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Exotic grasses and nitrate enrichment alter soil carbon cycling along an urban–rural tropical forest gradient

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Abstract

Urban areas are expanding rapidly in tropical regions, with potential to alter ecosystem dynamics. In particular, exotic grasses and atmospheric nitrogen (N) deposition simultaneously affect tropical urbanized landscapes, with unknown effects on properties like soil carbon (C) storage. We hypothesized that (H1) soil nitrate (NO_3^-) is elevated nearer to the urban core, reflecting N deposition gradients. (H2) Exotic grasslands have elevated soil NO_3^- and decreased soil C relative to secondary forests, with higher N promoting decomposer activity. (H3) Exotic grasslands have greater seasonality in soil NO_3^- vs. secondary forests, due to higher sensitivity of grassland soil moisture to rainfall. We predicted that NO_3^- would be positively related to dissolved organic C (DOC) production via changes in decomposer activity. We measured six paired grassland/secondary forest sites along a tropical urban-to-rural gradient during the three dominant seasons (hurricane, dry, and early wet). We found that (1) soil NO_3^- was generally elevated nearer to the urban core, with particularly clear spatial trends for grasslands. (2) Exotic grasslands had lower soil C than secondary forests, which was related to elevated decomposer enzyme activities and soil respiration. Unexpectedly, soil NO_3^- was negatively related to enzyme activities, and was lower in grasslands than forests. (3) Grasslands had greater soil NO_3^- seasonality vs. forests, but this was not strongly linked to shifts in soil moisture or DOC. Our results suggest that exotic grasses in tropical regions are likely to drastically reduce soil C storage, but that N deposition may have an opposite effect via suppression of enzyme activities. However, soil NO_3^- accumulation here was higher in urban forests than grasslands, potentially related to of aboveground N interception. Net urban effects on C storage across tropical landscapes will likely vary depending on the mosaic of grass cover, rates of N deposition, and responses by local decomposer communities.

Keywords: dissolved organic carbon, enzyme activities, invasive species, NAGase, nitrogen deposition, non-native species, phosphatase, seasonality, secondary rain forest, soil moisture, *β*-glucosidase

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Introduction

Urban expansion is occurring rapidly in tropical regions (Lambin *et al*., 2003) with significant potential to alter carbon (C) storage in nearby ecosystems via interacting disturbances (Kaye *et al*., 2006; Martinelli *et al*., 2006). For example, deforestation near urban centers is likely to be accompanied by the simultaneous spread of exotic plant species and elevated rates of atmospheric nitrogen (N) deposition, both of which are likely to alter soil C storage with unknown interacting effects. Humid tropical forests are the most C-rich ecosystems on the Earth, storing approximately 28% of aboveground C and 10% of soil C globally, so the effects of urbanization on C dynamics in this biome are of broad concern (Post *et al*., 1982; Schlesinger, 1997). Declines in tropical forest soil C with anthropogenic activity have been linked to elevated soil respiration, erosion, and reduced C inputs from plant biomass (Lugo & Brown, 1993). Overall, human disturbance to tropical forests is estimated to emit 0.2 Pg of C to the atmosphere per year, but the proportion of this flux coming from remote vs. urban-proximate sites is not well known (Achard *et al*., 2004; Baccini *et al*., 2012). As urban centers expand, a greater proportion of tropical forests will undergo a variety of disturbances that are absent from or do not occur in concert in more remote sites.

At a regional scale, urbanization may promote forest regeneration and C storage because of human migration to urban centers and abandonment of agricultural lands. For example, secondary forest growth followed widespread agricultural abandonment in Puerto Rico starting in the 1930s (Helmer, 2004; Kennaway & Helmer, 2007), which was an early case of an ongoing and increasingly widespread trend toward abandonment of small-scale agriculture in Latin America (Grau & Aide, 2008). With the regeneration of secondary forests, there is significant potential to restore aboveground C stocks in plants (Silver *et al*., 2000; Marin-Spiotta *et al*., 2008; Aryal *et al*., 2014; Gonzalez *et al*., 2014; Shimamoto *et al*., 2014). However, the potential for recovery of lost

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soil C, even after long-term forest regeneration, varies greatly across sites with no clear trends (Powers, 2004; Jimenez *et al.*, 2008; Marin-Spiotta & Sharma, 2013). Also, many abandoned lands are relatively near urban centers and are within the range of current urban expansion, such that secondary forests are subject to ongoing disturbances such as clearing, exotic species establishment, and N deposition (Chytry *et al.*, 2008; Cusack & McCleery, 2014).

The spread and establishment of exotic (i.e., non-native) herbaceous plant species in tropical sites is of particular concern. Urban centers are a major source of entry for exotic plants species to degraded or deforested areas (Cowie & Werner, 1993; McKinney, 2002, 2008). A loss of forest canopy has been linked to the subsequent spread of shade intolerant exotic grasses, especially in tropical sites where mature forest canopies are dense and native plants are adapted to low light for germination (Murphy *et al.*, 2008; Siddique *et al.*, 2008). Exotic grasses and other herbaceous species can persist indefinitely in cleared sites and interrupt successional trajectories (Kuusipalo *et al.*, 1995; Parrotta *et al.*, 1997; Powers *et al.*, 1997; Cusack & Montagnini, 2004), effectively converting tropical forests to exotic grasslands indefinitely. Conversion of tropical forest to grassland can result in lower soil C stocks as well as decreased aboveground biomass (Lucas *et al.*, 1996; Williams & Baruch, 2000; Ehrenfeld, 2003). Removal of forest canopy structure can also reduce nutrient and moisture inputs to soils because large forest canopies effectively ‘scrub’ moisture and nutrients from the atmosphere (Schulze, 1989; Clark *et al.*, 1998). Investigation of the landscape-scale effects of exotic grasslands on tropical soil C storage in urban-proximate systems has received little attention, despite widespread concern about the long-term stability of tropical C sinks.

A second global change factor, N deposition, generally overlays the complex mosaic of land covers around urban centers, and N deposition rates in tropical regions are expected to be the highest globally in the coming decades (Galloway *et al.*, 2004). Increased N deposition results from fossil fuel combustion, industrial activity, and agricultural fertilizer (Lara *et al.*, 2001; Krusche *et al.*, 2003; Martinelli *et al.*, 2006) and can acidify soils, stimulate leaching of base cations, and negatively affect water quality (Pouyat *et al.*, 1995; McDonnell *et al.*, 1997; Matson *et al.*, 1999; Lara *et al.*, 2001; Groffman *et al.*, 2009). In both temperate and tropical sites, urban-proximate forests can have higher soil mineral N levels, mineralization rates, and N leaching relative to rural reference sites (White & McDonnell, 1988; Zhu & Carreiro, 1999; Groffman *et al.*, 2009; Cusack, 2013). Unlike most temperate sites, however, tropical forests on highly weathered soils tend to have relatively high background N availability (Walker & Syers, 1976; Chestnut *et al.*, 1999; Martinelli *et al.*, 1999; Hedin *et al.*, 2009), such that added N does not generally stimulate plant growth (Mirmanto *et al.*, 1999; Harrington *et al.*, 2001; Ostertag, 2001; Kaspari *et al.*, 2008; Cusack *et al.*, 2011b). Thus, the effects of N deposition on tropical forest C storage are likely to occur via changes in belowground processes.

In particular, soil C storage can be sensitive to N addition in N-rich tropical ecosystems because microbial decomposers on plant litter are spatially segregated from N available in mineral soils (Berg & Matzner, 1997). In general, microbial decomposers have high N : C requirements relative to the N : C available in senesced plant tissues (Cleveland & Liptzin, 2007), suggesting a fundamental N limitation to decomposition. Still, N fertilization studies across tropical forest sites have found variation in the direction of the N effect on soil C cycling, including positive and negative responses of decomposition enzyme activities, production of dissolved organic C (DOC), soil respiration rates, and soil C levels (Cleveland & Townsend, 2006; Cleveland *et al.*, 2006; Mo *et al.*, 2008; Cusack *et al.*, 2010, 2011a,b). Variation in the effect of N on microbial enzyme activities may be related to local litter quality. For example, a multisite study in temperate forests found that added N suppressed enzyme activities in forests with low-quality litter (i.e., high lignin) and increased activities in forests with high-quality litter, with related changes in soil C stocks (Waldrop *et al.*, 2004). Nonetheless, a recent review concluded that N fertilization generally increased the activity of certain enzymes across temperate and tropical sites, in part because of the high N requirement for enzyme production (Marklein & Houlton, 2012). Despite the growing impact of N deposition on tropical regions, the effect of elevated N on soil C storage is still poorly understood, and interactions with other global change factors such as invasive plants are completely unexplored.

This research sought to understand the combined effects of exotic grass establishment and N deposition on tropical soil C cycling in an urban setting. We used six paired exotic grassland/secondary forest sites along a tropical urban-to-rural gradient to address the following hypotheses (H1): Soil nitrate (NO_3^-) is elevated in forest and grassland sites nearer to the urban core, reflecting an N deposition gradient. (H2) Exotic grasslands have relatively drier soils, elevated soil NO_3^-, and decreased soil C relative to secondary forests, with higher N promoting decomposer activity. (H3) Exotic grasslands have greater seasonality in soil NO_3^- vs. secondary forests, due to higher sensitivity of grassland soil moisture to rainfall. In general, we predicted that elevated soil NO_3^- would have a positive effect on microbial decomposer enzyme activities because of the high N : C requirements of microbial biomass relative to plant tissues. We expected that higher decomposer activity in exotic grasslands would be linked to lower soil C storage compared with secondary forests. We also predicted that soil NO_3^- would be negatively related to DOC production because of more complete decomposition of organic matter at high N levels, with decreased DOC indicating reduced transport of C from decomposing tissues to mineral soil. We assessed temporal and spatial variability in soils of unmanaged exotic grasslands relative to paired adjacent secondary forests, examining soil C, nutrients, and microbial activity.

Materials and methods

Study sites

The main site for this study was an urban–rural (hereafter 'urban') watershed in Puerto Rico, with additional reference forest sites in a more remote watershed (Fig. 1). In the urban Río Piedras watershed, six secondary forest stands were paired with nearby exotic grasslands. These sites were all within the San Juan Urban Long Term Research Area (ULTRA-Ex), and none of the sites were under active management. The six sites were in six subwatersheds (i.e., 'watershed synoptics') and represented all of the major forested areas of the urban watershed, spanning from the low-elevation urban core to higher elevation rural and suburban areas (Fig. 1). The urban 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, and has mean annual temperature (MAT) of 25.7 °C. Averages from 18 years of stream discharge data for the Rio Piedras show a distinct dry season (Dry) from February to March (0.3–0.37 $m^3\ s^{-1}$), an early wet season (Wet) from April to July (0.4–0.6 $m^3\ s^{-1}$), a peak in rainfall during the hurricane season (Hurricane) from August to December (0.7–1 $m^3\ s^{-1}$), and a transition back to drier conditions during January (0.5 $m^3\ s^{-1}$) (USGS 2015). Sixteen weeks of preliminary N deposition data in the mid-urban watershed (near site 3.0, Table 1) 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 urban watershed has also shown large river outputs of N compared with nearby rural watersheds (Ortiz-Zayas *et al.*, 2006), suggesting substantial N deposition within the watershed.

The remote forest sites were located in the USFS Cubuy Annex (18°16′ N, 65°52′ W), which is also secondary forest in the subtropical moist forest life zone and has elevation from 300 to 500 masl, MAP of 2500 mm, and MAT of 26 °C (Silver *et al.*, 2004). The remote watershed was used only to provide general reference comparisons of overall trends with the urban watershed. Rates of wet N deposition near the remote forest site were 3.9 kg-N $ha^{-1}\ yr^{-1}$ (NADP/NTN 2013).

All forest stands were secondary regeneration following widespread agricultural abandonment in Puerto Rico starting in the 1930s (Kennaway & Helmer, 2007). Both the Rio Piedras and Cubuy forests contained novel mixtures of native and non-native tree species (Helmer, 2004; Lugo, 2004; Kennaway & Helmer, 2007; Cusack & McCleery, 2014). Abundant exotic grass species in the urban watershed included *Panicum maximum* Jac q. and *Pennisetum purpureum* Schumach., which are both African grasses and can establish in open areas (Lugo *et al.*, 2001). Soils at all sites were volcaniclastic Oxisols (Beinroth, 1982; Huffaker, 2002).

Within the urban watershed, the site at watershed synoptic 0.5 (Fig. 1, Table 1) is nearest to the urban core in the Bosque San Patricio, which is managed by the Puerto Rico Department of Natural Resources and the Environment (DNRE). This site has had forest regeneration since the 1930s, although part of the forest was used as US military housing until the 1960s (DNRE 2003). The Bosque San Patricio also contains an area of exotic grassland. A larger forest fragment in the mid-watershed (3.0, Table 1) is in the Jardín Botánico of the University of Puerto Rico and has an adjacent area of exotic grass cover. Forest plots in this area have had secondary regeneration since the 1930s (Lugo *et al.*, 2005). Site 4.3 was previously a private coffee plantation that was allowed to reforest starting in the 1930–1940s, with an adjacent empty lot of exotic grass cover. Stands 5.1 and 6.0 are on unprotected government land with natural forest regeneration of unknown age near Las Curias Reservoir and surrounding a power station, respectively, with adjacent exotic grassland areas. Site 5.0 is on private land belonging to the Betancourt family, with a nearby empty lot covered in exotic grasses.

Landscape-scale urban factors were measured to characterize each site. Forest and grassland fragment sizes, distance to the urban center, and distance to the nearest major road were measured using Arc GIS 10 with a land-cover classification map for the area (Kennaway & Helmer, 2007) (Table 1). Forest fragments were defined as areas of continuous forest and were generally bounded by roads, grasslands, or urban cover. Four broad land-cover classes were modified from Kennaway & Helmer (2007) for the Rio Piedras watershed, and total area was calculated for each cover type, including high-density urban cover (2509 ha), low-density urban cover (2372 ha), grassland (782 ha), and forest (829 ha), leaving 185 ha as 'other' (waterways, wetlands, etc.).

Field collections

Soils were collected during the three dominant seasons from 2010 to 2011, including hurricane season (October 2010), the dry season (March 2011), and the early wet season (July 2011). For all measurements, soils were collected from paired grassland and forest sites on the same day. Soils were collected from 0 to 10 cm depth using a 2.5 cm diameter soil corer. This depth showed the largest changes in soil C cycling with N fertilization in remote forests in Puerto Rico (Cusack *et al.*, 2011b). At each of the six paired exotic grassland/secondary forest sites, soils were collected from three 20-m transects under each of two cover types, for a total of 36 transects in the

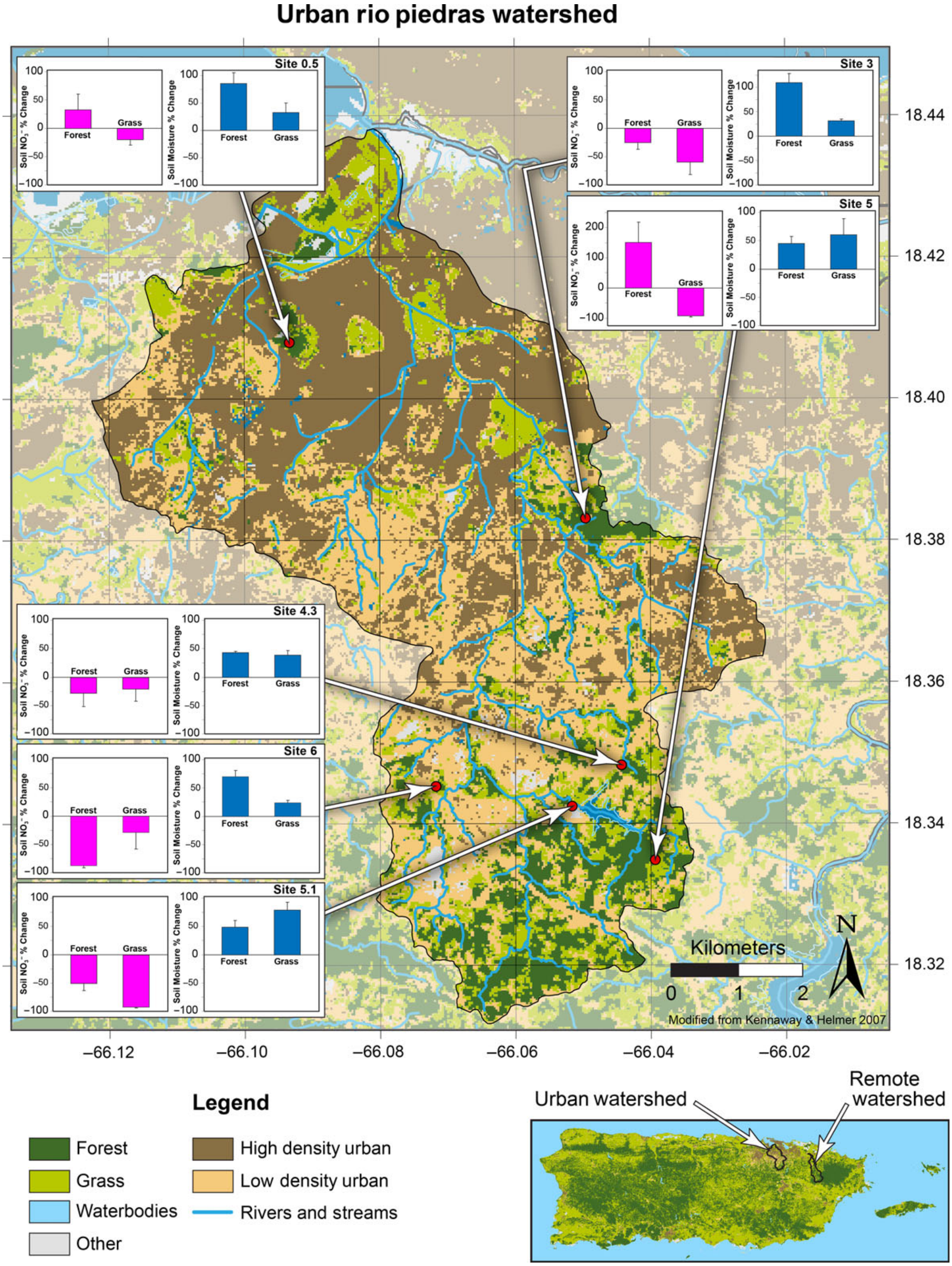

Fig. 1 Six paired exotic grassland/secondary forest sites are shown for the urban Rio Piedras watershed, spanning from the lower elevation urban core (north), to the suburban and rural upper elevation (south). The remote reference watershed that includes the Cubuy Annex to the USFS is also shown (inset). Four main cover classes are shown plus waterways. Graphs for each site give the seasonal shift in soil NO_3^- and soil moisture, showing the percent change from the dry season to the following wet season for exotic grasslands and secondary forests (mean % difference ± 1 s.e.). Overall, exotic grasslands showed greater and more consistent seasonal declines in soil NO_3^- than did forests, whereas secondary forests showed greater seasonal increases in soil moisture than did grasslands.

Table 1 Information for six paired exotic grassland/secondary forest sites, and remote secondary forest sites

Watershed*	Site ID	Elevation (masl)	Latitude N	Longitude W	Forest fragment size (km^2)	Grass fragment size (km^2)	Distance to urban center (km)	Shortest distance to major road (km)	Forest basal area m^2 ha^{-1}
Urban RP	0.5	20	18.407850	−66.093383	0.27	0.03	3.6	0.6	38 ± 1
Urban RP	3.0	40	18.383082	−66.049615	1.79	0.10	6.8	2.1	30 ± 2
Urban RP	4.3	100	18.348346	−66.044362	0.21	0.04	10.5	2.6	20 ± 3
Urban RP	5.0	160	18.334904	−66.039383	0.56	0.05	12.1	1.2	29 ± 6
Urban RP	5.1	120	18.342533	−66.051667	0.06	0.02	10.9	1.3	37 ± 9
Urban RP	6.0	88	18.345326	−66.071781	0.07	0.01	10.3	0.2	33 ± 3
Remote C	R	577	18.276670	−65.868970	0.67*	n/a	28.3	4.6	37 ± 1

*The two locations are the urban Rio Piedras watershed in the San Juan Metropolitan area (Urban RP), and the remote secondary forests in the USFS Cubuy Annex to El Yunque National Forest (Remote C). The remote secondary forest sites (n = 6) are an annex to nearby El Yunque National Forest (11 270 ha of forest).

urban watershed. Along each transect, four soil cores were collected at each of four points (total of 16 cores per transect) and then pooled to give one representative sample for each of the 36 transects. Six forest stands were sampled similarly in the remote Cubuy forest. Bulk density was measured from 0 to 10 cm back from the undisturbed face of a 20-cm-deep pit in each site using a 6.5-cm-inner-diameter corer as described in Cusack (2013). Standing grass biomass was collected on each transect using a 323-cm^2 quadrant, dried at 60 °C until weight stabilized, and weighed. Grass heights at all sites ranged from 2 to 3 m.

Soil respiration and temperature were measured at all sites once during the wet season in July 2011 with a LiCor (Lincoln, NE, USA) 6400XT fitted with a soil CO_2 flux chamber. At each site, four 10-cm PVC collars were inserted directly into mineral soil along each transect leaving a 2-cm lip aboveground and allowed to equilibrate for 1 h prior to measurements. Paired forest and grassland plots were measured sequentially on the same day at approximately midday, avoiding heavy rain events.

Soil analyses

For soil nutrient analyses, fresh soils were extracted using 2 M potassium chloride (KCl) for ammonium (NH_4^+), NO_3^-, DOC, and total dissolved N (TDN) on the day of collection in Puerto Rico to minimize storage effects on mineral N pools (Turner & Romero, 2009) as described in Cusack (2013). Frozen extracts were shipped for analysis to the University of California – Los Angeles. Extractable NH_4^+ and NO_3^- were analyzed using standard colorimetric techniques in 96-well plates (Rhine *et al.*, 1998; Doane & Horwath, 2003). Analyses included standard curves, check standards, and four analytical replicates per sample. Mineral N levels were measured on a BioTek Instruments Inc. (Winooski, VT, USA), Synergy HT absorbance spectrometer. Extracted DOC and TDN were measured in duplicate together with standard curves and check standards on a Shimadzu TOC-L CSH, with a TNM-L unit for TN detection. Separate soil subsamples were air-dried and ground using a mortar and pestle for total C and N analyses, and grass biomass was ground on a Wiley Mill. Dry soils and grass biomass were analyzed for C and N content on a Costech Elemental Analyzer (Valencia, CA) using atropine and acetanilide as standards.

All microbial enzyme activity and biomass data reported here are for fresh pooled soil samples shipped overnight to the University of California – Los Angeles, and analyzed within 48 h. Standard methods for soil enzyme assays followed Sinsabaugh *et al.* (2003) with changes as described in Cusack (2013). Hydrolytic enzymes were measured using fluorometric assays on soils incubated at 27 °C for 0.5–8 h, with incubation times based on initial tests. Carbon acquisition was measured as cellulose-degrading β-glucosidase activity, N acquisition was measured as chitin-degrading *N*-acetylglucosaminidase (NAGase) activity, and P acquisition was measured as acid phosphatase activity. These enzymes were previously observed to have the highest activities for acquisition of C, N, and P in this watershed (Cusack *et al.*, 2011a). Plates with 96-well plates were read on a Bio-Tek Instruments Inc., Synergy HT absorbance/fluorescence spectrometer at 365 nm excitation/450 nm emission for fluorescence. Microbial biomass C and N were measured using a modified chloroform fumigation-slurry extraction as described in Fierer & Schimel (2003), similarly assuming an extraction efficiency of 0.45 (Beck *et al.*, 1997; Dictor *et al.*, 1998). Extracted microbial biomass C and N were compared to non-chloroform extractions on a Shimadzu TOC-L CSH, with a TNM-L.

Total rock-derived soil nutrients were measured from 0 to 10 cm to compare site characteristics for grasslands vs. forests and to include as predictors in statistical analyses. Air dried soils were prepared as above and then digested with concentrated HNO_3, 30% H_2O_2, and concentrated HCl at the US Forest Service International Institute of Tropical Forestry laboratory in Puerto Rico, in a modification of a standard digestion method (Luh Huang & Schulte, 1985). Total acid extraction values of aluminum (Al), calcium (Ca), iron (Fe), magnesium (Mg), manganese (Mn), potassium (K), sodium

(Na), phosphorus (P), and sulfur (S) were determined using a Spectro Plasma Emission Spectrometer, Spectro, Kleve, Germany (model Spectro Ciros CCD – ICP). The certified reference material Montana Soil (NIST – 2711) was analyzed to verify the completeness of elemental recovery. Subsamples dried at 105 °C were used for moisture correction. These measures were conducted on soils collected during the wet season only, because total rock-derived nutrient content of soils is relatively stable over annual timescales.

Statistical analysis

Soil C and N pool sizes were analyzed for significant differences between exotic grassland and secondary forest sites over three seasons in the urban watershed. Repeated-measures multivariate analysis of variance (MANOVA) was used to assess differences between cover types across the three time points, using *post hoc* tests to compare parameters for specific seasons or sites. We used preliminary backward stepwise linear regression analyses including all factors to identify potential predictors of soil C and N measures. We then included only potentially significant factors identified by these stepwise regressions in final linear models. Microbial biomass C and N, NO_3^-, NH_4^+, total mineral N, TDN, enzyme activities, soil moisture, pH, bulk density, and total rock-derived nutrients were included as covariates in initial stepwise tests. Final tests included MANOVA for soil measures across the three seasons and ANCOVA for wet season CO_2. We repeated these analyses to identify predictors of soil C (C concentrations, C stocks, DOC, CO_2), soil N (NO_3^-, NH_4^+, total mineral N), and enzyme activities. We assessed spatial variability in soil C and N (H1) by including distance to the urban core, distance to a major road, watershed synoptic, and elevation as predictors. We also calculated paired plot differences in soil C and N measures to assess the relationship between grassland and forest spatial variability. To assess effects of cover type and soil N on soil C (H2), we used the MANOVA above to identify significant predictors of soil C and then ran *post hoc* Student's *t*-tests to compare between cover types. To explore seasonal patterns in exotic grasslands vs. secondary forests (H3), we used the MANOVA above to identify when time was a significant factor, and then ran *post hoc* means separation Tukey HSD tests to compare biogeochemistry by season. To further assess the magnitude of seasonal changes in exotic grasslands vs. secondary forests, we calculated the percent change in soil properties for each individual plot from the dry season to the subsequent wet season (Fig. 1) and compared the averages of percent change. We used separate tests to compare the remote watershed forests with urban watershed secondary forests and grasslands. For site-level comparisons, we used $n = 6$ per vegetation type, and for initial stepwise regression analyses, we used the total observations for each parameter $n = 36$. Where necessary, values were log-transformed to meet assumptions of normality. These general linear analyses were used to test the three hypotheses individually and to explore specific relationships and trends.

We also analyzed all of the above relationships simultaneously using structural equation modeling (SEM) path analysis to assess the relative importance of the main driving factors from each of the individual hypotheses (i.e., distance to the urban core, cover type, and seasonality). We used distance to the urban core, cover type, and seasonality as our exogenous factors, and soil NO_3^-, moisture, microbial enzyme activity, and soil C concentrations as endogenous factors. Because of high autocorrelation among enzyme activities, we used only β-glucosidase activity in the model, which was also the most significant enzyme predictor in the general linear models. We used SEM modification indices to add or remove specific pathways from the model. An initial model included microbial biomass C as an endogenous factor, but this was removed because of poor model similarity to the saturated model. Season and cover type were rank-transformed and converted to binary data, respectively, for SEM. The final default model (i.e., our model) was tested for overall similarity to the saturated model (i.e., ideal model) using common metrics of comparison (i.e., no significant difference from saturated model, chi-square $P > 0.05$, GFI > 0.9, NFI > 0.9, RMSEA < 0.05). The model used 123 observations.

General linear models were performed using 11.0.0 JMP PRO software (SAS Institute Inc., Cary, NC, USA), and SEM path analysis was performed using SPSS AMOS 23 software (IBM, Armonk, NY, USA). Statistical significance for all relationships and models was $P < 0.05$ unless otherwise noted, and means are reported ± one standard error.

Results

Spatial trends in soil N and C in an urban watershed

Spatial trends for soil NO_3^- and C relative to urban features were strongest for exotic grasslands, with greater variability in secondary forests. Soil NO_3^- in both cover types was generally higher nearer to the urban core than in more suburban and rural sites, supporting H1 (Fig. 2). However, only grasslands had a clear and significant decline in soil NO_3^- with distance to the urban center ($R^2 = 0.33$, $P < 0.05$) and with increased subwatershed remoteness (i.e., watershed synoptic, $R^2 = 0.37$, $P < 0.05$). Soil NO_3^- in forests was more variable in the lower watershed and diminished more gradually in the upper watershed. Still, the most rural forests in the urban watershed had NO_3^- concentrations down near background levels found in the remote secondary forest (Fig. 2). Differences in soil NO_3^- for forest–grassland paired sites were positively related to distance to a major road ($R^2 = 0.35$, positive correlation, $P < 0.05$), showing greater accumulation of NO_3^- in forests vs. grasslands in more remote areas. Also, soil NO_3^- levels were not significantly correlated between paired forest–grassland sites, suggesting that distinct factors governed spatial variability in soil NO_3^- for the two cover types.

Spatial trends in soil C concentrations were positively related to patterns for soil NO_3^-. Grassland soil C was

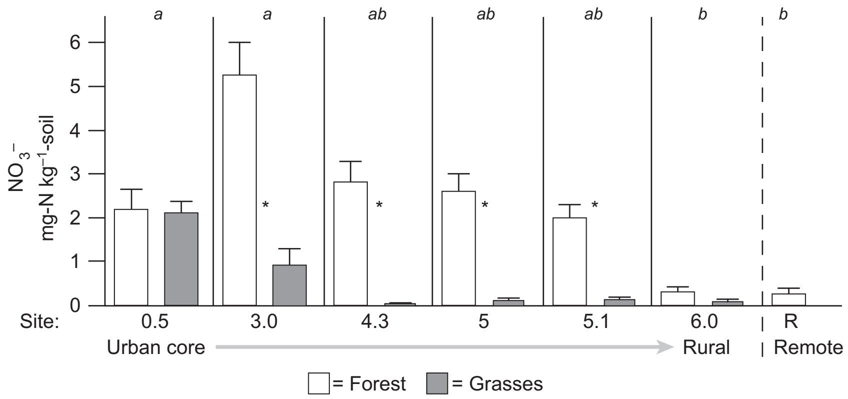

Fig. 2 Soil NO_3^- levels are shown for six paired exotic grassland/secondary forest sites along an urban–rural gradient and for remote secondary forests (R) for reference. Soil NO_3^- in both cover types showed general declines with distance from the urban core, with greater variability in forested sites. Letters show significant differences among the six urban sites and the remote site using a Tukey HSD test ($P < 0.05$). * indicates significant difference between grassland and secondary forests at a given site ($P < 0.05$).

negatively associated with distance to the urban center ($R^2 = 0.28$, $P < 0.05$) and with increased subwatershed remoteness (i.e., watershed synoptic, $R^2 = 0.26$, $P < 0.05$). Soil C concentrations in forests were more variable than in grasslands, but tended to be lowest in the upper watershed. Similar to NO_3^-, there was not a significant correlation for soil C between paired forest–grassland sites.

Soil NO_3^- and C storage in exotic grasslands vs. secondary forests

Exotic grasslands had consistently lower soil C storage than urban secondary forests, which was linked to elevated soil respiration and higher decomposer enzyme activities, providing partial support for H2. On average, soil C concentrations were significantly lower in exotic grasslands than in secondary forests (Fig. 3a). There were also significantly higher CO_2 losses via soil respiration for grasslands vs. forests during the wet season measurement (Fig. 3b), and no difference in soil temperature (25.8 ± 0.4 °C in grasslands and 26.5 ± 0.2 °C in forests). Significant factors in the analysis of soil respiration included cover type, soil moisture, and total hydrolytic soil enzyme activities ($P < 0.05$). Interestingly, despite the higher soil respiration rates in grasslands, soil microbial biomass C and N during the wet season were lower in grasslands than in forests (Fig. 3c, Table 2), and microbial biomass C : N was significantly lower in grasslands (7.2 ± 0.5) versus forests (8.8 ± 0.6). C : N is a ratio and thus singular. Nonetheless, grasslands had higher β-glucosidase and NAGase enzyme activities relative to secondary forests (Fig. 3d, Table 2). Thus, β-glucosidase activity when normalized per unit of microbial biomass C was approximately twice as high in grasslands (4.1 ± 0.1 activity-nmol/μg-microbial C/hr) vs. secondary forests (2.0 ± 0.3, $P < 0.05$ activity-nmol/μg-microbial C/hr) for this time point, suggesting a fundamental difference in microbial function. Similarly, β-glucosidase, NAGase, and phosphatase enzyme activities when normalized to soil C content were much higher in exotic grasslands vs. secondary forests (Table 2). Overall, the higher microbial activity in grasslands vs. secondary forests was strongly associated with lower soil C stocks in grasslands.

Contrary to our prediction, soil C and NO_3^- were significantly positively correlated overall ($R^2 = 0.53$, $P < 0.05$), with a somewhat weaker relationship in forests than in grasslands ($R^2 = 0.52$ vs. 0.63, respectively, $P < 0.05$). Also contrary to our prediction, soil NO_3^- was strongly negatively related to soil enzyme activities. Soil NO_3^- was the only significant biogeochemical factor in the analyses of normalized soil enzyme activities, with negative relationships between normalized β-glucosidase, NAGase, and phosphatase activities ($R^2 = 0.13$, 0.22, 0.09, respectively, $P < 0.05$), as well as with total hydrolytic enzyme activities (Fig. 4b, $R^2 = 0.26$, $P < 0.05$). Finally, and also contrary to our prediction, forests had higher soil NO_3^- concentrations than exotic grasslands, and forest soils were relatively more enriched in NO_3^- per unit of TDN compared to grasslands (Fig. 4a). Other soil nutrients did not show strong differences between cover types except for total K, which was significantly higher in grasslands than in urban forests ($P < 0.05$) (Table 3, Table S1). These data indicate greater accumulation of NO_3^- in secondary forests vs. paired grasslands across the urban–rural gradient and negative effect of NO_3^- enrichment on decomposer enzyme activities. Soil NO_3^- levels were positively linked to soil C levels via suppression of decomposer enzyme activities.

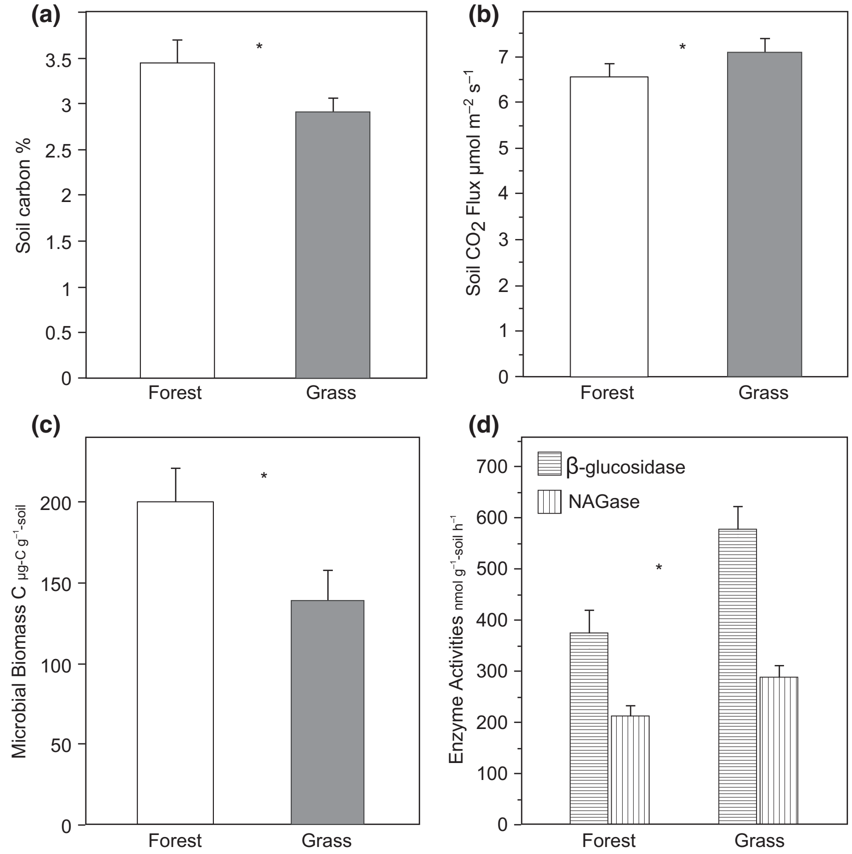

Fig. 3 Carbon levels and fluxes illustrate overall changes in C cycling for exotic grasslands vs. secondary forests in an urban tropical watershed: (a) Soil C concentrations were significantly higher in urban secondary forests vs. grasslands; (b) soil respiration (CO_2 fluxes) were significantly lower in urban forests vs. grasslands, with overall lower respiration rates in these urban sites than in remote secondary forests in Cubuy (7.0 ± 0.2 μmol m^{-2} s^{-1}, not shown); (c) microbial biomass C was significantly higher in forest vs. grassland soils; and (d) potential activities of two hydrolytic decomposition enzymes were lower in forest vs. grassland soils. *indicated significant difference between cover types ($P < 0.05$).

Seasonal variation in soil properties for forests and exotic grasslands

Exotic grasslands had somewhat less seasonality in soil moisture but more seasonality in soil NO_3^- compared to paired secondary forests, partially supporting H3. Assessing the percent change in soil moisture from the dry season to the subsequent wet season, forests had a $67 \pm 7\%$ increase in soil moisture, vs. a $42 \pm 7\%$ increase in grassland soil moisture (Fig. 1, $P < 0.05$). These seasonal shifts in soil moisture were significant for both cover types (i.e., percent change significantly different from 0), with generally drier soils in the dry vs. wet season, but overall wetter conditions in grasslands (Fig. 5a).

In contrast, exotic grasslands showed greater and much more consistent seasonality in soil NO_3^- than did secondary forests. Assessing the percent change in soil NO_3^- from the dry season to the wet season, forests had an average of $-1 \pm 20\%$ shift in soil NO_3^- (n.s.) vs. grasslands which had a $-54 \pm 9\%$ decline in soil NO_3^- (Fig. 1, $P < 0.05$). The lack of significant seasonality in forest soil NO_3^- resulted from high spatial variability in seasonal trends among forest sites (Fig. 1). Also, secondary forests had no seasonal shift in soil NO_3^- from the hurricane season to the dry season, whereas soil NO_3^- in grasslands nearly doubled during the dry season relative to both wet seasons (Fig. 5b). Overall, soil moisture was negatively but relatively weakly correlated with soil NO_3^- levels in exotic grasslands ($R^2 = 0.27$, $P < 0.05$), and there was no significant correlation in secondary forests. These data show a difference in the strength of seasonality for soil moisture and NO_3^- with highly variable temporal trends in forest soil NO_3^-, and only a weak link between moisture and NO_3^-.

Seasonal trends in decomposer enzyme activities generally tracked soil moisture. β-glucosidase and

Table 2 Microbial biomass levels and soil enzyme activities for two cover types across three seasons

Watershed	Season	Cover type	Microbial Biomass C μg-C/ g-soil*,†	Microbial Biomass N μg-N/ g-soil*,†	β-Glucosidase Enzyme Activity nmol/ g-soil/h*,†,‡	Phosphatase Enzyme Activity nmol/ g-soil/h*	NAGase Enzyme Activity nmol/ g-soil/ h*,†,‡	Enzyme Activity of C : N Acquisition*	Enzyme Activity of C : P Acquisition *,‡	Enzyme Activity of N : P Acquisition*	Normalized β-glucosidase Enzyme Activity nmol/ g-C/h*,†,‡	Normalized Phosphatase Enzyme Activity nmol/ g-C/h*,‡	Normalized NAGase Enzyme Activity nmol/ g-C/h*,‡
Urban RP	Hurricane	Forest	200 ± 30	24 ± 4	347 ± 76	825 ± 155	202 ± 20	1.7 ± 0.3	0.42 ± 0.05	0.27 ± 0.04	11037 ± 1033	25038 ± 2508	6704 ± 489
Urban RP	Hurricane	Grass	141 ± 9	20 ± 2	582 ± 53	936 ± 161	294 ± 4	2.0 ± 0.2	0.66 ± 0.07	0.34 ± 0.06	20105 ± 1846	32419 ± 5896	10199 ± 475
Urban RP	Dry	Forest	236 ± 23	28 ± 3	333 ± 52	812 ± 71	224 ± 29	1.6 ± 0.3	0.43 ± 0.07	0.30 ± 0.06	11496 ± 1170	28277 ± 2199	7798 ± 902
Urban RP	Dry	Grass	192 ± 28	29 ± 5	345 ± 53	888 ± 111	242 ± 25	1.5 ± 0.2	0.42 ± 0.07	0.32 ± 0.06	12174 ± 945	35221 ± 6966	9510 ± 651
Urban RP	Wet	Forest	142 ± 31	26 ± 4	429 ± 44	588 ± 78	376 ± 43	1.2 ± 0.03	0.78 ± 0.09	0.67 ± 0.07	14725 ± 2114	17503 ± 2409	12771 ± 2020
Urban RP	Wet	Grass	157 ± 26	27 ± 4	447 ± 68	545 ± 44	382 ± 38	1.2 ± 0.1	0.87 ± 0.16	0.74 ± 0.11	18017 ± 2699	22308 ± 2589	15576 ± 1926
Remote C	Wet	Forest	661 ± 54	83 ± 10	891 ± 43	2406 ± 148	740 ± 51	1.2 ± 0.02	0.37 ± 0.02	0.31 ± 0.01	14326 ± 568	38676 ± 1437	11849 ± 631

Microbial measures for exotic grasslands vs. secondary forests are shown for the urban Rio Piedras (Urban RP) watershed (unshaded). Secondary forests in the remote Cubuy (Remote C) watershed are also given for comparison (shaded). Data are presented as averages ± one standard error (n = 6). Significant differences across cover types or seasons are given for each column.

*Significant effect of time.

†Interaction of time*cover type ($P < 0.1$).

‡Significant effect of cover type.

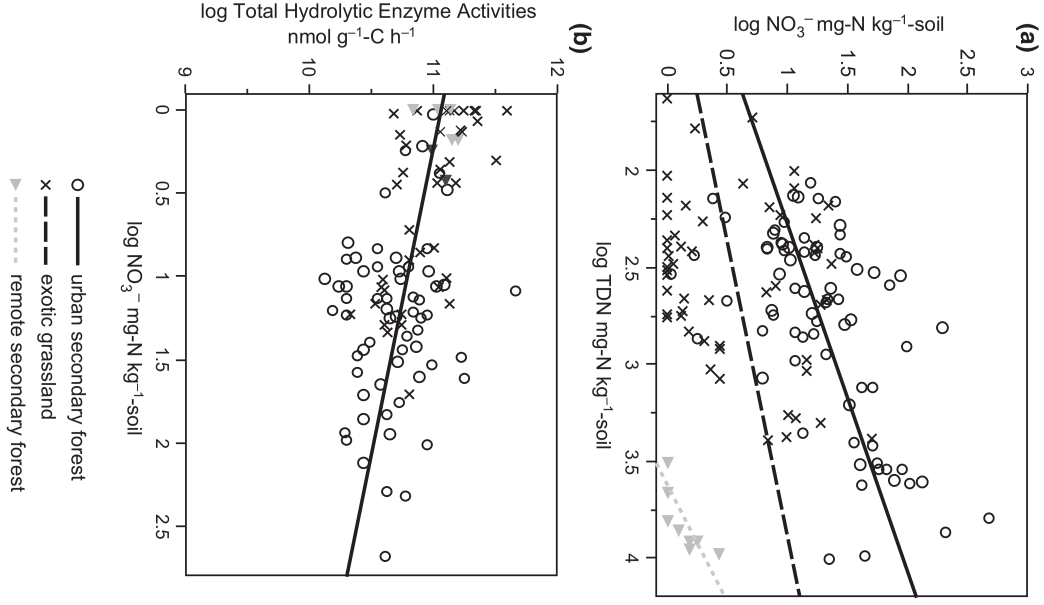

Fig. 4 (a) Soil NO_3^- levels were more enriched in secondary forests (circles, solid line) vs. exotic grasslands (× and dashed line), relative to total dissolved N (TDN) in the urban watershed. The remote Cubuy secondary forest sites are shown for reference (grey triangles and dotted line), with extremely low NO_3^- and high TDN. (b) Overall, soil NO_3^- was the strongest predictor normalized hydrolytic decomposer enzyme activities (per unit of soil C), showing a negative relationship ($P < 0.05$ for all relationships, R^2 values given in text).

NAGase enzyme activities were significantly lower in the dry vs. wet season in both cover types, and seasonal shifts were greater in forests than in grasslands, similar to patterns for soil moisture. Assessing the percent change in β-glucosidase and NAGase activities from the dry season to the subsequent wet season, forests had averages of 48 ± 18% and 77 ± 14% increases, respectively, vs. 26 ± 21% and 40 ± 12% increases in grasslands (Table 2, $P < 0.05$). In contrast, phosphatase activities were relatively high during the hurricane and dry seasons in both cover types and declined 35 ± 3%

in both during the final wet season (Table 3), following patterns for forest soil NO_3^-. Thus, seasonality in soil moisture was most closely linked to seasonality in C and N acquisition activities, whereas temporal patterns in soil NO_3^- were positively linked to seasonality of P acquisition activity.

Notably, there was no seasonal variation in DOC, in stark contrast to trends for soil moisture, NO_3^-, and enzyme activities (Fig. 5c). Rather, extractable DOC closely reflected total soil C stocks, with higher levels in secondary forests vs. exotic grasslands. Soil C concentrations and content did not vary seasonally, nor did soil N concentrations or TDN (Table 3). Thus, total soil organic matter and dissolved organic matter were stable through time in each cover type, suggesting a temporal decoupling in the processes driving soil C vs. NO_3^- levels.

Other factors showed some seasonal variation. Seasonal trends for microbial biomass C and N were opposite of enzyme activities, with the highest overall biomass during the dry season and an interaction with cover type ($P < 0.1$), such that microbial biomass was lower in grasslands than in forests only during the hurricane and dry seasons (Table 2). Interestingly, soil bulk density also changed seasonally, with higher bulk density in the dry vs. wet season (Table 3), and a negative correlation with soil moisture ($R^2 = 0.41$, $P < 0.05$). Aboveground grass biomass was marginally lower during the dry season, with 2.2 ± 0.4 kg m^{-2} in the dry season and 3.6 ± 0.7 kg m^{-2} in the wet seasons ($P = 0.07$). Foliar C and N concentrations in grass biomass did not vary seasonally and averaged 42.5 ± 0.3% C and 1.2 ± 0.06% N across sites.

Urban soil properties vs. remote forests

Comparing the urban watershed sites to the remote Cubuy secondary forests showed general differences in soil properties. The urban forests had lower soil moisture, NH_4^+, DOC, TDN, and soil C and N concentrations than the remote forests, and higher soil NO_3^-, bulk density, and pH (Table 3). Notably, remote forests had much lower enrichment in NO_3^- relative to TDN than urban forests (Fig. 4a). Soil respiration rates in the remote forest during the wet season were similar to overall rates in the urban sites (Fig. 3b), whereas soil temperatures were lower (23.3 ± 0.1 °C). The remote forest also had higher microbial biomass C and N, and higher overall soil enzyme activities than the urban sites, although normalized enzyme activities were similar (Table 2). Interestingly, the C : N and C : P enzyme ratios were lower in the remote forest than in urban sites, while N : P enzyme ratios were similar (Table 2), suggesting that N and P were more scarce relative to C

Table 3 Soil nutrient content and chemistry for two cover types across three seasons

Watershed	Season	Cover type	NH_4^+ mg-N/kg-soil	TDN mg-N/kg-soil†	Soil C %†	Soil N %†	Soil C : N	C stock mg cm^{-2} to 10 cm depth†	Bulk Density g cm^{-3}*	Soil pH
Urban RP	Hurricane	Forest	2.26 ± 0.38	18.2 ± 3.9	3.4 ± 0.4	0.27 ± 0.02	11.9 ± 0.3	313 ± 28	1.05 ± 0.07	6.38 ± 0.30
Urban RP	Hurricane	Grass	2.85 ± 0.32	14.6 ± 1.1	2.9 ± 0.1	0.24 ± 0.01	12.0 ± 0.5	264 ± 26	0.91 ± 0.11	6.81 ± 0.49
Urban RP	Dry	Forest	2.32 ± 0.24	14.5 ± 3.2	3.3 ± 0.3	0.27 ± 0.02	11.8 ± −0.3	329 ± 18	1.07 ± 0.07	6.06 ± 0.26
Urban RP	Dry	Grass	2.44 ± 0.56	12.6 ± 2.9	2.6 ± 0.3	0.21 ± 0.03	12.3 ± 0.3	254 ± 28	1.04 ± 0.05	5.91 ± 0.37
Urban RP	Wet	Forest	2.88 ± 0.20	15.6 ± 2.7	3.5 ± 0.4	0.29 ± 0.02	11.9 ± 0.4	268 ± 44	0.82 ± 0.09	6.31 ± 0.27
Urban RP	Wet	Grass	3.01 ± 0.60	12.6 ± 2.2	2.7 ± 0.3	0.23 ± 0.03	12.1 ± 0.4	196 ± 31	0.75 ± 0.07	6.39 ± 0.30
Remote C	Wet	Forest	3.05 ± 0.22	45.3 ± 3.5	6.2 ± 0.2	0.46 ± 0.02	13.4 ± 0.2	280 ± 9	0.48 ± 0.02	5.46 ± 0.12

Soil chemistry measures for exotic grasslands vs. secondary forests are shown for the urban Rio Piedras (Urban RP) watershed (unshaded). Secondary forests in the remote Cubuy (Remote C) watershed are also given for comparison (shaded). Data are presented as averages ± one standard error ($n = 6$). Significant differences across cover types or seasons are given for each column.

*Significant effect of time.

†Significant effect of cover type.

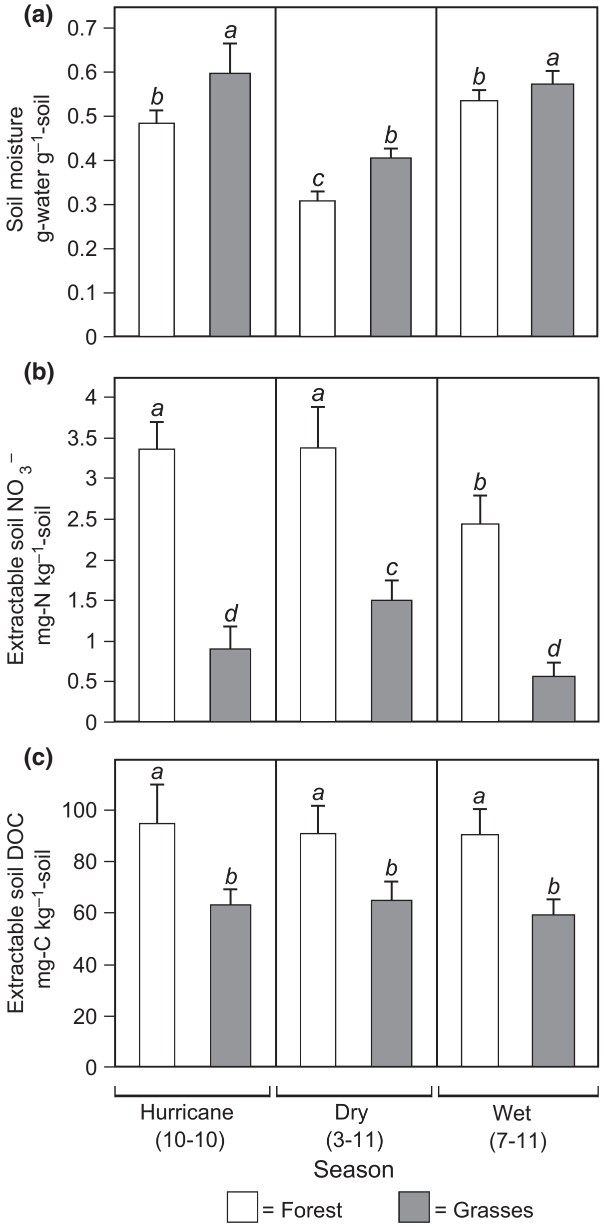

Fig. 5 Seasonal shifts in soil biogeochemical parameters are shown for the urban watershed. (a) Soil moisture showed significant declines during the dry season for both secondary forests (F white) and exotic grasslands (G shaded). Soil moisture was significantly lower in forests vs. grasslands, and soil moisture across the urban sites was lower than in the remote Cubuy secondary forests (0.91 ± 0.02 g-water/g-soil, not shown). (b) Soil NO_3^- showed significant accumulation during the dry season in exotic grasslands, which had overall lower soil NO_3^- than secondary urban forests. Urban sites in general had significantly higher soil NO_3^- than the remote Cubuy secondary forests (0.16 ± 0.06 mg-N/kg-soil, not shown). (c) Soil dissolved organic C (DOC) levels showed no seasonality for either cover type, and DOC was significantly lower in exotic grasslands vs. urban forests. Urban sites had overall significantly lower DOC levels than the remote Cubuy secondary forests (377 ± 19 mg-C/kg-soil, not shown). Letters show significant differences among seasons and cover types using Tukey HSD tests ($P < 0.05$).

for decomposers in the remote vs. urban sites. Remote forest parameters were generally more similar to urban secondary forests than to exotic grasslands, but the largest differences were at the watershed scale. These data provide further evidence for general NO_3^- enrichment in the urban watershed relative to more remote sites.

Relative importance of urbanization, cover type, and seasonality

Testing the three hypotheses simultaneously in SEM path analysis revealed that seasonality was a fairly weak predictor of soil biogeochemical properties relative to cover type and distance to the urban center (Fig. 6). Assessing path coefficients between pairs of factors, distance to the urban center was significantly correlated with all of the endogenous factors in this analysis, including a negative relationship with NO_3^-, further supporting H1 (Table 4). Cover type was also significantly related to NO_3^- and soil moisture, while the direct path from cover type to soil C was not significant. Rather, soil NO_3^- was the strongest direct predictor of soil C. Thus, the effect of cover type on soil C appeared to be mediated by changes in soil NO_3^-, partially supporting H2. Interestingly, season was only a marginally significant predictor of soil moisture ($P = 0.1$) and NO_3^- ($P = 0.06$) in these analyses (Fig. 6, Table 4), suggesting that although seasonality was significant when analyzed alone (above), the effects of human disturbance (i.e., cover type and distance to the urban center) were much stronger drivers of urban soil C and N levels. Overall, the final default model was not significantly different from the saturated model (df = 4; $\chi^2 = 2.883$, $P = 0.578$; GFI = 0.994; NFI = 0.989; RMSEA < 0.001), indicating that our model was close to an ideal fit for the data. The full model predicted 66% of the variability in soil C concentrations and 45% of the variability in soil NO_3^- (Fig. 6).

Discussion

Spatial trends in urban soil N enrichment

Atmospheric N deposition is the most likely source contributing to the relatively large soil NO_3^- pools in these urban-proximate ecosystems, similar to other urban sites (Fenn *et al.*, 2003; Martinelli *et al.*, 2006). The strong decline in grassland soil NO_3^- with distance to

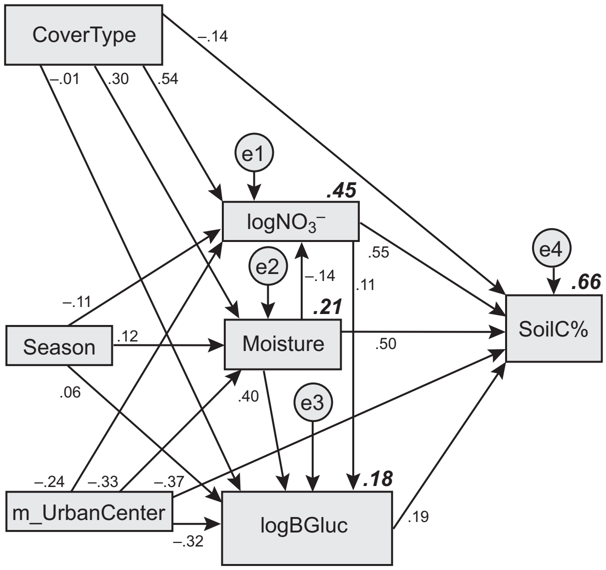

Fig. 6 An SEM path analysis is shown for simultaneous assessment of the three hypotheses in the study. Cover type, season, and distance to the urban center (m_UrbanCenter) were used as exogenous factors for predicting the endogenous factors: soil NO$_3^-$ (logNO$_3^-$), soil moisture, β-glucosidase soil enzyme activities (logBGluc), and soil C concentrations (SoilC%). Values near the base of each arrow show standardized estimates of correlations among pairs of factors (see Table 4 for significance levels). Bold values in italics above each endogenous factor show the squared multiple correlation for that factor (i.e., R^2). Factors in rectangles represent measured values, and circles represent unmeasured error terms for each endogenous factor (e.g., e1). Our model was not significantly different from the ideal saturated model, indicating a good fit for the data.

Table 4 Structural equation model (SEM) path coefficients and significance levels

Path	Estimate	SE	CR	*P**
Moisture ← Season	0.024	0.015	1.555	0.120
Moisture ← CoverType	0.087	0.023	3.741	*
Moisture ← m_UrbanCenter	0.000	0.000	4.080	*
logNO$_3^-$ ← Moisture	−0.262	0.141	−1.861	0.063
logNO$_3^-$ ← Season	−0.040	0.024	−1.690	0.091
logNO$_3^-$ ← m_UrbanCenter	0.000	0.000	−3.298	*
logNO$_3^-$ ← CoverType	−0.289	0.038	−7.560	*
logBGluc ← Moisture	0.699	0.162	4.315	*
logBGluc ← logNO3	0.107	0.103	1.040	0.298
logBGluc ← CoverType	−0.007	0.053	−0.132	0.895
logBGluc ← Season	0.019	0.027	0.681	0.496
logBGluc ← m_UrbanCenter	0.000	0.000	−3.518	*
SoilC% ← logBGluc	0.734	0.221	3.323	*
SoilC% ← Moisture	3.252	0.424	7.674	*
SoilC% ← logNO3	1.932	0.250	7.741	*
SoilC% ← m_UrbanCenter	0.000	0.000	−6.030	*
SoilC% ← CoverType	−0.263	0.128	−2.060	*

The unstandardized regression weights (Estimate), standard error (SE), critical ratio (CR), and *P* values are shown for each path coefficient between pairs of variables in the SEM analysis (Fig. 6). Standardized estimates for each path are shown in Fig. 6. When the CR is > |1.96| for a regression weight, that path is significant at the 0.05 levels or better.
P value of *denotes $P < 0.05$.

the urban center in particular suggests an N deposition gradient. Previous work in the Puerto Rican urban forests showed that they were enriched in NO$_3^-$ and depleted in NH$_4^+$ compared to two remote primary forests (Cusack, 2013), like the comparison here with secondary remote forests, indicating external inputs of NO$_3^-$ to urban ecosystems. Grassland soil NO$_3^-$ levels were significantly lower than in secondary forests, contrary to our predictions and opposite of a temperate urban study that showed higher soil NO$_3^-$ in grasslands vs. forests (Groffman *et al.*, 2009). Here, canopy scrubbing of atmospheric pollution by forest canopies [i.e., higher effective N deposition (Clark *et al.*, 1998; Schulze, 1989)] could contribute to the higher soil NO$_3^-$ that we observed in secondary forests vs. grasslands. Forest fragment size was previously shown to be a strong predictor of soil NO$_3^-$ levels among these urban forests (Cusack, 2013), suggesting that larger canopies 'scrub' more N from atmospheric pollution. This mechanism could explain why soil NO$_3^-$ levels were higher but also more variable among forest sites, whereas exotic grasslands showed more predictable declines in soil NO$_3^-$ with distance to the urban center.

Other factors may also have contributed to the variability in soil mineral N levels among sites and between cover types. For example, there are exotic N-fixing tree species distributed unevenly among the forest fragments, which have been associated with elevated soil N levels (Cusack & McCleery, 2014), and could help explain some of the variability in soil NO$_3^-$ among forest stands. Comparing the two cover types, higher soil moisture in grasslands vs. forests could also have led to greater leaching losses or denitrification of NO$_3^-$ (Corre *et al.*, 2014), reducing grassland soil NO$_3^-$ pools. However, soil moisture and NO$_3^-$ were only weakly correlated here. Similarly, a lack of relationship between soil moisture and redox-sensitive nutrient concentrations was observed in a remote forest moisture manipulation in Puerto Rico (Hall *et al.*, 2013). Together, these data suggest that drivers of temporal patterns in tropical forest soil NO$_3^-$ are complex and are somewhat decoupled from soil moisture. It appears likely that the higher soil NO$_3^-$ in secondary forests vs. exotic grasslands observed here resulted from both the aboveground structural properties of forests, as well as soil processes.

Effects of exotic grasslands and N deposition on soil C

Our results show that the spread of non-native grasses in tropical forest regions is likely to drastically reduce soil C storage. Here, differences in normalized enzyme activities and microbial biomass C : N point to a compositional and/or metabolic shift in the decomposer community between the two cover types, potentially driving lower soil C in grasslands. The higher respiration rates per unit of microbial biomass in the grasslands in particular indicate reduced microbial carbon use efficiency (CUE). This result for grasslands is somewhat contradictory to a recent review which suggested that microbial CUE increases with lower C : N ratios of litter and with higher soil fertility, due to a shift in resource stoichiometry to more closely match requirements of microbial biomass (Manzoni *et al.*, 2012). In these urban Puerto Rican sites, grass tissues had relatively low C : N ratios of ~35, which according to the above review should lead to high microbial CUE. While not measured directly, litter C : N ratios in the urban forests were most certainly higher than in the grasslands, and likely similar to litter C : N ratios of ~41 in lower elevation remote Puerto Rican forests (Cusack *et al.*, 2009). In terms of soil fertility, grasslands had higher soil K, but lower soil N availability and lower apparent N deposition levels than did secondary forests. Rather than a simple shift in CUE due to changes in resource availability, the apparent reduction in microbial CUE in grasslands vs. secondary forests could reflect a broader shift in microbial community composition toward more fast-growing, low-efficiency microbes (Blazewicz *et al.*, 2014), which may result from the greater availability of more labile C compounds produced by grasses vs. woody species (Filley *et al.*, 2008; Liao & Boutton, 2008). Similar to our results, a temperate study found lower soil C and microbial biomass C levels in urban grasslands vs. forests (Groffman *et al.*, 2009). Our data strongly suggest that a change in the microbial processing of soil C is responsible, at least in part, for smaller soil C stocks in exotic tropical grasslands vs. urban secondary forests.

In contrast to the effects of exotic grasses, it appears that NO_3^- enrichment in these urban ecosystems suppressed decomposer enzyme activities and may have had a positive effect on soil C storage, potentially contributing to the higher soil C levels in forests vs. grasslands. Effects of N fertilization on soil microbial activity in remote mature tropical forests have been mixed, with some studies observing increased soil respiration (Cleveland & Townsend, 2006), and others observing declines in soil respiration and increased soil C stocks with elevated N (Mo *et al.*, 2008; Cusack *et al.*, 2011b). In theory, increased N availability should promote the production of microbial enzymes that acquire C and P from organic matter (β-glucosidase and phosphatase, respectively), and suppress enzymes that acquire N (NAGase) (Sinsabaugh & Moorhead, 1994). However, we saw an overall decline in total hydrolytic enzyme activities with elevated soil NO_3^-, suggesting that N enrichment in urban-proximate ecosystems may suppress decomposition. Thus, there appear to be interacting effects of cover type and NO_3^- accumulation on soil C cycling.

Seasonal variation in urban soil biogeochemistry

While canopy capture of N deposition may help explain forest vs. grassland spatial patterns in soil NO_3^-, temporal patterns are likely related to seasonal shifts in microbial activity. Both cover types had generally high soil NO_3^- during the dry season. Nitrate accumulation during dry periods is common in tropical seasonal sites and has been attributed to declines in microbial denitrification and reduced plant uptake (Yavitt *et al.*, 1993; Miambo *et al.*, 2007). While the overall dry-to-wet season pattern for NO_3^- was similar in both cover types, secondary forests also showed surprisingly high levels of soil NO_3^- during the hurricane season preceding the dry season. This unexpected seasonal trend could be related to episodic increases in soil nutrients in these tropical forests following large storms during the hurricane season, which transfer nutrient-rich green biomass from trees to soils (Scatena *et al.*, 1993; Silver *et al.*, 1996; Heartsill-Scalley *et al.*, 2010). In general, temporal and spatial trends in soil NO_3^- for secondary forests were complex, whereas grassland soil NO_3^- and enzyme activities more clearly followed predicted seasonal effects.

The lack of a relationship between seasonality in soil NO_3^- and DOC levels was surprising. Instead, DOC levels closely reflected total soil C stocks. Global trends have shown a positive relationship between NO_3^- accumulation and DOC levels, with these two solutes linked via decomposition rates and microbial biomass requirements for C and N (Taylor & Townsend, 2010). If NO_3^- accumulation depended primarily on mineralization of OM followed by nitrification in these soils, then we would have expected a strong positive relationship of NO_3^- with DOC (DOC production from incomplete decomposition of OM), or a strong negative relationship of NO_3^- with DOC (evidence for complete decomposition of C during mineralization) (Kalbitz *et al.*, 2000). For example, N fertilization in remote

Puerto Rican forests showed that decomposer activity was positively related to DOC production (Cusack *et al*., 2010, 2011b). Here, the seasonality of soil enzyme activities did indicate a shift in C cycling, but DOC levels did not reflect this. These results may suggest that salt-extractable DOC is not as microbially responsive a pool of C as expected, and/or that DOC production in these systems is decoupled from NO_3^- levels. A decoupling between soil NO_3^- and DOC seems particularly likely if NO_3^- levels most strongly reflect N deposition rather than mineralization and nitrification processes.

As indicated by the SEM path analysis, seasonality in general did not emerge as a dominant driver of soil biogeochemistry at these sites, with spatial effects of cover type and the urban core providing clearer controls on soil dynamics. Overall, our results suggest that exotic grasses and atmospheric N deposition may have opposite or interacting effects on tropical soil C stocks. Net changes in soil C storage across urbanizing tropical landscapes are likely to vary according to the spread and establishment of exotic grasslands, local N deposition rates, and interacting urban effects on decomposer communities.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Total concentrations of soil elements are given for 0–10 cm depth for secondary forest and exotic grassland sites in an urban tropical watershed and a remote watershed. Elements include major nutrients (P, S, Ca, K, Mg, Fe, Mn), and aluminum (Al). Averages are given for each site ±1 standard error.