses. Variables were kept in the model at p < 0.05, checked for multicollinearity and removed according to the following criteria: climatic (e.g., mean annual temperature [MAT] and MAP) variables were selected over landscape (e.g., elevation, distance from coast) variables and MAP was selected over MAT. This was done because MAP is the major source of climatic variation in the tropics (Townsend et al. 2007) and often a strong driver of ecological processes (e.g., Rentería and Jaramillo 2011). Similarly, across the 14 forest assemblages, mean fallen leaf C, N, P concentrations and their ratios were examined for relationships with mean climate, stand, and landscape variables. Here, we used the geology index (see Methods: Island wide classifications) to assess the effect of substrate. For these analyses, age classes were converted to numeric variables using the midpoint age for each of five age classes.

Aggregate fallen leaf chemistry.—Because litter nutrients are often correlated with each other, and we had a large number of variables, we used a principal components analysis (PCA) on aggregate fallen leaf chemistry (C, N, P, S, Ca, Mg, K, Mn, Al, and Fe) to reduce the number of variables for exploratory analyses. Also, we conducted separate analyses for karst and non-



Fig. 1. Map of Puerto Rico showing significant differences in fallen leaf chemistry for the subtropical dry, moist and wet forest Holdridge life zones; the subtropical lower montane forest life zone was not included in analyses (see Table 2). When values did not differ between two life zones (but did for a third), means are shown for the statistically equal values.

karst plots due to widespread differences between these substrates (see *Results*). Component scores, calculated separately for karst (n = 37) and non-karst (n = 103), were correlated with climate (MAP, MAT), landscape (elevation, aspect, distance from the coast, percent slope, slope position), and stand-scale (basal area, density, Nfixer basal area, N<sub>2</sub>-fixer density, and stand age) variables, using Pearson's r. SAS 9.3 (SAS Institute, Cary, NC) was used for analyses.

# RESULTS

### Island-wide classifications

*Fallen leaf chemistry.*—Fallen leaf C, Ca, Mg, Na, and Mn concentrations differed significantly (p < 0.05) across the three main Holdridge life zones (Fig. 1, Table 2). After controlling for substrate, the subtropical wet forest life zone had the highest fallen leaf C and Mn and the lowest Ca and Mg concentrations compared to the moist and dry forest life zones (note: all of the life zones on the island are subtropical). Fallen leaf Na concentrations were lowest for the dry forest life zone. There were no other significant differences in fallen leaf chemistry among the three life zones (Tables 2 and 3). The lower montane forest life zone, not included in formal analyses due to low replication, had low fallen leaf P, Ca, Mg, K, and Fe, and high C, Na, Mn, and S concentrations (Table 2), and the highest (by rank) C/N, C/P, and N/P ratios (Table 3).

Within the forest types, six elements (C, P, Ca, Mn, Al, and Fe) in fallen leaves differed significantly by geologic type and two (C, Ca) by leaf longevity (Fig. 2, Table 2). Fallen leaf C concentration was slightly greater on karst (SD and evergreen combined) versus non-karst plots (Fig. 2, Table 2). Fallen leaf Ca concentrations on karst exceeded those on non-karst by nearly 50% while fallen leaf P, Mn, Fe, and Al concentrations were lower on karst (testing H2). C and Ca

Table 2. Fallen leaf chemistry by Holdridge life zone, forest type, and forest assemblages. Different superscripts indicate different means (least squares) from linear contrasts for forest type and from post hoc Tukey tests for life zones and forest assemblages. For forest type, 'a' and 'b' indicate differences between karst and non-karst and '1' and '2' indicate differences between leaf longevity classes. Groups with <5 plots (in parentheses) were excluded from statistical analyses.

Landscape classification	n	С	Ν	S	Ρ†	Ca†	Mg†	K†	Na†‡	Mn†	Al†	Fe†
Holdridge life zone												
Subtropical dry	12	42.9 <sup>c</sup>	1.61	0.25	0.51	32.0 <sup>a</sup>	3.9 <sup>a</sup>	1.76	0.11 <sup>b</sup>	0.14 <sup>b</sup>	1.75	1.83
Subtropical moist	73	47.7 <sup>b</sup>	1.87	0.27	0.76	26.3 <sup>a</sup>	3.3 <sup>a</sup>	2.40	0.28 <sup>a</sup>	0.19 <sup>b</sup>	1.77	1.89
Subtropical wet	56	49.8 <sup>a</sup>	1.85	0.29	0.72	20.5 <sup>b</sup>	2.7 <sup>b</sup>	2.48	$0.27^{a}$	$0.26^{a}$	2.17	1.83
Subtropical lower montane	(2)	51.8	1.80	0.32	0.42	9.4	2.1	1.55	0.35	0.63	1.70	1.45
Forest type												
SD Karst	14	46.9 <sup>a,2</sup>	1.72	0.27	0.49 <sup>b</sup>	45.3 <sup>a,1</sup>	3.2	2.09	0.20	$0.08^{b}$	1.32 <sup>b</sup>	1.48 <sup>b</sup>
Evrg Karst	24	$51.2^{a,1}$	1.70	0.27	0.57 <sup>b</sup>	$31.5^{a,2}$	2.3	1.83	0.34	0.09 <sup>b</sup>	1.13 <sup>b</sup>	1.19 <sup>b</sup>
SD Nonkarst	11	43.2 <sup>b,2</sup>	1.73	0.23	0.93 <sup>a</sup>	$28.0^{b,1}$	4.0	2.76	0.15	$0.26^{a}$	3.94 <sup>a</sup>	4.12 <sup>a</sup>
Evrg Nonkarst	90	48.1 <sup>b,1</sup>	1.92	0.29	$0.77^{a}$	20.1 <sup>b,2</sup>	3.3	2.54	0.23	$0.30^{a}$	2.17 <sup>a</sup>	2.02 <sup>a</sup>
Cloud Forest	(4)	51.0	1.67	0.29	0.49	9.3	2.3	1.96	0.27	0.40	1.71	1.28
Forest assemblage												
CasSyl/PsiGua	14	48.7 <sup>ab</sup>	$1.90^{a}$	0.28	$0.71^{\text{abc}}$	21.0 <sup>ab</sup>	3.2	2.63	0.21	$0.27^{ab}$	2.00	1.80
CecSch/CitSin	9	52.1 <sup>a</sup>	2.14 <sup>a</sup>	0.32	$0.74^{\text{abc}}$	17.7 <sup>b</sup>	2.6	2.41	0.20	0.34 <sup>ab</sup>	1.68	1.32
GuaGui/DenArb	35	$48.5^{ab}$	$2.04^{a}$	0.29	$0.82^{ab}$	27.7 <sup>ab</sup>	3.3	2.52	0.24	0.23 <sup>ab</sup>	2.00	2.03
CitFru/ExoCar	(3)	42.8	1.82	0.23	0.70	54.2	3.9	4.08	0.54	0.16	1.47	1.61
BurSim/BucBuc	9	48.1 <sup>ab</sup>	1.83 <sup>ab</sup>	0.27	0.57 <sup>abc</sup>	32.4 <sup>a</sup>	2.7	1.70	0.27	0.17 <sup>ab</sup>	1.19	1.52
ThoStr	(2)	49.8	1.08	0.22	0.47	28.9	2.2	0.83	0.23	0.07	1.01	0.86
SpaCam	22	47.8 <sup>ab</sup>	1.96 <sup>a</sup>	0.29	$0.95^{a}$	30.7 <sup>a</sup>	3.4	2.78	0.19	0.14 <sup>b</sup>	1.35	1.52
NeoRes/GueSca	(2)	42.1	1.27	0.19	0.65	20.7	4.5	1.36	0.32	0.12	2.78	2.63
SyzJam	11	49.9 <sup>ab</sup>	1.58 <sup>ab</sup>	0.27	0.50 <sup>bc</sup>	18.7 <sup>ab</sup>	2.6	2.38	0.37	0.34 <sup>a</sup>	2.77	2.51
CasGui/AndIne	7	47.9 <sup>ab</sup>	$1.60^{ab}$	0.23	0.60 <sup>abc</sup>	24.2 <sup>ab</sup>	3.1	3.43	nd	$0.26^{ab}$	3.28	2.61
TabHet/CasArb	9	48.9 <sup>ab</sup>	1.20 <sup>b</sup>	0.24	$0.40^{\circ}_{1}$	17.7 <sup>b</sup>	2.4	1.50	0.35	$0.15^{ab}$	1.28	1.19
LeuLeu	10	45.3 <sup>b</sup>	2.11 <sup>a</sup>	0.29	$0.84^{\text{abc}}$	31.5 <sup>a</sup>	4.1	2.45	0.16	0.21 <sup>ab</sup>	2.60	2.86
PreMon/MirGar	(2)	53.4	1.44	0.25	0.31	6.1	1.6	1.57	0.77	0.31	1.79	1.25
AcaFar/ProPal	5	43.0 <sup>b</sup>	1.73 <sup>ab</sup>	0.22	0.63 <sup>abc</sup>	26.2 <sup>ab</sup>	3.2	1.90	0.12	0.13 <sup>ab</sup>	3.35	3.80

*Notes:* Forest assemblage codes are explained in Appendix: Table A2. Values for C, N, and S are percentages; all others are in mg/g. The abbreviation "nd" indicates no data.

<sup>+</sup> Back-transformed least-square means of In-transformed data.

‡ Sample sizes for Na approximately half that of the other elements.

concentrations were also related to leaf longevity: fallen leaves from SD forests had lower C and higher Ca than evergreen forest litter. Substrate also influenced element stoichiometry. Fallen leaf C/P and N/P ratios were higher on karst (1.5 and 1.3 times greater, respectively) compared with non-karst plots (Fig. 2, Table 3); the former being due to higher C and lower P concentrations on karst, while the latter was driven primarily by lower P concentrations on karst rather than any differences in N, which were not detected at this scale.

For forest assemblages, there were notable differences in fallen leaf C, N, P, Ca, and Mn concentrations (Table 2), and for fallen leaf C/N and C/P ratios (Table 3), the latter reflecting gradients in all three elements. Fallen leaf N concentrations only differed by forest assemblage, not showing any effects due to climate, geology, or leaf longevity (indicated by lack of significant differences among life zones and

forest types).

Fallen leaf N concentrations were highest in the successional CecSch, GuaGui, SpaCam, and LeuLeu forest assemblages and fallen leaf P was highest for the SpaCam and GuaGui assemblages (Table 2). (Forest assemblages are denoted by a single species code in the text. Full names of assemblages are found in the Appendix: Table A2 and are further explained in Brandeis et al. [2009].) CecSch had the highest litter C and the lowest Ca concentrations. Fallen leaves from the TabHet assemblage, also successional, had the lowest N and P concentrations, indicating relatively low fertility, and the highest C/N and C/P ratios and a high N/P ratio (by rank). The more fertile successional assemblages LeuLeu and SpaCam had the lowest C/P ratios and LeuLeu had the lowest C/N ratio. Despite having one of the highest fallen leaf N concentrations, the SpaCam assemblage also had the highest P concentrations, yielding one of the lowest N/P

Table 3. Fallen leaf element ratios (mass-based) by Holdridge life zone, forest type, and forest assemblages. Different superscripts indicate different means from linear contrasts. See Table 2 for an explanation of superscripts. Groups with <5 plots (in parentheses) were excluded from statistical analyses.

Landscape classification	n	C/N	C/P†	N/P†
Holdridge life zone				
Subtropical dry	12	30.5	888	30.7
Subtropical moist	73	28.1	675	25.7
Subtropical wet	56	28.9	711	25.2
Subtropical lower montane	(2)	33.4	1223	40.4
Forest type	. ,			
SD Karst	14	30.3	982 <sup>a</sup>	34.2 <sup>a</sup>
Evrg Karst	24	31.6	935 <sup>a</sup>	29.5 <sup>a</sup>
SD Nonkarst	11	26.8	473 <sup>b</sup>	18.8 <sup>b</sup>
Evrg Nonkarst	90	26.3	648 <sup>b</sup>	24.8 <sup>b</sup>
Cloud Forest	(4)	34.5	1113	32.3
Forest assemblage				
CasSyl/PsiGua	15	28.0 <sup>bc</sup>	725 <sup>abc</sup>	27.7
CecSch/CitSin	9	25.9 <sup>bc</sup>	724 <sup>abc</sup>	27.7
GuaGui/DenArb	35	25.9 <sup>bc</sup>	615 <sup>bc</sup>	24.1
CitFru/ExoCar	(3)	25.6	639	28.6
BurSim/BucBuc	9	27.4 <sup>bc</sup>	869 <sup>abc</sup>	35.8
ThoStr	(2)	48.3	1190	24.0
SpaCam	22	26.0 <sup>bc</sup>	522 <sup>c</sup>	20.6
NeoRes/GueSca	(2)	32.7	633	19.9
SyzJam	11	33.5 <sup>ab</sup>	1036 <sup>ab</sup>	31.6
CasGui/AndIne	7	32.1 <sup>abc</sup>	819 <sup>abc</sup>	26.6
TabHet/CasArb	9	41.3 <sup>a</sup>	1334 <sup>a</sup>	31.8
LeuLeu	10	21.6 <sup>c</sup>	547 <sup>bc</sup>	25.9
PreMon/MirGar	(2)	39.1	1822	49.7
AcaFar/ProPal	5	25.6 <sup>bc</sup>	699 <sup>abc</sup>	27.9

*Note:* Forest assemblage codes in Appendix: Table A2. † Back-transformed least-square means of In-transformed data.

ratios (by rank) among the assemblages. The cloud forest assemblage PreMon, not included in formal analyses, had the highest C/P and N/P ratios and the lowest fallen leaf P (by rank), indicating extremely low P availability.

Fallen leaves from the oldest age class had significantly greater C and lower P (testing H4), Al, and Fe concentrations than the other age classes (Table 4). Older forests also had the highest litter C/P and N/P (testing H4) ratios (Table 4), driven by high C and low P.

Forest floor, leaf litter, and element masses.— Across the Holdridge life zones, fallen leaf litter mass was nearly two times greater in the wet forest life zone compared with the subtropical moist and dry forest life zones (Table 5). Masses of Na and Mn in the forest floors were the only element pools that differed among the life zones (Table 5), reflecting their lower concentrations in the drier life zones. When forest type classified the plots, the evergreen forests (karst and non-karst together) tended to have greater fallen leaf mass than SD forests (p = 0.07; Table 5). Calcium content in the forest floor was on average double the amount in karst versus non-karst forests, while the opposite was true for total pools of Mn and Al. Although there were no detectable differences in forest floor mass among forest assemblages, there were differences in the masses of forest floor P, Ca, Mn, and Al (Table 5), reflecting differences in fallen leaf concentrations (Table 2).

### Sources of variation

In comparing the variation among the three scales-the divisions within life zone, forest type, and forest assemblage, plots within the divisions, and subplots within plots (i.e., sampling error)most of the variation for fallen leaf C was at the subplot scale and at the plot scale for fallen leaf N and P concentration (Table 6). For Ca concentration, considerable variation was also captured at the division scales, suggesting the classifications are useful for this element. For fallen leaf N and P concentrations, >15% of total variation was captured by the divisions within forest assemblage, suggesting some utility in this classification for these elements. For fallen leaf C concentration, the amount of variation captured by the divisions within forest type and forest assemblage was low (<15%).

In contrast, masses of forest floor and leaf litter, and forest floor C and Ca mass showed the greatest variability at the subplot scale (Table 7). This variation was also very high, ranging from 68-94% of the total variation, and nearly double the subplot variation for element concentrations (Table 7). This indicates more sampling is needed at this scale (i.e., increased replication) to reduce the variation in the estimates of forest floor, litter, and C and Ca masses. Thus, in this dataset, total pools of C in particular, where variation is focused at the subplot scale, are largely driven by the variation in forest floor masses rather than by concentrations (Table 7). When the other element pools are classified by life zone (data not shown), the pattern of variation is the same as for C pools except for Mn and Na, which are more similar to Ca pools showing some variation among the divisions. Similarly, when classified by forest type, the pattern of variation for



Fig. 2. Map of Puerto Rico showing least square mean concentrations in fallen leaves for variables differing by forest type using contrast statements (see Table 2). Cloud forests were not included in data analyses and values are not mapped. Uncolored areas indicate forests on ultramafic soils or non-forested areas such as crops and pastures, urban or bare lands, and water bodies.

element pools other than Ca, Mn, and Al (data not shown) is similar to that for C pools. For Ca, Mn, and Al pools, variation is greater among the forest types than among the plots (as shown for Ca), reflecting some utility of mapping by forest types for these element pools (and indeed we noted significant differences for Ca, Mn, and Al element pools across forest types; Table 5). Overall, because of the extreme subplot variation in fallen leaf, total forest floor, and total element

Table 4. Fallen leaf chemistry (least square means) by age class for island-wide plots. Only elements and ratios (mass-based) showing significant differences between the oldest age class and all others (tested by non-orthogonal contrasts) are shown. Where significant (with  $p \le 0.05$ ), geologic substrate and mean annual precipitation were included as factors in the ANOVAs.

Age class†	Age in 2000 (years)	n	C (%)	P (mg/g)‡	Al (mg/g)‡	Fe (mg/g)‡	C/P‡	N/P‡
0	variable§	28	47.6	0.78	2.70	2.87	668	24.3
1	1–9	20	47.9	0.72	1.56	1.70	679	24.5
2	10-22	39	49.4	0.69	1.56	1.51	768	28.4
3	23-49	34	49.0	0.67	1.77	1.83	776	26.9
4	50-64+	20	51.1	0.53	1.12	0.98	990	32.5

 $\dagger$  Ages are biased low for plots in the oldest age class (50–64+ yr); stands > 64 yr could not be accurately dated and were assigned an age of 64 yr.

‡ Back transformed of In-transformed data.

§ Low density forest, mapped as pasture.

Table 5. Masses (g/m<sup>2</sup>) of forest floor, fallen leaves, and elements in the forest floor by Holdridge life zone, 2000 forest type, and forest assemblages. Geology was included as a factor in the ANOVAs for life zone and forest assemblage (see text). See Table 2 for an explanation of superscripts. Groups with <5 plots (in parentheses) were excluded from statistical analyses.

		Total					Ma	ass in fo	rest flo	oor for				
Landscape classification	n	forest floor†	Fallen leaves†	C†	N†	S	P†	Ca†	Mg†	K†	Na†	Mn†	Al†	Fe†
Holdridge life zone														
Subtropical dry	13	543	145 <sup>b</sup>	253	6.7	1.4	0.21	14.1	1.22	0.69	$0.04^{b}$	$0.05^{b}$	0.76	0.82
Subtropical moist	73	467	226 <sup>b</sup>	218	7.1	1.4	0.32	10.4	1.22	0.94	$0.10^{a}$	0.07 <sup>b</sup>	0.75	0.78
Subtropical wet	56	553	397 <sup>a</sup>	264	8.7	1.5	0.35	8.8	1.41	1.23	$0.14^{a}$	$0.17^{a}$	0.99	0.86
Subtropical lower montane	(2)	298	222	154	4.9	0.9	0.12	3.1	0.55	0.42	0.09	0.14	0.37	0.33
Forest type														
SD Karst	15	498	$210^{2}$	328	6.9	1.5	0.23	16.2 <sup>a</sup>	1.13	0.80	0.09	0.03 <sup>b</sup>	0.51 <sup>b</sup>	0.59 <sup>b</sup> ‡
Evrg Karst	24	536	322	257	7.5	1.5	0.27	14.5 <sup>a</sup>	1.01	0.82	0.19	0.04 <sup>b</sup>	0.51 <sup>b</sup>	0.57 <sup>b</sup>
SD Nonkarst	11	437	$127^{2}$	200	5.9	1.1	0.30	8.6 <sup>b</sup>	1.28	0.77	0.05	$0.08^{a}$	$1.30^{a}$	1.39 <sup>a</sup>
Evrg Nonkarst	90	505	294 <sup>1</sup>	237	8.0	1.4	0.35	8.6 <sup>b</sup>	1.40	1.15	0.10	0.13 <sup>a</sup>	$0.98^{a}$	0.89 <sup>a</sup>
Cloud Forest	(4)	469	307	240	6.5	1.3	0.20	4.2	0.90	0.81	0.08	0.15	0.59	0.57
Forest assemblage														
CasSyl/PsiGua	14	465	234	224	7.5	1.4	0.29 <sup>ab</sup>	8.1 <sup>ab</sup>	1.29	1.06	0.09	$0.1^{ab}$	$0.98^{ab}$	0.83
CecSch/CitSin	9	668	384	315	11.5	2.1	$0.44^{a}_{1}$	10.3 <sup>ab</sup>	1.34	1.38	0.10	$0.15^{ab}$	$0.85^{ab}$	0.70
GuaGui/DenArb	35	533	312	249	8.9	1.4	$0.40^{ab}$	10.5 <sup>ab</sup>	1.46	1.18	0.11	$0.10^{ab}$	0.83 <sup>ab</sup>	0.96
CitFru/ExoCar	(3)	285	94	131	4.2	1.2	0.18	7.9	0.79	0.55	0.07	0.04	0.49	0.65
BurSim/BucBuc	9	666	434	333	10.1	1.9	0.26 <sup>ab</sup>	19.2 <sup>a</sup>	1.39	0.90	0.23	0.09 <sup>ab</sup>	$0.87^{ab}$	0.63
ThoStr	(2)	732	383	362	7.7	1.5	0.33	16.3	1.53	0.52	0.15	0.05	0.72	0.62
SpaCam	22	394	220	183	6.3	1.1	$0.35^{ab}$	9.7 <sup>ab</sup>	1.09	0.94	0.08	0.06 <sup>b</sup>	$0.47^{b}$	0.54
NeoRes/GueSca	(2)	767	316	344	6.7	1.1	0.36	11.3	2.71	0.61	0.19	0.07	1.67	1.59
SyzJam	11	515	361	256	7.6	1.3	$0.24^{ab}$	8.5 <sup>ab</sup>	1.27	1.12	0.17	$0.17^{a}_{1}$	1.15 <sup>ab</sup>	1.37
CasGui/AndIne	7	787	417	356	10.2	1.6	$0.41^{ab}$	16.6 <sup>ab</sup>	1.98	2.27	0.38	$0.14^{ab}$	1.77 <sup>ab</sup>	1.24
TabHet/CasArb	9	456	303	210	4.6	1.0	$0.17^{ab}$	5.0 <sup>b</sup>	0.91	0.66	0.16	$0.07^{ab}$	$0.50^{ab}$	0.43
LeuLeu	10	691	225	325	11.7	1.9	$0.44^{a}$	$16.7^{a}$	2.05	1.33	0.08	0.11 <sup>ab</sup>	$1.67^{a}$	1.54
PreMon/MirGar	(2)	430	331	221	5.5	1.1	0.13	2.6	0.61	0.64	0.15	0.11	0.69	0.46
AcaFar/ProPal	5	325	89	154	4.8	1.2	0.15 <sup>b</sup>	7.6 <sup>ab</sup>	0.66	0.44	0.03	0.03 <sup>b</sup>	0.68 <sup>ab</sup>	0.70

Note: Forest assemblage codes in Appendix: Table A2.

† Back-transformed least-squares means of In-transformed data.

p = 0.07.

Table 6. Variance component analysis showing percentage of variance in each component for fallen leaf C, N, Ca, and P concentrations by three landscape classification schemes. (Divisions with <5 plots were excluded from statistical analyses.)

	Land	dscape cl	assification
Variable and variance components	Life zone	Forest type	Forest assemblage
Fallen leaf C			
Among divisions	<1	15	10
Among plots within divisions	46	35	38
Within plots	53	49	52
Fallen leaf N			
Among divisions	0	3	23
Among plots within divisions	58	55	36
Within plots	42	41	41
Fallen leaf Ca			
Among divisions	25	39	22
Among plots within divisions	31	20	31
Within plots	44	40	47
Fallen leaf P			
Among divisions	$<\!\!1$	1	16
Among plots within divisions	77	77	56
Within plots	22	22	28

mass, we present their data for descriptive purposes only.

# C, N, P, and their ratios

Landscape, climate, and plot-scale variables all had significant effects on fallen leaf C, N, P concentrations and their ratios. Across all plots, multiple regression analysis indicated fallen leaf C concentration increased with MAP and was greater on karst and in the oldest forest age class (adjusted  $r^2 = 0.19$ , p < 0.001, n = 141) as shown above. Fallen leaf N concentration was positively related to the basal area (ln) of N<sub>2</sub>-fixing tree legumes (testing H3) (r = 0.37, p < 0.001, n = 138). A multiple regression on fallen leaf N concentration included the basal area of N<sub>2</sub>-fixers, slope position, and a positive effect of percent slope (adjusted  $r^2 = 0.19$ , p < 0.001).

Both fallen leaf N concentration and the basal area of  $N_2$ -fixers were higher in valley bottom

Table 7. Variance component analysis showing percentage of variance in each component for fallen leaf mass, total forest floor C and total forest floor Ca masses by three landscape classification schemes. (Divisions with <5 plots were excluded from statistical analyses.)

	Land	dscape cl	assification
Variable and variance components	Life zone	Forest type	Forest assemblage
Forest floor mass			
Among divisions	0	0	6
Among plots within divisions	6	6	0
Within plots	94	94	94
Fallen leaf mass			
Among divisions	0	0	<1
Among plots within divisions	14	15	12
Within plots	86	85	87
Total C mass			
Among divisions	0	0	6
Among plots within divisions	8	8	0
Within plots	92	92	94
Total Ca mass			
Among divisions	9	19	14
Among plots within divisions	19	13	12
Within plots	72	68	74

and mid-slope positions than on ridgetops (oneway ANOVA for litter N, F = 5.9, p = 0.004; for (ln) basal area of N<sub>2</sub>-fixers, F = 11.8, p = 0.0001; Fig. 3). Fallen leaf C/N ratio was the only other chemical variable that differed by slope position (see H5) (F = 6.6, p = 0.0019, Fig. 3), and was greater on karst, positively related to MAP and tree density and negatively related to the basal

area of N-fixers (adjusted  $r^2 = 0.21$ , p < 0.0001). In contrast to fallen leaf C concentration and C/N, which increased with increasing MAP, fallen leaf P concentration significantly decreased with MAP (testing H1) as well as with tree density, and was positively related to distance from the coast and, as expected, differed by geology (testing H2) and in the oldest versus younger age classes (testing H4) (adjusted  $r^2 = 0.26$ , p < 0.001). Fallen leaf C/P ratios were related to factors influencing C and P together: C/P ratios were highest in older stands, greater in karst, increased with MAP and tree density, and showed a negative effect of distance from the coast.

Similarly, fallen leaf N/P increased with MAP (largely due to decreasing P), was positively related to the basal area of N<sub>2</sub>-fixers and stand age, negatively related to distance from the coast, and was greater in karst versus non-karst (adjusted  $r^2 = 0.22$ , p = 0.001). Although the contribution of N<sub>2</sub>-fixers was significant in the multiple regression model, across plots fallen leaf N/P was highly correlated with leaf P (r = -0.84, p < 0.0001) but not with fallen leaf N (p = 0.35).



Fig. 3. Mean (+ 1 SE) fallen leaf N concentration, C/N ratio, and basal area of  $N_2$ -fixing trees of plots by landscape position. Different letters indicate significant differences across positions. For basal area of  $N_2$ -fixers, values are back-transformed means of log (natural) transformed data. Untransformed means of  $N_2$ -fixer basal areas are 11.3, 7.8, and 2.8 m<sup>2</sup>/ha for toeslope, midslope and ridgetop, respectively.



Fig. 4. Fallen leaf N concentration as a function of (A) mean basal area of putative  $N_2$ -fixing tree species, and (B) mean stand age, and (C) the relation between basal area of  $N_2$ -fixers and stand age for 14 forest assemblages across Puerto Rico. Fallen leaf P is also shown as a function of mean stand age (B). Older mean ages are biased low as forest stands > 64 yr could not be accurately dated and were assigned an age of 64 yr.

Across the 14 forest assemblages, similar to the plot-scale (n = 141) analyses, mean fallen leaf C concentration for the assemblages was positively related to MAP (r = 0.73, p = 0.003) and negatively with MAT (r = -0.80, p = 0.006). Mean litter N concentration for the assemblages, however, was not related to climate but was related to stand characteristics: litter N concentration increased with the basal area of N<sub>2</sub>-fixing tree legumes (testing H3) (r = 0.66, p = 0.01; Fig. 4A) and decreased with increasing stand age (testing H4) (r = -0.66, p = 0.01; Fig. 4B),

reflecting the fact that legume basal area in the assemblages also decreased with stand age (r = -0.83, p < 0.001; Fig. 4C). (Note: legume basal area across all plots was lowest in the oldest age class compared to the others, p = 0.002.) Moreover, plots within forest assemblages with the highest legume dominance were found in mid to low topographic positions (Appendix: Table A3). N<sub>2</sub>-fixing legumes within several forest assemblages also correlated with fallen leaf N: fallen leaf N was positively related to the basal area of fixers in CecSch (r = 0.72, p = 0.03)



Fig. 5. Fallen leaf C/N, P, and C/N ratio as a function of the basal area of putative N<sub>2</sub>-fixing tree species for 14 forest assemblages across Puerto Rico. Lines are shown where relationships are significant (p < 0.05).

and GuaGui (r = 0.39, p = 0.02), to the density of fixers in GuaGui (r = 0.52, p = 0.001) and SpaCam (r = 0.51, p = 0.02), and to the relative density of fixers in SpaCam (r = 0.53, p = 0.01) and CasGui (r = 0.78, p = 0.04). Importance values of the functional groups did not correlate with fallen leaf N at any scale. As with fallen leaf N, the C/N ratio was also related (negatively) to the basal area of legumes (r = -0.64, p = 0.01; Fig. 5A) in the assemblages and to their mean age (r = 0.69, p = 0.007).

Table 8. Variable loadings (eigenvectors) from principal component analysis of fallen leaf chemistry for karst and non-karst plots across Puerto Rico. PC axes shown had eigenvalues >1. Boldface indicates loadings > 0.37, considered relevant.

Variable	PC1	PC2	PC3
Karst			
Cumulative variance (%)	42.7	66.5	82.2
Percent C	-0.35	0.21	0.39
Percent N	0.24	0.49	0.20
Percent S	0.09	0.59	0.13
Ln (P mg/g)	0.36	0.24	0.06
Ln (Ca mg/g)	0.21	< 0.01	-0.63
Ln (Mg mg/g)	0.18	0.36	-0.50
Ln (K mg/g)	0.38	-0.01	0.19
Ln (Mn mg/g)	0.39	-0.03	0.27
Ln (Al mg/g)	0.38	-0.34	0.12
Ln (Fe mg/g)	0.41	-0.26	0.13
Non-karst			
Cumulative variance (%)	28.8	54.3	68.4
Percent C	0.13	0.49	0.25
Percent N	0.48	0.03	0.31
Percent S	0.44	-0.15	0.37
Ln (P mg/g)	0.43	0.29	0.02
Ln (Ca mg/g)	0.35	0.25	-0.27
Ln (Mg mg/g)	0.31	0.29	-0.24
Ln (K mg/g)	0.17	0.19	0.19
Ln (Mn mg/g)	-0.18	0.08	0.65
Ln (Al mg/g)	-0.24	0.44	0.31
Ln (Fe mg/g)	-0.19	0.51	0.14

Fallen leaf N and P concentrations were highly correlated across the assemblages (r = 0.79, p <0.001). So similar to N, fallen leaf P concentration increased with the basal area of legumes (testing H3) (r = 0.59, p = 0.03, Fig. 5B) and decreased in the older assemblages (testing H4) (r = -0.66, p =0.01, Fig. 4B). No other elements or ratios, in particular the N/P ratio (p = 0.44), were related to the mean basal area of N<sub>2</sub>-fixing legumes for the assemblages (Fig. 5C). Fallen leaf P concentrations did not correlate with any additional climate, stand, or landscape-scale attributes across assemblages. Similarly, assemblage N/P ratios were not related to these variables and showed no significant trend with mean fallen leaf N or P concentrations.

# Modeling with landscape, climate, and site variables for aggregate fallen leaf chemistry

For karst (n = 37), the first three PCA axes explained 82% of the variation in fallen leaf chemistry (Table 8). The first PCA axis was dominated by K, Mn, Al, and Fe and correlated negatively with forest age (r = -0.33, p = 0.04) and tree density (r = -0.38, p = 0.02). The second

axis identified a strong positive contribution by N and S but did not correlate with the measured variables. The third axis was driven by a negative contribution from Ca and Mg and a positive contribution from C; this axis correlated positively with MAP, distance from the coast, and elevation, and negatively with MAT.

For non-karst (n = 104), the first three axes explained 68% of the variation in the data (Table 8). The first axis identified a N, P, and S gradient that correlated negatively (r = -0.25, p = 0.01) with MAP. The second axis was dominated by Fe and Al (and negative with C) and was negatively correlated with MAP and forest age. The third axis was driven largely by Mn, and similar to the third axis for karst, correlated positively with elevation, distance from the coast, and MAP, and negatively with MAT.

# Discussion

#### Island-wide classifications: life zones and forest type

Aggregating similar ecological systems into map units is useful when features differ among the divisions. The three landscape classifications depict unique variations in fallen leaf chemistry across Puerto Rico. Holdridge life zones, reflecting a climatic gradient, identified substantial variation in fallen leaf C, Ca, Mg, Na, and Mn concentrations after accounting for a co-occurring gradient in geology across the zones. MAP more than doubles across the zones (MAT decreases only by about 10%; Table 1), and appears to be a major driver for most of these litter elements.

Forest type, incorporating variation in both climate and geology, shows a climate effect on fallen leaf C and Ca concentrations; C is higher and Ca is lower in the wetter evergreen forests compared with their drier SD counterparts (Table 1: compare MAP between SD and evergreen forests on karst and non-karst). Long-lived leaves, such as those found in the wetter evergreen forests, typically have high leaf mass per unit area, with more C-rich cell wall components like lignin and structural carbohydrates (Poorter and Villar 1997, Wright et al. 2004, Fyllas et al. 2009, Poorter et al. 2009), likely explaining the greater fallen leaf C in evergreen forests and more mesic life zones. The lower fallen leaf Ca concentration in the wetter life zones and evergreen forests suggests limited Ca available for plant uptake compared to drier life zones. Calcium, a mobile cation in soil, is easily leached in more humid environments (Bohn et al.1985). Magnesium, also mobile as a cation, follows a similar pattern to Ca across the life zones and forest types (though the differences between evergreen and SD litter were not significant for Mg, p = 0.09). Notably, Na shows the opposite pattern to Mg and Ca in these classifications (and in contrast to H1). Perhaps marine inputs of Na are greater in the wetter life zones and forest types; though a confirmation would require more analyses. Unlike C and Ca concentrations, which differed by both geology and leaf longevity in the forest types, Mn concentrations only differed by geology, suggesting stronger geologic than climatic control.

Karst soils originate from limestone and have high pHs that regulate the availabilities of several elements, including Mn, P, Fe and Al, all of which were significantly lower in fallen leaves from karst compared with non-karst. At pH > 7 (the exact pH depends on the element), P and Mn complex with carbonates and Fe and Al complex with hydroxides (Bohn et al. 1985), and are unavailable for plant uptake. Base rich soils in the tropics are often low in aluminum (Quesada et al. 2010). Given their similar geochemical controls, it is not surprising that the PCA grouped Fe and Al (and Mn for karst) together. Karst sites also show the expected effect of high fallen leaf Ca concentration. However, we currently lack an explanation for the higher C concentrations in fallen leaves from karst vs. non-karst plots and note this effect is independent of MAP.

We anticipated that rainfall would correlate inversely with fallen leaf concentrations of the mobile cations Ca, Mg, K, Na, and of P (H1), and that fallen leaves on karst substrates would have reduced P, Fe, Mn, and Al concentrations and greater Ca compared with leaves on non-karst substrates (H2). Our findings are consistent with the predictions, except for: (1) rainfall effects on P (but see below), (2) fallen leaf Na concentrations were opposite what was predicted (as above), and (3) fallen leaf K concentrations did not show any patterns. Perhaps the exceptionally high mobility of K in leaf litter (e.g., Schreeg et al. 2013) contributed to high variation in our data and an inability to detect differences.

Biogeographic patterns of plant stoichiometry remain under explored (Elser et al. 2010). Here we show that element stoichiometry in fallen leaves shows strong substrate control: C/P and N/P ratios were higher on karst compared with non-karst plots, the former being due to higher C and lower P on karst, while lower P on karst rather than differences in N drives the latter. Townsend et al. (2007) also noted that differences in foliar N/P ratios between soil types were a function of changes in P rather than N. Similarly, Posada and Schuur (2011) found the increases in litter N/P and C/P (along an increasing precipitation gradient) were due to decreasing P. Changes in P also appear to drive global variations in foliar and litter N/P ratios (McGroddy et al. 2004, Reich and Oleksyn 2004, Ordoñez et al. 2009).

Because foliar and litter N from tropical forests are often insensitive to regional changes in precipitation (Read and Lawrence 2003, Fyllas et al. 2009, Posada and Schuur 2011), the lack of differences across the climatically defined life zones or forest types was not surprising. This is also consistent with global scale analyses (Elser et al. 2010), where foliar N showed no pattern with MAT and only a weak pattern with precipitation.

# Island-wide classifications: forest assemblages and age class

To our knowledge, this is the first study to demonstrate unique biogeochemical signals for replicated tropical forest communities at the regional scale: fallen leaf C, N, P, Ca, and Mn concentrations, and C/N and C/P ratios show significant differences across the forest assemblages. Tree species growing together often vary widely in their nutrient concentrations (Townsend et al. 2007, Hättenschwiler et al. 2008). We hypothesized that, all else being "equal" (accounting for differences in geology and MAP), forest communities differing in species composition would vary in litter nutrients.

Indeed, our analyses show that the community scale is important for fallen leaf N, which did not differ by life zone or forest type. Foliar N, while insensitive to growing conditions, often varies by taxonomy (Townsend et al. 2007, Fyllas et al. 2009). Across forest assemblages, 44% of the

variation in fallen leaf N concentration is explained by the dominance of potentially Nfixing legumes (H3), suggesting that forest community composition can be used to predict litter chemistry and associated ecosystem processes (see below). We also found a substantial decline in legume dominance with increasing age of forest assemblages (Fig. 4C). Consequently, although stand age did not predict significant variation in fallen leaf N concentration of individual plots, when averaged by community type, fallen leaf N concentration declined with mean age (H4).

The positive relationship between fallen leaf N concentration (negative for C/N ratio) and the basal area of N2-fixing tree species occurred at multiple scales: across all plots and within and across forest assemblages (H3). Although we limited our analyses to N<sub>2</sub>-fixing legumes known to nodulate, nodulation can be facultative depending on environmental constraints (Pearson and Vitousek 2001, Barron et al. 2011) and perhaps tree size (Gehring et al. 2005). Hedin et al. (2009) propose a model of biological  $N_2$ fixation in tropical forests where facultative fixation by tree legumes increases after forest disturbance, including such relatively small disturbances as the creation of tree fall gaps. While this mechanism provides a plausible explanation for the low inputs of N via fixation in older tropical forests, the model does not consider changes in the abundances of N2-fixers as forests age. The decline in legume dominance with increasing age of forest assemblages (Fig. 4C), suggests that demographic changes need to be addressed when considering the potential contribution by legumes to tropical forest N dynamics.

In contrast to other studies (Nardoto et al. 2008, Álvarez-Clare and Mack 2011, Inagaki et al. 2011) reporting higher foliar N/P ratios in legumes compared with non-legumes, we did not find an effect of legumes on fallen leaf N/P ratios at the community level. This was likely because litter P and N concentrations were correlated across communities (as well as across plots). Our finding is consistent with Townsend et al. (2007) who showed that legumes as a group did not predict differences in canopy N/P values, and suggests legumes may have an inconsistent effect on N/P ratios.

Forest assemblages (after accounting for geologic substrate) also differed in fallen leaf P concentrations, which unlike C concentrations (see below), were not related to broad climate differences among the communities. To our knowledge, this is the first time differences in fallen leaf or leaf litter P concentration have been shown for communities across a large region (see Condit et al. 2013 for individual tree species responses to soil P), and suggests that community types can serve as indicators for fallen leaf P concentrations. Among the stand characteristics we measured, fallen leaf P concentration was positively related to the basal area of N2-fixers in the assemblages (H3). In other words, these observations suggest that community differences in the dominance of N<sub>2</sub>-fixers, rather than MAP (see below), drive substantial community-scale variation in fallen leaf P concentration. Phosphorus availability has been shown to increase with legume dominance (Hughes and Denslow 2005); we confirm this relation across multiple community types. Exceptions among the assemblages are worth noting: SpaCam and TabHet are found in similar climates with comparable mean stand ages, substrate conditions, and legume dominance (Tables 1 and 6), and both are common in abandoned pastures (Weaver 2000, Chinea and Helmer 2003, Brandeis et al. 2009), yet show extremes in fallen leaf P concentration (SpaCam = 0.95 mg/g, TabHet = 0.40 mg/g), indicating that other factors contribute to differences in soil P availability across communities. SpaCam communities, but not TabHet communities, are also found on former sugar plantations on rich alluvial soils (Abelleira-Martínez and Lugo 2008) and more often in lower landscape positions that TabHet (Appendix: Table A3).

Fallen leaves from the oldest age class were depleted in P compared to the four younger classes (H4; Table 4) and had significantly greater N/P (H4) and C/P ratios. This reflects findings from two studies in Brazil where litterfall or litter N/P ratios were greater in old versus younger forests (Davidson et al. 2007, Amazonas et al. 2011). In these studies the shifts in the ratios were interpreted as recovery of the N cycle during either succession or forest restoration. In contrast, and similar to several age sequences in the Yucatán (Read and Lawrence 2003), we found no significant changes in fallen leaf N concentra-

tions across our time sequence, unless stand age and fallen leaf N were averaged by community type (and a negative relationship ensued). The higher N/P ratios in the oldest age class, averaged across all communities, are apparently due to a decrease in fallen leaf P rather than changes in N concentrations. The decrease in mean fallen leaf N (as well as P) with average age of the forest communities suggests that the age trends for N need more exploration. While we lack data to infer dynamics, N cycling in Puerto Rico may recover earlier in succession than in Brazil and decline in older communities. Rapid recovery (within 7 years) of N cycling after agriculture has been found elsewhere in the Caribbean (Templer et al. 2005), though in aggrading dry forests of the Yucatán both N and P limited production (Campo and Vázquez-Yanes 2004). Whether N availability is low enough to limit production in the older communities would need testing; recent experiments demonstrate limitation (or co-limitation) by N in mature lowland forests (cf. Wright et al. 2011, Sayer et al. 2012, Fisher et al. 2013). The relatively high fallen leaf P and lower element/P ratios in the younger age classes also suggest that P cycling is currently much faster on the island than in the past when forests > 50 years old dominated the landscape.

We also found increased fallen leaf C and decreased Al and Fe concentrations in the oldest age class. Lohbeck et al. (2013) found an increase in leaf thickness with succession in dry and wet forests in Mexico, which presumably reflects increased C concentrations and is likely associated with leaf longevity. The lower concentrations of Al and Fe in oldest stands (supported by the PCA analyses for both karst and non-karst) suggest a high degree of weathering has occurred in the older and presumably stable forests (Chadwick et al. 2003). In the non-karst plots, the PCA analyses also showed that fallen leaf Al and Fe concentrations were correlated negatively with MAP. High rainfall on older lava flows in Hawaii was related to substantial Al and Fe (and P) loss from soil (Porder and Chadwick 2009). Although MAP did not vary across the age classes in our dataset, we can assume that the older stands are less disturbed than the younger stands, allowing for prolonged in situ weathering.

# Additional patterns for fallen leaf C, N, P, and their ratios

We previously demonstrated significant differences among life zones and forest types for fallen leaf C concentration, which we interpreted as being effects of precipitation. Furthermore, at both plot and forest assemblage scales, fallen leaf C increases with increasing MAP. Posada and Schuur (2011) also found C concentrations of leaf litter increased significantly with MAP, which likely contributed to low soil C turnover in their wettest tropical forests. Overall then, and unlike for N and P, fallen leaf C appears to be under strong climatic control.

We expected that fallen leaf P concentration would be negatively related to MAP (H1): regional and global-scale analyses (Austin and Vitousek 2000, Santiago et al. 2005, Ordoñez et al. 2009, Yuan and Chen 2009, Elser et al. 2010) show decreases in foliar and senesced-leaf P with increasing MAP, as well as decreases in soil extractable P (Porder and Chadwick 2009). In a multiple regression of all plots, increasing MAP contributed to decreasing fallen leaf P concentrations. However, we did not find a difference among the life zones or longevity classes for fallen leaf P, nor did the average of fallen leaf P concentration correlate with average MAP for the assemblages. The lack of a strong effect across this climatically diverse landscape could result from differential effects of rainfall on P availability in wet versus dry forests. Rainfall in tropical dry forests may increase P availability (Read and Lawrence 2003, Rentería and Jaramillo 2011) and MAP likely has non-linear effects on available P (Porder and Chadwick 2009). Phosphorus concentrations also decreased with tree density, which could be related to greater competition for limited amounts of P.

Because of the active erosional environment in Puerto Rico, we expected to find greater P availability in less stable or depositional landscape positions (H5) but did not. Porder et al. (2006) failed to find a topographic effect on foliar nutrients for a dominant species in Costa Rica, and speculated this could be due to high erosion rates on ridgetops, among other possibilities.

We did find that the basal area of  $N_2$ -fixing trees was greater in lower landscape positions, at both the plot scale and within the major forest assemblages, which corresponded with high

fallen leaf N concentrations and low C/N ratios. While physical processes may potentially influence nutrient distributions in landscapes, species distributions often follow topographic features (Clark et al. 1999, Condit et al. 2000) and could also contribute to the spatial patterning of nutrients. To our knowledge, this is the first time a preference for lower landscape positions by legume-dominated (and relatively young) forest communities has been documented. In Puerto Rico and elsewhere in the tropics, forests at higher elevations and on steeper slopes are often older than more accessible forests (Helmer et al. 2008). Our finding provides a biologically-based, community-scale explanation for greater fallen leaf N concentrations (and presumably N availability) in lower landscape positions.

Wide ranges in litter N/P ratios from tropical forests have been noted (cf. McGroddy et al. 2004) but are largely unexplained. The substantial differences for the major forest types (range: 19–34) and forest age classes (range: 24–33), with the highest ratios found in the karst and oldest forests (as above), provide two possible explanations for the high biome-scale variation. Across all plots we also found a positive effect of MAP and the abundance of N<sub>2</sub>-fixing legumes on fallen leaf N/P ratios, as indicated previously.

We also found wide ranges in fallen leaf C/P and C/N ratios across the communities. Globally, high litter C/P ratios suggest low P availability and resorption of P, typical of the tropics, and often leads to high N/P ratios, indicating N-rich and P-poor nutrient economies (McGroddy et al. 2004, Yuan and Chen 2009). This conclusion may be valid, but not where N and P are low and may be co-limiting. For example, the infertile TabHet community has high N/P (31.8, ranked third of 14, though N/P ratios did not differ significantly across the communities) and C/P (1334, ranked second of 14) ratios, yet has the lowest fallen leaf N and P concentrations of assemblages with n >3. Including the C/N ratio, which was highest (41.3) among all the assemblages, would reveal the low N economy in this case. In fact, across all 14 assemblages, fallen leaf C/P ratios were negatively correlated with litter N concentrations (r = -0.66, p = 0.01), indicating that "P-poor" economies equate with relatively N-poor economies. Similarly, the high elevation Cloud Forest has the highest C/P ratio among the forest types, the second highest N/P ratio, and the highest C/N ratio (all by rank), suggesting co-limitation by N and P, as previously proposed for montane tropical forests (Tanner et al. 1998). (Overall across Puerto Rico, MAT did not affect fallen leaf N concentrations or C/N and N/P ratios.)

# Forest recovery, novel communities, and island biogeochemistry

The community scale data show the effect of successional dynamics and the development of novel communities (cf. Lugo and Helmer 2004) on island wide biogeochemistry. For example, one of the most common forest assemblages in Puerto Rico, SpaCam (Table 1; see also Brandeis et al. 2009), dominated by the introduced tree Spathodea campanulata (Appendix: Table A2), has high fallen leaf N and P concentrations, and as mentioned previously is widespread on abandoned pastures and former sugar plantations. Another common assemblage, GuaGui (dominated by the native Guarea Guidonia and the nonnative Magnifera indica), which was previously managed for coffee, has high fallen leaf N and P concentrations, and has likewise increased across the island since deforestation 100 year ago (Brandeis et al. 2009). Although these two dominant successional assemblages may be fairly fertile, not every successional assemblage is necessarily nutrient-rich. For example, the Tab-Het assemblage, dominated by the native Tabebuia heterophylla, has some of the lowest fallen leaf nutrient concentrations and the highest C/ nutrient ratios of the assemblages.

Non-native legume trees can drastically alter the functioning of tropical ecosystems (Vitousek and Walker 1989, Hughes and Denslow 2005), including N and P cycling. The high litter N assemblage LeuLeu is dominated by the naturalized tree legume Leucaena leucocephala, while the high litter N assemblage CecSch is dominated by the native tree legume Inga vera (Appendix: Table A2), indicating that native and non-native legumes may function similarly. What is not known is whether the introduced legumes are outcompeting potential non-legume colonizers. As well, only  $\sim 8\%$  of the basal area in the dominant and fertile SpaCam assemblage is comprised of N2-fixers. Thus, the presence of N-fixing legumes provides only a partial explanation for the variation in fallen leaf N and P

concentrations across Puerto Rico. That SpaCam assemblages often occur on nutrient-rich soils may reflect specialization to the high levels of nutrients on those sites (cf. John et al. 2007).

The wide ranges in litter C/N and C/P ratios across forest assemblages also indicate that communities can also have significant and unique effects on island wide stoichiometry and related processes. For example, leaf litter C/N ratio is a strong regulator of decomposition and soil emissions of N oxide gases (nitrous oxide and nitric oxide combined) (Erickson et al. 2002). Forest assemblages with fallen leaf C/N ratios <27, indicating a high potential for large fluxes of these gases (Erickson et al. 2002), include those with high legume abundances (AcaFar and LeuLeu) as well as SpaCam, GuaGui, and CecSch. Given that these forest types are relatively young and currently widespread on the island, there is a strong possibility that N oxide emissions have increased across Puerto Rico over the previous century due to forest clearing and subsequent succession.

# Multi-scale variation in litter chemistry and mass: implications for future sampling and final considerations

The sampling design allowed us to quantify the proportion of the total variance in each classification stratum. The within-plot variation for litter mass was substantially greater than for litter chemistry and typically exceeded 80%, indicating increased subsampling is needed to reduce this variation. Because spatial variation in forest soil properties is notoriously high (cf. Conant et al. 2003), often requiring a logistically prohibitive number of samples, subsampling followed by compositing (bulking) is a common technique for soil analyses (e.g., Martin et al. 2011). Despite the high variation for forest floor masses, the range of values is within published values (cf. Brown and Lugo 1982). Nonetheless, future monitoring projects need to assess and respond to components with high variation.

Litter chemistry and mass often vary seasonally in tropical forests, especially in forests with pronounced dry seasons (Read and Lawrence 2003, Campo and Vázquez-Yanes 2004, Anaya et al. 2007, Townsend et al. 2007, Sayer and Tanner 2010). Here, some of the variation among plots could be because collection occurred over five-

months. Yet this period is considered dry for the island, when leaching and mass losses are likely low. Differential climate effects across the island could contribute another source of unaccounted for variation in chemistry. For example, low nutrient concentrations could be due to leaching and/or mineralization loss from fallen leaves rather than to a rainfall effect on site nutrient availability, as hypothesized for Ca, Mg, K, Na, and P (H1). A recent experiment on senesced leaves from 41 tropical woody species found that < 5% of total leaf litter Ca was soluble (Schreeg et al. 2013), suggesting leaching would have little impact on Ca concentrations in our study. However Mg has a rather high solubility ( $\sim 20\%$  of total litter Mg; Schreeg et al. 2013) and can be leached from canopy foliage (Wood et al. 2005), suggesting the negative relation between fallen leaf Mg concentrations and MAP (life zones and PCA results) may need to be interpreted with caution. Although Na solubility is substantial (28%; Schreeg et al. 2013), we still found higher concentrations of Na in fallen leaves in the wetter life zones. Moreover, P solubility was much greater than N solubility (35% vs. 5%, respectively; Schreeg et al. 2013) suggesting the possibility that the ranges in C/P ratios across our sites may also be influenced by leaching processes. Overall, we did not find a strong effect of MAP on fallen leaf P concentrations. Finally, immobilization by litter microbes may further modify fallen leaf element concentrations, though the effect may be more pronounced in wet seasons when nutrients are more likely to be leached (Lodge et al. 1994). Despite these potential limitations, we find patterns in fallen leaf element concentrations across Puerto Rico that are consistent with known drivers of nutrient availability in tropical environments. Furthermore, we identify new relationships among several nutrient elements, forest recovery, and associated novel forests across the island.

## Conclusions

Fallen leaf chemistry provides a window into the various and often complex factors affecting plant nutrient availability. Based on a landscapescale, systematic sample of fallen leaf litter across a tropical island, we found that three existing landscape classifications (Holdridge life zones, forest types, and forest assemblage) can be used to identify and map unique differences in fallen leaf chemistry. Many nutrients differed among all three classifications, indicating that climate (mainly precipitation), geological type, and tree species together influence their availabilities. Fallen leaf N concentration only differed across forest assemblages, highlighting the importance of species' influence on N availability. Analyses at plot and forest assemblage scales revealed new linkages among dominance of N<sub>2</sub>-fixing tree legumes, topographic position, and age of forest assemblages: fallen leaf N concentration correlated positively with the basal area of N<sub>2</sub>-fixing legumes and was highest in lower landscape positions and in younger, often novel, forest assemblages. Fallen leaf C concentrations were highest on karst and in the oldest forests and positively related to MAP. Fallen leaf P, Fe, and Al concentrations were lowest and C/P and N/P ratios highest in the oldest forest age class ( $\geq 50$ years), and C/N ratios increased with increasing age of forest assemblage. Taken together, results from this observational study suggest that N and P availability may currently be greater in the predominantly novel forests across much of Puerto Rico than when older and presumably native forests dominated the landscape. Variance analysis indicated that sampling was sufficient for fallen leaf chemistry but mostly inadequate for total masses of forest floors and forest floor elements.

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# SUPPLEMENTAL MATERIAL

#### Appendix

Table A1. N<sub>2</sub>-fixing capability (status) assigned to species in the Fabaceae present in the plots based on nodulation potential. Nodulation is usually consistent within a genus; if information was lacking for a certain species (e.g., *Acacia anegadensis*) assignment was based on consensus for that genus.

Genus	Scientific name	Status	Reference
Acacia	Acacia anegadensis	yes	
	Acacia farnesiana	yes	
	Acacia macracantha	yes	
	Acacia muricata	yes	
	Acacia nilotica	yes	Kirkbride 2002
	Acacia polyacantha	yes	Kirkbride 2002
	Acacia tortuosa	yes	
Adenanthera	Adenanthera pavonina	no	Kirkbride 2002, Sprent 2009
Albizia	Albizia adinocephala	yes	Sprent 2009
	Albizia carbonaria	yes	*
	Albizia lebbeck	yes	Sprent 2000
	Albizia procera	yes	*
Anadenanthera	Anadenanthera peregrina	yes	Kirkbride 2002
Andira	Andira inermis	yes	Sprent 2007
Bauhinia	Bauhinia monandra	no	Sprent 2007
	Bauhinia multinervia	no	*
	Bauhinia pauletia	no	
	Bauhinia purpurea	no	
	Bauhinia tomentosa	no	
	Bauhinia variegata	no	Kirkbride 2002
Caesalpinia	Caesalpinia coriaria	no	deFaria 1989
-	Caesalpinia pulcherrima	no	
Calliandra	Calliandra haematomma	yes	Sprent 2009
	Calliandra surinamensis	yes	*
Cassia	Cassia fistula	no	Sprent 2007
	Cassia grandis	no	*
	Cassia javanica	no	
	Cassia javanica var. indochinensis	no	
Cojoba	Cojoba arborea	yes	Kirkbride 2002, Sprent 2009
Cynometra	Cynometra portoricensis	no	1
Dalbergia	Dalbergia sissoo	yes	Sprent and Parsons 2000, Sprent 2009
Delonix	Delonix regia	no	De Faria et al. 1989
Enterolobium	Enterolobium cyclocarpum	yes	Sprent 2009
Erythrina	Erythrina berteriana	yes	Sprent 2009
	Erythrina corallodendron	yes	*
	Erythrina corallodendron var. connata	yes	
	Erythrina crista-galli	yes	
	Erythrina eggersii	yes	
	Erythrina fūsca	yes	

Genus	Scientific name	Status	Reference
	Erythrina poeppigiana	yes	Sprent and Parsons 2000
	Erythrina variegata	yes	-
	Erythrina variegata var. orientalis	yes	
Gliricidia	Gliricidia sepium	yes	Sprent 2009 (but see Kirkbride 2002)
Hebestigma	Hebestigma cubense	yes	Sprent 2009
-	Hebestigma cubense var. cubense	yes	-
Hymenaea	Hymenaea courbaril	yes	Pons et al. 2007
Inga	Inga laurina	yes	Sprent 2009
-	Inga nobilis ssp. quaternata	yes	Kirkbride 2002
	Inga vera	yes	
Leucaena	Leucaena leucocephala	yes	Sprent 2009
	Leucaena leucocephala ssp. leucocephala	yes	1
Lonchocarpus	Lonchocarpus domingensis	yes	Sprent 2009
1	Lonchocarpus glaucifolius	yes	1
	Lonchocarpus heptaphyllus	yes	
Machaerium	Machaerium lunatum	yes	Sprent 2009
Myrospermum	Myrospermum frutescens	no	1
Ormosia	Ormosia krugii	ves	Sprent 2009
Parkinsonia	Parkinsonia aculeata	no	Sprent and Parsons 2000 (but see Kirkbride 2002)
Peltophorum	Peltophorum pterocarpum	no	Bryan et al. 1996
Pictetia	Pictetia aculeata	ves	Sprent 2009
Piscidia	Piscidia carthagenensis	ves	Sprent 2009
	Piscidia piscipula	ves	1
Pithecellobium	Pithecellobium dulce	ves	Sprent 2009
	Pithecellobium unguis-cati	ves	1
Poitea	Poitea florida	ves	Kirkbride 2002
Prosopis	Prosopis pallida	ves	Kirkbride 2002, Sprent 2009
Pterocarpus	Pterocarpus indicus	ves	Sprent 2009
1	Pterocarpus macrocarpus	ves	Kirkbride 2002
	Pterocarpus officinalis	ves	
Samanea	Samanea saman	ves	Sprent 2009
Senna	Senna bicapsularis	no	Sprent 2007, 2009
	Senna polyphylla	no	1 ,
	Senna siamea	no	
	Senna spectabilis	no	
Sesbania	Sesbania grandiflora	ves	Sprent 2009
Tamarindus	Tamarindus indica	no	Dommergues 1995

Assemblage code in text	Full assemblage code	Dominant species
CasSyl	CasSyl/PsiGua	Casearia sylvestris, Psidium guajava,† Coffea arabica†
CecSch	CecSch/CitSin	Cecropia schreberiana, Citrus sinensis,† Inga vera
GuaGui	GuaGui/DenArb	Guarea guidonia, Dendropanax arboreus, Mangifera indica†
CitFru	CitFru/ExoCar	Citharexylum fruticosum, Exostema caribaeum, Ardisia obovata
BurSim	BurSim/BucBuc	Bursera simaruba, Bucida buceras
ThoStr	ThoStr	Thouinia striata
SpaCam	SpaCam	Spathodea campanulata†
NeoRes	NeoRes/GueSca	Neolaugeria resinosa, Guettarda scabra, Eugenia monticola, Tabebuia haemantha
SyzJam	SyzJam	Syzygium jambos†
ČasGui	CasGui/AndIne	Casearia guianensis, Andira inermis, Guazuma ulmifolia
TabHet	TabHet/CasArb	Tabebuia heterophylla, Casearia arborea
LeuLeu	LeuLeu	Leucaena leucocephala†
PreMon	PreMon/MirGar	Prestoea montana, Micropholis garciniifolia, Sloanea berteriana, Micropholis chrysophylloides, Henriettea squamulosum
AcaFar	AcaFar/ProPal	Acacia farnesiana,† Prosopis pallida†

Table A2. Codes and dominant species for the fourteen forest assemblages.

Notes: Assemblage codes are after Brandeis et al. 2009. Codes are based on the first three letters of the genus and species of the dominant two species. † Introduced species.

Table A3. Basal area of N2-fixing legumes (m2/ha) by forest assemblage and slope position within forest assemblage. Sample size in parentheses.

Forest assemblage	Mean	Toeslope	Midslope	Ridgetop
CasSyl/PsiGua	9.9	12.8 (6)	9.6 (6)	2.2 (2)
CecSch/CitSin	9.6	17.8 (1)	11.2 (6)	0(2)
GuaGui/DenArb	9.7	10.9 (17)	11.6 (6)	2.9 (6)
CitFru/ExoCar	4.1		1.4 (2)	9.6 (1)
BurSim/BucBuc	2.5	8.8 (2)	1.2(2)	0.5(5)
ThoStr	1.3	2.5(1)		0(1)
SpaCam	5.6	6.8 (6)	5.6 (11)	4.1 (Ś)
NeoRes/GueSca	0.8	,	/	0.8(2)
SyzJam	3.2	6.0(4)	2.9(4)	0 (3)
CasGui/AndIne	3.7	1.0(2)	0(1)	5.9 (4)
TabHet/CasArb	5.7	′	10.0 (4)	2.1 (5)
LeuLeu	13.5	18.3 (5)	10.1 (3)	7.8 (2)
PreMon/MirGar	0	`	`	0(2)
AcaFar/ProPal	15.0	31.6 (2)	3.9 (3)	

Note: Forest assemblage codes in Appendix: Table A2.