Soil nitrogen levels are linked to decomposition enzyme activities along an urban-remote tropical forest gradient

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Urban areas in tropical regions are expanding rapidly, with significant potential to affect local ecosystem dynamics. In particular, nitrogen (N) availability may increase in urban-proximate forests because of atmospheric N deposition. Unlike temperate forests, many tropical forests on highly weathered soils have high background N availability, so plant growth is unlikely to respond to increased N inputs. However, microbial activity and decomposition of carbon-rich plant tissue can respond positively to added N in these forests, as has been observed in a growing number of fertilization studies. The relevance of these controlled studies to landscape-scale dynamics in urban-proximate moist tropical forests requires further investigation. I used ten forest stands in three watersheds along an urban-remote gradient in Puerto Rico to test the hypotheses that urban activity has a positive effect on soil N availability, and that decomposition enzyme activities vary with soil N. I found that mineral N, total dissolved N (TDN), and ammonium:nitrate (NH₄⁺:NO₃⁻) ratios varied by nearly one order of magnitude across the urban-remote gradient, and variability among urban sites was high. On average, urban forests had higher soil NO₃⁻, lower NH₄⁺, and lower C:N values than remote forests, suggesting high nitrification rates and/or external inputs of NO₃⁻ to the urban forests, and enrichment in N relative to C. Total mineral N and total dissolved N were positively correlated with the activities of enzymes that acquire carbon (C) and phosphorus (P) from organic matter. Across this gradient soil N levels were stronger predictors of enzyme activities than soil C or pH, which drive enzyme activities globally. The ratio of NH₄⁺:NO₃⁻ was the strongest predictor of oxidative enzyme activities. Compared to global averages, ratios of C:N:P enzyme activities across these tropical forests indicated lower relative N-acquisition and higher relative P-acquisition, with N-acquisition lowest in the urban watershed, and P-acquisition highest in the upper-elevation remote watershed. These results suggest a strong urban effect on forest soil N levels, and show a link between changes in N availability and microbial processing of soil organic matter.

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1. Introduction

Urban and industrial expansion are occurring rapidly in tropical regions (Lambin et al., 2003), with significant potential to alter biogeochemical processes in urban-proximate forests (Kaye et al., 2006; Martinelli et al., 2006). In particular, atmospheric nitrogen (N) deposition, until recently considered a North Temperate Zone problem, is expected to be highest in tropical regions in the coming decades (Galloway et al., 2004). Unlike many temperate forests, tropical forests on highly weathered soils tend to be rich in N, with high N availability and rapid rates of internal N cycling (Walker and Syers, 1976; Chestnut et al., 1999; Martinelli et al., 1999; Hedin et al., 2009). In temperate regions, urban-proximate forests can have higher soil N levels, mineralization rates, and N leaching relative to rural reference sites (White and McDonnell, 1988; Zhu and Carreiro, 1999; Groffman et al., 2009), providing an opportunity to assess human influences on the N cycle relative to background processes (Groffman et al., 2006). Similar urban-remote gradient studies are notably lacking for tropical ecosystems.

The extent to which added N may be retained in highly weathered tropical soils in urban-proximate forests is unknown. Because of high background soil N, deposition of this element in tropical regions could lead to large losses of N from soils, and lower retention than has been observed in temperate forests (Matson et al., 2002), such that soil N levels may not change drastically with N deposition. For example, an urban watershed in Puerto Rico showed large leaching losses of N compared with rural watersheds (Ortiz-Zayas et al., 2006). Even with large leaching losses and
relatively low plant demand, however, ecosystems within tropical urban watersheds may retain added N. An N fertilization experiment in Hawai‘i showed that added N can be retained in N-rich highly weathered soils, regardless of background N levels or plant demand, via sorption to charged mineral surfaces (Lohse and Matson, 2005). The retention of additional N in highly weathered, N-rich tropical soils near urban areas has yet to be examined at the landscape-scale.

Even in N-rich tropical forests, increased soil N availability has the potential to alter ecosystem dynamics. While N addition can stimulate plant growth in temperate forests (Townsend et al., 1996; Nadelhoffer et al., 1999; Churkina et al., 2010), a growing number of fertilization studies in N-rich tropical forests on highly weathered soils have demonstrated an apparent lack of N limitation to aboveground plant growth (Mirmanto et al., 1999; Harrington et al., 2001; Ostertag, 2001; Kaspari et al., 2008; Cusack et al., 2011b). Nonetheless, inputs of N to N-rich tropical forests have the potential to alter soil organic matter (SOM) cycling through a suite of microbially mediated mechanisms, including changes in decomposition enzyme activities, transport of dissolved organic C (DOC), and changes in soil respiration (Cleveland et al., 2006; Cleveland and Townsend, 2006; Mo et al., 2008; Cusack et al., 2010, 2011a, 2011b). Thus, changes in N availability in urban-proximate tropical forests may affect soil C storage and loss, with implications for global C cycling.

The sensitivity of microbial processes to added N in tropical forests is likely due in part to the high N:C requirements of decomposers relative to the range of N:C available in plant litter and soil organic matter (SOM) (Sylvia et al., 2004). However, N fertilization does not affect decomposition equally across sites (Berg and Matzner, 1997; Knorr et al., 2005), likely because of differences in litter tissue chemistry and varying responses to added N by different decomposition enzymes (Allison and Vitousek, 2004). For example, if N addition alleviates N limitation to decomposition, the activities of hydrolytic enzymes that acquire C and phosphorus (P) from organic matter are likely to increase, whereas enzymes that acquire N are likely to decrease (Sinsabaugh and Moorhead, 1994). Oxidative enzyme activities, which degrade complex C compounds and release physically occluded N, can also decrease with added N (Keyser et al., 1978; Fogg, 1988; Carreiro et al., 2000; Cusack et al., 2010), particularly in lignin-rich litter (Waldrop et al., 2004). Thus, the net effect of increased N availability on decomposition enzyme activities depends local substrate chemistry and the relative responses of different suites of enzymes.

I used ten forest sites along an urban-remote gradient in Puerto Rico to: (1) assess the urban influence on soil N levels, and (2) explore links between changes in soil N status and changes in decomposition enzyme activities. I hypothesized that urban-proximate tropical forests have elevated soil mineral N relative to remote forests, indicating retention of N deposition. I also hypothesized that soil mineral N levels are positively associated with the activity of enzymes that acquire C and P from SOM, but negatively associated with N acquisition and oxidative enzyme activities. I expected that ecosystem properties that drive enzyme activities in relatively undisturbed forests, such as soil C and pH (Sinsabaugh et al., 2008), would be secondary to mineral N in predictive power along an urban-remote gradient. Finally, I predicted that C:N:P ratios of acquisition activities would show lower relative N acquisition in forests closer to the urban center.

2. Materials and methods

2.1. Study sites

This study was conducted along an urban-remote forest gradient encompassing ten forest stands across three watersheds in Puerto Rico. Eight urban and suburban forest stands were located in the Rio Piedras watershed within the NSF San Juan Urban Long Term Research Area (ULTRA-Ex), with forest fragments spanning the watershed from the low-elevation urban core on the coast, to suburban areas in the upper watershed (Table 1). Two remote forests in a mid-elevation and an upper-elevation watershed in the Luquillo Experimental Forest, an NSF Long Term Ecological Research (LTER) site, were included (Fig. 1). Nitrogen fertilization experiments in both of the remote forests have demonstrated a lack of N-limitation to plant growth and litterfall productivity (Cusack et al., 2011b). Nitrogen deposition in the remote sites was ~3 kg-N ha⁻¹ yr⁻¹ (NADP/NTN, 2009). Sixteen weeks of preliminary N deposition data in the urban watershed showed high variability in N deposition rates, which when scaled up could represent inputs ranging from 10 to 40 kg-N ha⁻¹ yr⁻¹ (USFS-IITF 2011, unpublished data).

The Rio Piedras watershed is in the subtropical moist forest life zone (sensu Holdridge et al., 1971), ranges in elevation from 0 to 220 m above sea level (masl), has mean annual precipitation (MAP) of 1750 mm yr⁻¹, and mean annual temperature (MAT) of 25.7 °C. The Rio Piedras forests contain a mixture of native and non-native species, including exotic trees of the Fabaceae family in the canopy (Helmer, 2004; Lugo, 2004; Kennaway and Helmer, 2007). Forest fragment sizes, distance to urban center, and distance to the nearest major road were measured for urban forests using an Arc GIS cover classification map (Kaspari et al., 2011a, Table 1). Forest fragments were defined as areas of continuous forest, and were generally bounded by roads, grass sites or urban

<table>
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<tr>
<th>Watershed</th>
<th>Site ID</th>
<th>Elevation masl</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Forest fragment size km²</th>
<th>Distance to urban center km</th>
<th>Shortest distance to major road km</th>
<th>Basal area m² ha⁻¹</th>
<th>Exotics % of basal area</th>
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* Remote forest sites were in the Luquillo Experimental Forest within El Yunque National Forest, which covers 11,270 ha.
cover. The largest forested area was an ecological corridor traversing part of the mid-watershed (Sites 2.6 and 3.0; Table 1). The urban and suburban forests used in this study were secondary regeneration following widespread agricultural abandonment in Puerto Rico starting in the 1930s (Helmer, 2004; Kennaway and Helmer, 2007), providing an example of a widespread trend toward abandonment of small-scale agriculture in Latin America (Grau and Aide, 2008).

The two remote forests are dominated by native species, but differ significantly in tree species composition, forest structure, and canopy height (Weaver, 1991). The lower elevation remote site is a wet tropical rainforest (Bruijnzeel, 2001) in the Bisley Experimental Watersheds at 260 masl, receives 3500 mm MAP (García-Montino et al., 1996; Heartsill-Scalley et al., 2007), and has MAT of 23 °C. The upper elevation remote site is a lower montane forest characterized by abundant epiphytes and cloud influence (Bruijnzeel, 2001) in the Icacos watershed (McDowell et al., 1992) at 640 masl, has MAP of 4300 mm yr⁻¹ (McDowell and Asbury, 1994), and MAT of 21 °C. Soils in all forests were predominantly Ultisols, with Inceptisols on steep slopes. Soils in the urban watershed and lower elevation remote watershed are derived from volcanlastic material, whereas the upper elevation remote watershed is quartz diorite (Boccheciaiamp, 1978). Thus, ecosystem characteristics in the three watersheds are distinct, with differences between the two remote sites as great or greater than differences between the urban and remote sites. Clearly, the urban effect across this gradient cannot be isolated from background shifts in climate, plant species and soils. The main objective in using these distinct sites was to obtain a large range in soil N levels, and to assess variability within the urban watershed among forest stands, compared to the variability among the three watersheds and between two distinct remote forests.

2.2. Field collection

All soils were collected in October 2010 from 0 to 10 cm using a 2.5 cm diameter soil corer. This depth showed the largest changes in soil C cycling with N fertilization in the remote forests used here (Cusack et al., 2011b). At each of the eight urban sites, soils were collected from three 20-m transects for a total of 24 transects in the urban watershed. Six transects were used in each of the two remote forests, covering topographic and elevation variability. The greater number of sites in the urban watershed was used to assess variability among urban forest fragments, whereas the remote watersheds had continuous forest cover. Along each transect, four random points were sampled with five soil cores (20 cores per transect), and then pooled for one sample per transect.
Ecosystem properties were characterized at each site. Bulk density was measured from 0 to 10 cm back from the undisturbed face of a 20-cm pit in each forest stand using a 6.5 cm inner-diameter corer. A palate was first inserted at 10 cm depth, and then the corer was inserted and excavated to avoid loss of soil. Field-fresh wet weight was recorded, then each bulk density core was homogenized, and a subsample was oven dried at 105 °C until weight stabilized to calculate g dry soil cm⁻³. Tree basal area was measured along each transect using a handheld bottle-opener dendrometer (JIM-GEM Cruz-all, Forestry Suppliers, Jackson, MS). The fraction of non-native canopy trees was measured because of the potential relationship between N fixing exotic species and soil N status.

2.3. Enzyme activities and microbial biomass

The general methods in Sinsabaugh et al. (2003) were followed for soil enzyme assays with changes as noted. A preliminary test comparing the effects of freezing (−15 °C), refrigeration (5 °C), and ambient temperature (−25 °C) storage on hydrolytic enzyme activities in these tropical soils was conducted to explore the effect of soil storage on assay results, similar to DeForest (2009). This test showed that freezing suppressed activity for N- and P-acquisition hydrolytic enzymes after ten days, whereas refrigeration suppressed C- and P-acquisition activity non-linearly, suggesting irregular and unpredictable responses of these tropical soils to below-ambient storage temperatures, similar to tests on Panamanian soils (Turner and Romero, 2010). Thus, all data reported here are for fresh pooled soil samples shipped overnight to the University of California – Santa Barbara, and analyzed within 48 h.

Approximately 2.5 g of homogenized soil (1 g dry-weight equivalent) from each pooled sample was slurried in 100 mL of 50 mM, pH 5 acetate buffer on a stir plate for 2 min. This acidic pH buffer was used despite variability in native soil pH in an effort to obtain measures of maximum potential enzyme activities (Tabatabai, 1994), although it should be noted that this approach emphasized acid phosphatase over alkaline phosphatase activities. Oxidative enzymes were measured using colorimetric assays, and hydrolytic enzymes were measured using fluorometric assays. For fluorescent substrate, aliquots of 200 µL of soil slurry were pipetted into 96-well plates, using 8 analytical replicates per soil, and 200 µL of 200 µM fluorescing substrate was added to each assay. Background fluorescence of soils and substrates were measured, plus quench standards of soil plus the fluorescing standard (4-methylum-belliferone). The reaction was stopped using 10 µL of 1 N NaOH. For colorimetric assays 0.75 mL of soil slurry was measured as cellulose-degrading activity, and N-acetylglucosaminidase (NAG) activity. For peroxidase 10 µL of 0.3% H₂O₂ was added, and polyphenol oxidase activity was subtracted to obtain measures of peroxidase activity. Background absorbance of DOPA was measured, and an extinction coefficient was calculated using a standard curve of DOPA reacted with mushroom tyrosinase.

All assays were incubated at 27 °C for 0.5–8 h for fluorescence assays, and up to 24 h for colorimetric assays. Incubation times were based on initial tests. Carbon-acquisition activity was measured as cellulose-degrading β-glucosidase activity. N-acquisition was measured as chitin-degrading N-acetylglucosaminidase (NAG) activity. P-acquisition from acid phosphatase activity, and oxidative activity from polyphenol oxidase plus peroxidase activity. These enzymes were previously observed to have the highest rates of activities for each type of nutrient acquisition, and to be representative of a broader suite of enzymes for the remote Puerto Rican sites (Cusack et al., 2011a). Microbial biomass was measured using a modified chloroform fumigation-slurry extraction as described in Fierer and Schimel (2003), similarly assuming an extraction efficiency of 0.45 (Beck et al., 1997; Dibart et al., 1998). Plates were read on a PerkinElmer 1420 Victor3 at 365 nm excitation/450 nm emission for fluorescence, and 450 nm for absorbance.

2.4. Nutrient analyses

Soils were extracted using 2 M potassium chloride (KCl) for NH₄, NO₃, DOC, and total dissolved N (TDN) on the same day as collection in Puerto Rico to minimize storage effects on mineral N pools (Turner and Romero, 2009). Approximately 30 g fresh soil plus 75 mL of 2 M KCl were weighed into Nalgene bottles and shaken for 1 h. Soils plus blanks were then filtered through Whatman #1 filters pre-rinsed with 2 M KCl, collected into clean Nalgene bottles, and frozen. Frozen samples were shipped for analysis to the University of California – Santa Barbara, where NH₄ and NO₃ were measured on a Lachat QuickChem 8500, XYZ Autosampler ASX-520 series, and DOC and TDN were measured on a Shimadzu TOC. Soils were then air-dried and ground using a mortar and pestle. Total C and N concentrations were measured on air dried soils ground with a mortar and pestle at the University of California – Los Angeles on a Costech Elemental Analyzer using atropine as a standard.

2.5. Statistical analysis

All data for soil chemistry and enzyme activities were analyzed for means differences at two scales: among the three watersheds (urban, lower-elevation remote, and upper-elevation remote), and among the eight urban stands using Fishers least significant difference (LSD) tests. For comparisons among watersheds, stand-scale averages were used for replication (n = 8 for urban, n = 6 each for remote watersheds). Comparisons within the urban watershed used replicate transects within each forest stand (n = 3 per forest fragment). Regression analyses across the urban-remote gradient (n = 36) and within the urban watershed (n = 24) used data from each transect. Forward stepwise linear models were used to determine the relative importance of environmental and urban factors for predicting soil N levels and the activities of each enzyme. Only significant factors were retained in predictive models. Nitrogen response variables included NH₄, NO₃, NH₄:NO₃, total mineral N, TDN, microbial biomass N, N concentrations, and C:N ratios. Covariates in all tests included soil C concentrations, bulk density, soil pH, soil moisture, elevation, and basal area. For analyses within the urban watershed, forest fragment size, distance to the urban center, distance to nearest road, topographic position (ridge, valley or slope), and the percent of exotic trees of total basal area were additional factors. The areas of urban forest fragments were not normally distributed (Table 1), so they were grouped into three ordinal size classes (small <0.3 km², medium 0.3–1.5 km², and large >1.5 km²). Analyses were performed using Splus 8.0.2 JMP software (SAS Institute Inc., 2009). Statistical significance was p < 0.05, and means are reported ± one standard error. Where necessary values were log transformed to meet assumptions of normality.

3. Results

3.1. Urban-remote soil characteristics

Soil mineral N pool sizes were significantly different for the urban versus remote sites, with little difference between the two remote forests. Across the urban-remote gradient, soil mineral N pools varied by an order of magnitude. Extractable NH₄ pools were significantly larger in the two remote forests versus the urban forests, with an overall range of 0.8—8.5 mg-N kg-soil⁻¹ (Fig. 2A). In
contrast, soil NO3 pools were significantly larger in the urban watershed than in the remote sites, with an overall range of 0.5–9.2 mg-N kg-soil$^{-1}$ (Fig. 2B). Thus, the ratio of NH4$^{+}$:NO3 was much lower in the urban versus remote forests (Fig. 2C), with an overall range of 0.2–12.5. Because these two pools of mineral N varied inversely between urban and remote sites, total mineral N was not significantly different among the three watersheds (Table 2). Similarly, TDN (mineral + organic) was not significantly different among the watersheds, but had a broad range (8–54 mg-N kg-soil$^{-1}$) and tended to be lowest in the urban watershed, with similar patterns for DOC (Table 2). Microbial biomass C and N pools were highest in the mid-elevation remote watershed (Table 2). Total soil N concentrations were intermediate in the urban watershed, whereas soil C concentrations were significantly lower in the urban versus the two remote sites (Table 2). Thus, bulk soil C:N ratios were significantly lower in the urban forests versus the remote sites (Fig. 2D). Across all sites, soil C concentrations ranged from 1.8 to 9.9% and N ranged from 0.1 to 0.5%. In summary, the three watersheds provided a broad range of soil N and C levels, and the urban watershed was enriched in soil N relative to C, and enriched in NO3 relative to NH4 compared to the two remote watersheds.
Other soil chemical and physical properties also varied strongly along the gradient. Soil pH was significantly higher in the urban (6.4 ± 0.3, n = 8) versus the mid-elevation (4.9 ± 0.1, n = 6) and upper-elevation (4.6 ± 0.1, n = 6) remote forests, and overall pH ranged from 4.4 to 8.5. Soil moisture was lowest in the urban watershed (0.49 ± 0.04 g water-g dry-soil⁻¹, n = 8), and not significantly different between the mid-elevation (1.03 ± 0.05 g water-g dry-soil⁻¹, n = 6) and upper-elevation remote forests (0.85 ± 0.09 g water-g dry-soil⁻¹, n = 6), with an overall range of 0.3–1.3 g water-g dry-soil⁻¹. Bulk density was significantly higher in the urban watershed (1.05 ± 0.07 g cm⁻³, n = 8), and not significantly different between the mid-elevation (0.79 ± 0.07 g cm⁻³, n = 6), and upper-elevation remote forests (0.80 ± 0.1 g cm⁻³, n = 6). Within the urban watershed, bulk density was inversely correlated with soil moisture (R² = 0.46, n = 24). Similar to N pools, these parameters had much broader ranges within the urban watershed than between the two remote sites, with urban forest soil pH ranging from 4.5 to 8.5, soil moisture ranging from 0.30 to 0.98 g water-g dry-soil⁻¹, bulk density ranging from 0.72 to 1.4 g cm⁻³, and soil C content ranging from 1.8 to 5.9 kg-C m⁻².

3.2. Variability in soil N within the urban watershed

There was high variability in soil N and C among the eight urban forest sites. Total dissolved N ranged from 8.7 to 43.1 mg-N kg⁻¹ soil⁻¹ within the urban watershed, total mineral N ranged from 3.7 to 9.5 mg-N kg⁻¹ soil⁻¹, bulk soil N concentrations ranged from 0.14 to 0.50%, microbial biomass N ranged from 1.6 to 65.8 µg-N g⁻¹ soil⁻¹, microbial biomass C ranged from 61.7 to 567.5 µg-C g⁻¹ soil⁻¹, and NH₄⁺ and NO₃⁻ pool sizes and soil C:N all varied significantly among urban forest fragments (Fig. 1A, B and D). Many of the differences among urban sites were greater than the difference between the two remote forests (Fig. 2, Table 2), despite the relatively close proximity of the urban sites, and the significant ecosystem-scale differences between the two remote sites. In contrast, the ratio of NH₄⁺:NO₃⁻ was fairly constant among urban forests (Fig. 2C), suggesting some commonality in the nature of mineral N cycling in this human-dominated watershed.

3.3. Predictors of soil N pools

Within the urban watershed, soil mineral N pools were best predicted by factors not present in the remote forests, suggesting a strong urban influence on soil N at this scale. In particular, forest fragment size class was strongly related to soil N, with significantly higher levels of NO₃⁻, mineral N, TDN, DOC and C concentrations in the largest forest fragments (>1.5 km²). Distance to the urban center had a strong quadratic relationship with soil N and C, such that values were low in forest fragments nearest the urban center (i.e. the bottom of the watershed), high in the mid-watershed, and low in the upper elevation areas of the urban watershed (quadratic relationships for: total mineral N R² = 0.47, TDN R² = 0.33, N concentrations R² = 0.50, C concentrations R² = 0.51, n = 24 for each, p < 0.05). Distance to the nearest major road was positively correlated with microbial biomass N (R² = 0.36, n = 24, p < 0.05), such that there was larger microbial biomass farther from roads. The abundance of exotic trees in the canopy, which were absent in the remote forests, was significantly negatively correlated with soil N concentrations (R² = 0.43, n = 24, p < 0.05), microbial biomass N (R² = 0.39, n = 24, p < 0.05), and DOC (R² = 0.19, n = 24, p < 0.05). Thus, rather than potentially N-fixing Fabaceae trees driving increases in soil N levels, these and other exotic trees appeared to be most successful in low-N soils. Overall, patterns of soil N in the urban watershed were complex and related to a suite of urban factors.

In addition to these relationships with urban features, soil N pools varied along environmental gradients, with some differences in trends within the urban watershed versus across the larger urban-remote gradient. Soil NO₃⁻ in the urban watershed was positively correlated with soil C concentrations (R² = 0.44, n = 24, p < 0.05), but there were no strong predictors of NO₃⁻ at the broader scale. The best predictor of NH₄⁺ was soil moisture, both within the urban watershed (R² = 0.36, n = 24, p < 0.05), and at the broader scale (R² = 0.50, n = 36, p < 0.05). NH₄⁺ also declined significantly with soil pH at the broad scale (R² = 0.45, n = 36, p < 0.05), but not within the urban watershed. There were no significant predictors of NH₄⁺:NO₃⁻ ratios within the urban watershed, but at the broader scale these also declined strongly with pH (R² = 0.58, n = 36, p < 0.05). Within the urban watershed, microbial biomass N increased significantly with soil moisture (R² = 0.30, n = 24, p < 0.05) and declined with elevation (R² = 0.21, n = 24, p < 0.05), without similar trends at the larger scale. Soil C:N also declined with elevation in the urban watershed (R² = 0.24, n = 24, p < 0.05), but microbial biomass C:N increased with elevation and basal area (multiple regression R² = 0.44, n = 24, p < 0.05) in this watershed. At the broader scale there was no trend in microbial biomass C:N with elevation, and soil C:N increased with elevation, opposite of trends within the urban watershed. Thus, broad-scale trends in soil N pools and pH were not reflected within the urban watershed, and urban patterns in NO₃⁻, soil C:N and microbial C:N were not seen across the urban-remote gradient.

3.4. Soil enzyme activities

On average, the urban watershed had significantly lower N-acquisition, P-acquisition and oxidative enzyme activities compared to the two remote watersheds, and C-acquisition activity was highest in the mid-elevation remote forest (Fig. 3). The two remote forests were different from each other for oxidative and P-acquisition activity, but not for N-acquisition activity (Fig. 3). Within the urban watershed, only C-acquisition enzyme activities were significantly higher in the large forest fragment size class than in the mid-sized and small size classes (593 ± 105 nmol g⁻¹ soil⁻¹ h⁻¹ versus 274 ± 32 nmol g⁻¹ soil⁻¹ h⁻¹ and 336 ± 52 nmol g⁻¹ soil⁻¹ h⁻¹, respectively).

Nutrient acquisition ratios also varied among the watersheds, with significantly higher ratios of C:P and N:P acquisition activities in the urban versus the remote sites, suggesting relatively low P acquisition activity in the urban forests. The ratio of C:P activity was 0.48 ± 0.05 in the urban forest, 0.20 ± 0.03 in the mid-elevation remote forest, and 0.09 ± 0.01 in the upper elevation remote forest. The ratio of N:P activity was 0.30 ± 0.03 in the urban forest, 0.12 ± 0.01 in the mid-elevation forest, and 0.08 ± 0.01 in the upper elevation remote forest. While not significantly different, the urban forest tended to have higher C:N acquisition activity than the mid- and upper-elevation remote forests (1.79 ± 0.16, 1.64 ± 0.11, and 1.15 ± 0.07, respectively). There were no significant differences in nutrient acquisition ratios between the two remote forests, although all ratios tended to be lowest in the upper-elevation remote forest. Overall, the ratio of C:N:P acquisition activity was 1:0.6:2 in the urban watershed, 1:0.6:5 in the mid-elevation urban watershed, and 1:0.9:11 in the upper-elevation remote watershed, indicating relatively lower N acquisition in the urban watershed, and higher P acquisition in the upper-elevation remote watershed. Enzyme activities calculated per gram of soil C or per µg of microbial biomass, rather than per gram bulk soil, reduced the significance of differences among sites but did not greatly alter general trends shown here.
3.5. Predictors of enzyme activities

Soil N pools were the strongest predictors of oxidative, C-acquiring, and P-acquiring enzyme activities. For oxidative enzyme activities across the urban-remote gradient, the strongest predictors were the ratio of NH$_4^+$:NO$_3$ and elevation, together explaining 90% of the variability ($n = 36$, $p < 0.05$). Soil NH$_4^+$:NO$_3$ was the single strongest predictor of oxidative activities at this scale, with a positive log relationship (Fig. 4A). Oxidative enzyme activity was also negatively correlated with soil pH ($R^2 = 0.52$, $n = 36$, $p < 0.05$), and positively associated with soil C concentrations ($R^2 = 0.28$, $n = 36$, $p < 0.05$). Within the urban watershed, NH$_4^+$:NO$_3$ and soil C concentration were the strongest predictors of oxidative activities, together explaining 54% of the variability, with the ratio of NH$_4^+$:NO$_3$ the strongest single predictor (Fig. 4B). The correlation of oxidative activities with soil C concentration in the urban watershed was positive, but not as strong as the relationship with NH$_4^+$:NO$_3$ ($R^2 = 0.24$, $n = 24$, $p < 0.05$). Thus, increasing NO$_3$ and declining NH$_4^+$ appeared to stimulate oxidative enzyme activities at multiple scales in this study.

Carbon-acquisition enzyme activities across the three watersheds were best predicted by TDN (Fig. 4C), with soil moisture and elevation also significant factors (3-factor model, $R^2 = 0.67$). A positive relationship with soil C concentrations across the three watersheds was relatively weak ($R^2 = 0.18$, $n = 36$, $p < 0.05$), as was a negative relationship with pH ($R^2 = 0.12$, $n = 36$, $p < 0.05$). Within the urban watershed, total mineral N was the strongest single predictor of C-acquisition activities, explaining 61% of the variability (Fig. 4D). Carbon-acquisition activity was also positively correlated with soil C concentrations in the urban watershed ($R^2 = 0.54$, $n = 24$, $p < 0.05$), although the relationship was not as strong as for mineral N.

Soil pH was the single strongest predictor of P-acquisition enzyme activities at the broad scale, with a negative log relationship predicting 71% of the variability across the three watersheds (Fig. 4E). Elevation and soil moisture were also positively correlated with P-acquisition enzyme activity at this scale, and these two factors increased predictive power for the model including pH to 89%. P-acquisition activity was also positively correlated with soil C concentrations across all sites ($R^2 = 0.50$, $n = 36$, $p < 0.05$). In contrast to these broad-scale patterns, TDN was the single strongest predictor of phosphatase activity within the urban watershed, showing a strong positive correlation (Fig. 4F). Similar to the broad-scale patterns, soil C concentrations were also positively correlated with P-acquisition in the urban watershed ($R^2 = 0.33$, $n = 24$, $p < 0.05$), and there was a negative log relationship with soil pH ($R^2 = 0.39,$...
n = 24, p < 0.05). Thus, it appears that the broad-scale importance of soil pH in driving P-acquisition activities was secondary to the influence of soil N availability in the urban watershed.

Soil N pools were not strong predictors of hydrolitic N-acquisition enzyme activities. Soil moisture was the strongest predictor of N-acquisition activity across the three watersheds (positive relationship, \( R^2 = 0.63, n = 36 \)), and within the urban watershed (\( R^2 = 0.37, n = 24 \)), similar to the relationship between soil moisture and NH₄. Across the three watersheds, pH was also negatively correlated with N-acquisition activity (\( R^2 = 0.25, n = 36, p < 0.05 \)), and C concentrations were positively correlated (\( R^2 = 0.35, n = 36, p < 0.05 \)). Within the urban watershed C concentrations were positively correlated with N-acquisition activity (\( R^2 = 0.27, n = 24, p < 0.05 \)), and there was no relationship with pH.

Predictors of nutrient acquisition ratios at the broad scale reflected the influence of elevation, pH and N levels on individual enzyme activities. Both C:N and C:P acquisition activities decreased significantly with elevation (\( R^2 = 0.17 \) and 0.54, respectively, \( n = 36, p < 0.05 \)). Both C:P and N:P acquisition activities increased strongly with pH (\( R^2 = 0.65 \) and 0.77, respectively, \( n = 36, p < 0.05 \)), reflecting the negative relationship between P-acquisition and pH. Across the three watersheds, TDN, microbial biomass C, and elevation together predicted 40% of the variability in C:N acquisition activity. Soil pH plus TDN, microbial biomass C, and elevation predicted 81% of the variability in C:P acquisition activity. In these multiple regressions, the ratio of C:N acquisition tended to increase with TDN and microbial biomass C, whereas C:P acquisition decreased with TDN and microbial biomass C, such that higher TDN and microbial biomass appeared to shift enzyme acquisition activity from N-acquisition to C-acquisition, and from C-acquisition to P-acquisition.

Within the urban watershed, TDN and pH were the best predictors of nutrient acquisition ratios, with forest fragment size, distance to urban center, and NO₃ levels also significant factors. The best single predictor of C:N acquisition activity in the urban watershed was TDN (positive relationship, \( R^2 = 0.34, n = 24, p < 0.05 \)), and a three-factor model including forest fragment size and microbial biomass C increased predictive power to 75%, with C:N acquisition ratios positively related to both additional factors. Soil pH was the single best predictor of C:P and N:P acquisition ratios within the urban watershed (\( R^2 = 0.34 \) and 0.58, respectively, \( n = 24, p < 0.05 \)), with positive relationships similar to patterns at the broader scale (i.e. higher pH favoring C acquisition). However, adding NO₃ and distance to the urban center increased model predictive power for C:P acquisition activity to 76%, with a positive correlation to NO₃ (\( R^2 = 0.24, n = 24, p < 0.05 \)), and distance to urban center negatively correlated with C:P acquisition (\( R^2 = 0.25, n = 24, p < 0.05 \)). Thus, higher TDN appeared to shift enzyme acquisition activity from N-acquisition to C-acquisition in the urban watershed, similar to patterns at the broader scale, whereas urban factors like distance to the city center and soil NO₃ had apparently opposite effects on C:P acquisition ratios.

In total, the data presented here suggest a hierarchical conceptual model of causality between urban factors, soil nitrogen levels, and enzyme activities (Fig. 5). Across the urban-remote gradient, watershed location appeared to drive variability in soil chemistry, with subsequent effects on enzyme activities (Fig. 5A). Within the urban watershed, forest fragment size and distance to the city center apparently drove extreme small-scale variability in soil chemistry, which in turn drove enzyme activity levels (Fig. 5B).
4. Discussion

4.1. Urban effect on soil nitrogen

Urban forest soils were enriched in NO$_3^-$ and depleted in NH$_4^+$ compared to remote sites, indicating a significant urban effect on soil mineral N availability in these tropical forests. The smaller NH$_4^+$ pools in the urban versus remote forests could be indicative of lower N mineralization rates, as was observed in New York urban forests (White and McDonnell, 1988; Pouyat et al., 1997). However, since total mineral N levels (NH$_4^+$ plus NO$_3^-$) were similar across the urban-remote gradient in Puerto Rico, either N mineralization was high enough to supply significant nitrification, plant demand for NH$_4^+$ was lower, and/or there were external inputs of NO$_3^-$ to the urban forests. Similar to patterns here, NO$_3^-$ levels were higher in New York urban versus rural forests (Zhu and Carreiro, 1999).

Fig. 5. Conceptual models of hierarchical causality among urban factors, soil chemistry, and soil enzyme activities (EA) are shown for: (A) a broad-scale urban-remote tropical forest gradient across three distinct watersheds, and (B) forest fragments within an urban watershed. Arrows show the direction of hypothesized causality. Thicker arrows show the single strongest predictor of each measure, and narrow arrows show additional significant factors in ANOVA. Loops between factors in the same level show correlations. Positive relationships (black lines), negative relationships (dashed lines), and quadratic relationships (unfilled lines) are distinguished.
that study, the authors suggested that increased nitrification was due to the presence of exotic earthworms in urban sites. Although exotic earthworms are common in Puerto Rico and have been linked to accelerated decomposition rates (Liu and Zou, 2002; Dechaine et al., 2005), earthworms were ubiquitous across the entire urban-remote gradient. Some other mechanism may have promoted elevated nitrification in the Puerto Rican urban sites. For example, high proportions of exotic and potentially N-fixing canopy trees in the urban forests may have led to lower demand for soil ammonium than in remote sites, allowing for greater nitrification. Alternatively, atmospheric N deposition could explain the relatively large soil NO$_3^-$ pools in the urban–proximate ecosystems (Fenn et al., 2003; Martínez et al., 2006).

Preliminary N deposition data for San Juan (ITF 2011, unpublished data), and elevated N exports from the Rio Piedras/San Juan urban watershed relative to rural and remote watersheds (McDowell and Asbury, 1994; Ortiz-Zayas et al., 2006) indicate increased N fluxes at the watershed-scale. Similarly, higher N outputs from urban versus rural watersheds have been linked to atmospheric N deposition in USA temperate areas (Groppman et al., 2004). Nonetheless, there was not evidence for overall N enrichment for soil N concentrations, total mineral N, or TDN in the urban–proximate forests, and only NO$_3^-$ was elevated in urban versus remote soils. However, the significantly lower C:N ratios observed in the urban versus remote forests suggest a relative enrichment in N, with only C depleted in the urban sites. The relatively large NO$_3^-$ pools in the urban forests may be indicative of retention via high anion exchange capacity in these highly weathered clay soils (Lohse and Matson, 2005), or larger fluxes of NO$_3^-$ into urban sites may have maintained the larger pool sizes.

The high variability in soil N among sites within the urban watershed suggests a spatially complex effect of urbanization on N availability. Distance to the urban center and forest fragment size were significant factors in analysis of urban NO$_3^-$, mineral N, and TDN levels, with the highest values in the mid urban watershed and in larger forest fragments. The middle of the urban watershed is the main point of topographic inflection, with gradual terrain at lower elevations, and steep slopes at higher elevation. The mid watershed also had the highest proportion of large forest fragments as part of an ecological corridor. Canopy scrubbing of atmospheric pollution (i.e. higher effective N deposition; Schulze, 1989; Clark et al., 1998), combined with the point of inflection, could contribute to the soil N patterns observed in the urban watershed. Additionally, steeper topography in the upper watershed may have promoted N leaching losses and transport to the mid watershed. These spatial patterns in N cycling within the Rio Piedras watershed are typical of urban N cycles, where social and economic factors create complexities that are muted or absent in remote forests (Kaye et al., 2006; Pickett et al., 2008). Drivers of spatial and temporal variability in soil N availability within tropical urban watersheds merit further investigation.

4.2. Links between N availability and decomposition enzyme activities

Measures of soil N were generally the strongest predictors of decomposition enzyme activities across this urban–remote tropical gradient, and within the urban watershed. In particular, C-acquisition and P-acquisition enzyme activities were positively correlated with N availability, as predicted. This result is in agreement with controlled experiments in N-rich tropical soils that have shown positive effects of N fertilization on decomposition rates and hydrolytic enzyme activities (Hobbie and Vitousek, 2000; Allison and Vitousek, 2004; Cusack et al., 2011b). The positive relationship between soil mineral N levels and C acquisition enzyme activity fits with widely observed stoichiometric requirements of microbial biomass for higher C:N than is available in SOM (Cleveland and Liptzin, 2007), such that increased N availability could promote greater investment in C acquisition. Links between N availability and P-acquisition activity are of particular interest in tropical forests on highly weathered soils, where P can be a relatively scarce nutrient (Vitousek and Sanford, 1986; Harrington et al., 2001). Here, it appears that excess N may have been allocated to produce the N-rich enzymes that acquire P as well as C (Vitousek et al., 2010), similar to observations across a number of N fertilization studies (Marklein and Houlton, 2012).

Oxidative enzyme activities were most strongly related to NH$_4^+$:NO$_3^-$ ratios, with smaller ratios and lower oxidative activities in the urban versus remote forest soils. It is well known that N fertilization has the potential to suppress oxidative enzyme activities (Keyser et al., 1978; Kirk and Farrell, 1987; Fog, 1988; Carreiro et al., 2000; Frey et al., 2004; Cusack et al., 2010, 2011a), but the effect of specific forms of mineral N are poorly understood. Here, a shift from NH$_4^+$-dominated mineral N pools toward NO$_3^-$-dominated pools corresponded to a reduction in oxidative enzyme activities, suggesting that NH$_4^+$ in excess of plant and microbial demand (i.e. high nitrification rates), and/or external inputs of NO$_3^-$, may reduce microbial investment in oxidative enzymes. These data are in contrast with a study showing that microbial respiration rates responded similarly to different chemical forms of added mineral N (Ramirez et al., 2010). Nonetheless, the data presented here suggest that the relative abundance of NO$_3^-$ versus NH$_4^+$ may play a role in driving oxidative enzyme activities.

Soil pH and soil moisture were also important predictors of N-acquisition and P-acquisition enzyme activities across the urban–remote gradient. Soil pH spanned four orders of magnitude, despite similar volcaniclastic parent material in the urban and lower elevation remote forests, with greater acidity in remote soils. This is in contrast to temperate forest studies that have found increased acidity in urban soils relative to remote forest sites (Pouyat et al., 1995). A global study across a broad range of biomes and soil pH levels found that P-acquisition enzyme activities declined with pH (Sinsabaugh et al., 2008), similar to results here. It should be noted that an acidic buffer was used here with the aim of standardizing and maximizing enzyme activities across sites (Tabatabai, 1994), but soils with neutral and alkaline pH may have higher pH optima, particularly for alkaline phosphatase enzyme activities (Turner, 2010). Thus, the lower phosphatase activities reported here for higher pH soils reflect acid phosphatase activity only, whereas alkaline phosphatase activity may be higher than reported. Unlike the global study, oxidative enzyme activities also declined at higher pH across the Puerto Rican gradient, suggesting that fungal species native to acidic tropical soils may be poorly adapted to increases in pH. Surprisingly, N-acquisition activities were not correlated to soil N levels, but rather responded strongly to soil moisture. The lower soil moisture in the urban sites may have been related to a heat island effect around San Juan (Velasquez-Lozada et al., 2006), and/or lower rainfall in the lower-elevation urban sites. In general, it appears that there was a significant urban effect on a number of soil physical and chemical factors, which in turn drove landscape-scale variability in soil enzyme activities.

Certainly, additional factors not measured here could have simultaneously influenced soil N levels and enzyme activities in the urban watershed. For example, deposition of other atmospheric pollutants and particulates can occur simultaneously with N deposition (Lara et al., 2001). Land-use history also varied across the gradient, with agricultural abandonment and secondary regeneration predominant in the urban watershed (Helmer, 2004; Kennaway and Helmer, 2007), and historic human and hurricane disturbance frequent throughout rural and remote Puerto Rican
forests (Foster et al., 1999). Soil disturbance as well as variability in canopy tree species composition and litter chemistry could also have contributed to the variability in enzyme activities across the gradient (e.g. via fungal host preference) (Lodge and Cantrell, 1995; Lodge, 1997; Usioho et al., 2008; Soval-Perez et al., 2009). Some of these factors were likely captured in part with the measures of soil bulk density, canopy openness, tree species composition, basal area, and elevation. Despite this complexity of factors, the consistent strong relationships between soil enzyme activities and soil N levels provide evidence that N is a dominant control on SOC cycling, even in N-rich tropical soils.

4.3. Global context

Compared to global averages, total enzyme activities across this urban-remote gradient were very high for P-acquisition, near global averages for N- and C-acquisition, and in the lower range for oxidative enzyme activities (Sinsabaugh et al., 2008). Enzyme activities across the urban-remote tropical gradient spanned over an order of magnitude, covering approximately 1/3 of the global range, with much of this variability present over a relatively small spatial scale in the urban watershed. The low rates of C- and P-acquisition activity in the urban watershed were more similar to rates in a tropical pineapple plantation (Waldrop and Firestone, 2004) than to rates in the remote Puerto Rican forests, possibly suggesting a role of the agricultural history of these forests in driving current enzyme activities. Ratios of C:P acquisition and N:P acquisition activities were well below global averages for soils, whereas C:N acquisition activities were near or above global averages (Sinsabaugh et al., 2008), indicating P limitation to SOM processing in general, particularly in the remote watersheds. Here, only NAGase was used as a measure of N-acquisition activity, whereas the global study also included leucine aminopeptidase (LAP), which may contribute to the somewhat higher C:N acquisition activities reported here. However, prior work in Puerto Rican forests showed that LAP activity was <15% of total N-acquisition activity (Cusack et al., 2011a). Thus, the higher C:N acquisition ratios reported here more likely resulted from high soil N availability relative to C across these tropical forests, especially in the urban sites. The ratios of C:P:N enzyme activity in all of the watersheds showed lower N acquisition activity and higher P acquisition activity compared to the global average of 1:1:1 (Sinsabaugh et al., 2008). Overall, these results show that urbanization has great potential to alter ecosystem processes, providing enormous gradients in soil properties over relatively short distances. Furthermore, the potential positive urban effect on soil mineral N pools is significant in N-rich tropical forests, with consequences for microbial activity and SOM cycling.

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