**Title Page**

**Authors**

Lee H. Dietterich*[1, 2], Jason Karpman[2], Avishesh Neupane[2], Mark Ciochina[2], Daniela F. Cusack[1, 2, 3]

**Title**

# Carbon content of soil fractions varies with season, rainfall, and soil fertility across a lowland tropical moist forest gradient

**Author Affiliations and Corresponding Author**

*[1] Department of Ecosystem Science and Sustainability, Colorado State University, Campus Delivery 1476, Fort Collins, CO, 80523, USA.*

*[2] Department of Geography, University of California, Los Angeles, 1255 Bunche Hall Box 951524, Los Angeles, CA 90095, USA*

*[3] Smithsonian Tropical Research Institute, Apartado 0843-03092, Balboa, Ancon, Republic of Panama*

**Corresponding Author: lee.dietterich@gmail.com*

**Author ORCIDs**

Lee H. Dietterich: 0000-0003-4465-5845

Jason Karpman: 0000-0002-5948-3559

Avishesh Neupane: 0000-0003-4401-2161

Mark Ciochina: NA

Daniela F. Cusack: 0000-0003-4681-7449

## Declarations

**Funding**

Funding was provided by NSF GSS grant #BCS-1437591 and DOE grant DE-SC0015898 to D. F. Cusack.

**Conflicts of interest**

The authors declare no conflict of interest.

**Availability of data and material**

The raw data from this project is being submitted as Online Resource 1.

**Code availability**

The R script used in analyzing the data is being submitted as Online Resource 2.

**Authors' contributions**

The study was conceived and samples were collected by DFC. Laboratory analyses were performed by LHD, JK, AN, and MC. LHD analyzed the data and wrote the paper. All authors contributed to reviewing and editing the manuscript.

**Abstract**

Tropical forests contain some of the largest soil carbon (C) stocks on Earth, making them broadly relevant to terrestrial-climate feedbacks, yet our understanding of how their soil organic C (SOC) fractions vary over space and time is limited. We studied effects of season, fertility, and mean annual precipitation (MAP) on the C contents of soil fractions across 14 lowland forests in Panama. We measured free-debris, occluded-debris, and mineral-associated SOC fractions, as well as soluble C associated with each fraction. We hypothesized that mineral-associated SOC would be greatest in infertile, strongly weathered soils with large reactive mineral contents. We also hypothesized that the debris SOC fractions would accumulate during the dry season, reflecting seasonal increases in litterfall. To address this, we compared soil fractions in wet and dry seasons from fertile and infertile soils across a range of 1809 – 2864 mm MAP. The C content (mg C / g soil) of all soil fractions varied with fertility and MAP: specifically, free-debris SOC was greatest in wet, high-fertility soils, and occluded-debris SOC was greater in high-fertility than low-fertility soils. The mineral-associated SOC fraction, which contained the majority of bulk soil C, showed increasing C content with greater MAP in infertile sites, presumably driving similar spatial patterns in the bulk soil. Only the free-debris SOC fraction showed strong seasonal variation, increasing in mass during the dry season. Nitrogen behaved similarly to C. In summary, soil C contents increased with MAP in infertile sites but not fertile sites, driven by the mineral-associated SOC fraction. The dry season had greater free-debris SOC, but this seasonal trend was not apparent in bulk soil C, likely because of the small size of the free-debris SOC fraction. Thus, changes in the quantity and seasonality of precipitation, which are projected for tropical forests, might shift spatial and temporal patterns of soil C storage, which would in turn influence forest-climate feedbacks for this C-rich biome.

**Keywords:** Soil organic carbon (SOC), dissolved organic carbon (DOC), gradients, nitrogen, density fractionation, Panama.

## Introduction

Tropical forests account for 30% of global aboveground carbon (C) stocks (Field et al. 1998), and 30% of global soil organic C (SOC) (Jobbágy and Jackson 2000), and these aboveground and belowground SOC stocks are vulnerable to global change (Cusack et al. 2016). Soil organic C is comprised of a diverse variety of organic molecules that are stored for varying lengths of time, from months to millenia, via numerous mechanisms (Schmidt et al. 2011). In upland, mineral soils, the majority of long-term SOC storage takes place in organo-mineral associations (Trumbore 1993; Marin-Spiotta et al. 2009), while the majority of nutrient cycling is likely tied to the rapid turnover of organic matter recently originated from plant tissues (here referred to as "debris") (Swanston et al. 2005; Torn et al. 2013). Thus, understanding dynamics of different SOC fractions could help us predict effects of global change on multiple time scales. Belowground dynamics, particularly in tropical forests, remain a major source of uncertainty in Earth systems models (Ciais et al. 2013; Todd-Brown et al. 2014).

In particular, precipitation will likely be a major component of global change in the tropics (Kharin et al. 2007). Models predict longer, more severe dry seasons and fewer, more intense rainfall events for some tropical regions (Kharin et al. 2007; Joetzjer et al. 2013; Magrin et al. 2014; Boisier et al. 2015), and increased overall rainfall for other tropical regions (Seidel et al. 2008; Xie et al. 2010; Feng et al. 2013; Rodell et al. 2018). Assessing responses of SOC fractions to variation in precipitation among seasons or across rainfall gradients could help us improve predictions of how climate change might alter SOC storage in these C-rich ecosystems.

Tropical forest soils also vary widely in weathering status and fertility level (Quesada et al. 2011), which could affect how changes in rainfall might alter SOC storage. For instance, increasing weathering typically increases soil clay content, which is often related to greater SOC stocks (Jobbágy and Jackson 2000). Soil fertility can also directly influence SOC stocks via effects on plant productivity (Tanner et al. 2016; Cusack et al. 2018b), community composition or litter stoichiometry (Jandl et al. 2007; Schulp et al. 2008), or decomposer activity (Nottingham et al. 2013; Nottingham et al. 2015). Fertility can also alter the form or function of mycorrhizal symbioses (Johnson et al. 2010; Phillips et al. 2013; Sheldrake et al. 2017), which can in turn affect SOC cycling (Tedersoo and Bahram 2019). Thus, it is important to understand interacting effects of rainfall, seasonality, and soil fertility in order to predict how climate change might alter soil C storage across diverse tropical forest landscapes.

Conceptual models divide bulk SOC into functionally different fractions, which can be approximated by physical fractionation techniques. We used a four-pool approach, building on Sollins et al. (1999) and Cusack et al. (2018a) to measure the following fractions: (1) free-debris SOC (i.e. free light fraction), which typically contains the most rapidly cycling SOC in tropical soils (Marín-Spiotta et al. 2008) and is comprised largely of unattached particles of leaf and root litter; (2) occluded-debris SOC (i.e. occluded light fraction), which is chemically similar to free-debris SOC but has been incorporated into physical soil structures such as aggregates; (3) mineral-associated SOC (i.e. heavy or dense fraction), which is made up of organic compounds sorbed to mineral surfaces (Kleber et al. 2015); and (4) soluble C released during the isolation of each solid fraction. The debris fractions are likely most important for nutrient cycling and availability, while the mineral-associated SOC is generally the largest SOC fraction in clay-rich tropical soils (Trumbore 1993; Glaser et al. 2000; Marin-Spiotta et al. 2009) and can contain the oldest SOC (Trumbore and Zheng 1996; Torn et al. 1997; Lützow et al. 2006), although recent work has shown that portions of the mineral-associated SOC can also be dynamic in response to environmental change (Sollins et al. 2006; Cusack et al. 2011). Understanding how these fractions change over natural variability in MAP, seasonality, and soil fertility can provide insight into how climatic change might influence SOC storage and cycling across complex tropical forest landscapes.

We assessed seasonal variation in four SOC fractions in 14 lowland Panama moist forest sites across natural gradients in MAP and soil fertility, in which soil fertility varies primarily with geological substrate and is largely independent of MAP. We hypothesized that the debris fractions, which are derived from recent leaf and root litter inputs, vary with rainfall seasonality in tropical forests, reflecting changes in litterfall. We also hypothesized that mineral-associated SOC increases in wetter, more clay-rich soils, which have a high density of charged clay surfaces for sorption. Finally, we hypothesized that SOC would be more potentially soluble during the wet season when it is in more advanced stages of decomposition. Among fractions, we predicted that mineral-associated SOC would be the least mobile and therefore release the smallest amount of soluble C.

## Methods

### *Study sites*

The Isthmus of Panama spans a MAP gradient of ~1750 mm $y^{-1}$ MAP on the Pacific coast, to ~4000 mm $y^{-1}$ MAP on the Caribbean coast (Pyke et al. 2001; Engelbrecht et al. 2007). The forests have a tropical monsoon climate. The wetter Caribbean coast has a shorter dry season (~115 days), compared with the drier Pacific coast (~150 days). The Isthmus of Panama also includes soils varying widely in fertility, arising primarily from variation in geological substrate (Turner and Engelbrecht 2011; Condit et al. 2013; Cusack et al. 2018b; Turner et al. 2018).

Because of the strong influence of geology, soil nutrient levels are weakly if at all correlated with MAP (Stewart et al. 1980; Pyke et al. 2001; Turner and Engelbrecht 2011).

We studied 14 distinct forest stands across these gradients in the central lowlands of Panama spanning the Panama Canal region (Fig. 1, Table 1). The sites are a subset of a larger network of >50 1-ha plots maintained by the Smithsonian Tropical Research Institute (STRI) in lowland tropical forests (elevation 10 – 410 meters above sea level) (Pyke et al. 2001). All sites are on flat topography, and span a range of soil orders and textures (Cusack et al. 2018b). Sites' MAP values were calculated using the nearest available long-term rainfall data from Panama Canal Authority (ACP) sites (Engelbrecht et al. 2007). We note that these calculations tend to underestimate STRI's MAP measurements for the wetter Caribbean sites (STRI 2020). The mean annual temperature at all sites is ~26°C and mean monthly temperature varies by <1°C during the year (Windsor et al. 1990). All sites are classified as tropical moist forest (Holdridge et al. 1971), though tree species composition varies markedly among sites (Condit et al. 2013; Turner et al. 2018; Umaña et al. 2020).

The 14 sites were grouped into four MAP ranges and two levels of soil fertility, with sites selected to include fertile and infertile soils within each MAP range. The MAP ranges were: 1800 – 1900 mm MAP (3 sites), 2000 – 2350 mm MAP (4 sites), 2450 – 2600 mm MAP (4 sites), and 2800 – 3050 mm MAP (3 sites), hereafter referred to as 1850, 2200, 2500 and 3000 mm MAP ranges (Cusack et al. 2019). Fertile and infertile sites within each MAP range were selected based on prior data for total C, resin-extractable P, and base cations (Cusack et al. 2018b), and corresponded to cutoffs of approximately 2.4 mg/kg resin-extractable P, 2000 mg/kg extractable base cations, and pH 5.9. We had a total of seven fertile sites and seven infertile sites distributed across the MAP gradient.

*Sample collection*

Soils were collected at each site once during the late wet season (October – early December 2015), and once during the dry season (January – March 2016). At each site, three 50-m transects were established with 4 sampling points along each transect at 10, 20, 30 and 40 m. Soils were collected from 0-10 cm depth, collecting five soil cores at each sampling point along each transect using a 2.5 cm diameter constant volume soil corer (i.e. 20 cores per transect). Organic horizons are absent from most of these sites, which is typical for tropical soils with rapid litter decomposition. Where present, organic horizons were included except for intact surface litter, which was gently removed from the soil surface prior to sampling. Samples were then pooled and homogenized by hand for each transect, for three replicates per site. We did not observe charcoal in these samples, and $^{13}C$ NMR of soils near the middle of our rainfall gradient revealed no microscopic charcoal (Cusack et al. 2018a). To measure soil bulk density, one core per transect was collected with a 6 cm diameter constant volume corer; the corer was pounded into the undisturbed face of a small soil pit (30 cm depth) at the end of each transect, and carefully excavated by inserting a painter's pallet underneath it to prevent soil loss while removing the corer from the pit.

*SOC and DOC fractionation*

Soils were stored at 4 ºC and then sieved (<4 mm) one week or less before performing density fractionation. Density fractionation largely followed Swanston et al. (2005) and Cusack et al. (2018a). In brief, 30 g of fresh soil was weighed into a centrifuge tube, gently inverted by hand with ~100 ml of C-free sodium polytungstate (SPT, 1.7 g/ml; GeoLiquids, Inc., Prospect

Heights, IL, USA), and centrifuged for 1 h at 3500 rpm. This density was selected based on preliminary tests and visual inspection, with the aim of floating debris organic matter and not floating mineral soil. Material floating after this step was considered free-debris SOC, which was aspirated and rinsed five times with DI water over a 0.4 µm membrane filter to remove SPT. All filtrate solution was collected for soluble C analyses, with no material discarded at any point. The remaining soil was resuspended in 1.7 g/ml SPT and mixed with a SPX Lightnin G3U05R mixer for 60 s at 75% power, then given 450 J/ml of energy (Kaiser and Berhe 2014) with a Branson 450 sonifier with the following settings: power 10 (400 W), time 3 min, pulse 70%, solution volume ~110 ml. This energy level was used by Cusack et al. (2018a) for soils from this region, who found this energy level to be optimal for recovering occluded material with minimal contamination by mineral particles; occluded material recovered had a chemical signature of strongly decomposed organic matter. An ice bath was used during sonication to counteract the warming effects of the sonifier. Samples were centrifuged again for 1 h at 3500 rpm to isolate the occluded-debris SOC, which was aspirated and rinsed over a filter in the same way as the free-debris SOC, retaining all filtrate solution for soluble C analyses. The remaining pellet was considered mineral-associated SOC. We rinsed the mineral-associated SOC to remove SPT by aspirating the supernatant, adding DI water, stirring and shaking vigorously, then centrifuging for 1 h at 3500 rpm and repeating this process five times before drying the mineral-associated SOC at 105 ºC. All aspirated supernatant was retained for soluble C analyses.

We measured the dry mass of each solid fraction and compared the total to the moisture-corrected dry mass of the original fresh soil to ensure that minimal material was gained or lost during the fractionation procedure. On average, we recovered 99.74 ± 0.56% of the original moisture-corrected sample mass (n = 84, min = 86.39%, max = 117.19%). Each dried fraction was then ground, either in a ball mill (debris fractions) or with mortar and pestle (mineral-associated SOC) and C and N concentrations were measured on a Costech Elemental Analyzer (Valencia, CA).

*Soil C stability and soluble C leaching*

The supernatants associated with each SOC fraction were analyzed for total dissolved organic C (DOC) and total dissolved N (TDN) on a Shimadzu TOC-L with TDN analyzer (Columbia, MD). Soluble C extracted in SPT resembles the results of the commonly used potassium chloride (KCl) extractions for salt-extractable C and N (Swift 1996). The concentration of SPT used was greater than the standard concentration of KCl used for similar extractions, so it is likely that we recovered more soluble C than we would have in a KCl extraction. The C concentrations of supernatants associated with each fraction were analyzed separately, and total C was calculated using the volume of solution recovered from separating and rinsing each fraction. It should be noted that the initial collection of SPT contained C not only from the free-debris fraction, but also included salt-extractable C from the initial bulk soil sample. Similarly, the soluble C collected during the separation and rinsing of the occluded-debris fraction could include soluble C released from the mineral-associated fraction that was still present in the samples prior to separation of the occluded-debris fraction. Thus, the soluble C we present is associated with each of the three SOC fractions, but is not an isolated extraction from that fraction alone. Rather, the soluble C that we present represents a sequential extraction. Thus, soluble C reported for each fraction should be considered loosely related to that fraction, but also reflecting any other fraction still included in the sample up until that step. Still, our sequential extraction of soluble C represents release of increasingly protected or sorbed C over the course of the density fractionation process. Importantly, we collected and assessed all C released during the process, rather than discarding supernatant, as is commonly done for this method; this allows us to account for all of the C and N in the original bulk soils.

*Data processing and statistical analysis*

To account for changes in effective sampling depth due to seasonal shifts in bulk density (Table 1, Turner et al. 2015), we standardized effective dry season sampling depths to wet season sampling depth using seasonal ratios of bulk density. We used depth distributions for bulk soil C from Cusack and Turner (2020) and depth profiles for SOC fractions from Cusack et al. (2011) and Finstad et al. (in prep.) to adjust our dry season density fractionation data to match the depth sampled in the wet season (Fig. S1). We fit logarithmic models to the published depth profiles, and integrated over them to standardize our SOC fraction masses and C and N concentrations (data in Online Resource 1, calculations in Online Resource 2). We present statistical results for the standardized data in Table S1, and statistical results for the raw data in Table S2. Overall, standardizing for effective depth sampled in the dry season relative to the wet season resulted in few qualitative changes to statistical results, but was included to ensure robust comparisons across seasons.

We conducted statistical analyses using the following response factors: proportion of soil mass (g fraction / g bulk soil), C content (mg C / g bulk soil), C concentration (mg C / g fraction), and C:N ratio. We also calculated C stocks to 10 cm depth (mg C / $cm^2$ bulk soil) but did not run statistical analyses on stocks to this shallow depth. For soluble C extracted while separating and rinsing each fraction, we analyzed fraction soluble C (mg C soluble / g C in fraction) and total soluble C (mg C soluble / g bulk soil). We conducted the same statistical analyses for parallel measures of N. Measures for N were highly correlated with those for C in these fractions, so we discuss N results in the main text only where they differ from those for C.

For statistical analyses, our study was replicated at the level of experimental sites. Sites were chosen for their variation in rainfall and soil fertility because these have been shown to be the dominant factors driving plant distributions, root biomass, soil C stocks, and other ecosystem properties across these sites (Turner and Engelbrecht 2011; Condit et al. 2013; Cusack et al. 2018b; Turner et al. 2018; Cusack et al. 2019). Therefore, for the response factors above, we performed two-way analysis of variance (ANOVA) with season and site as fixed effects, and then used planned contrasts to test for effects of season (categorical nominal factor), fertility (categorical nominal factor), MAP range (categorical ordinal factor), and their interactions using sums of squares and degrees of freedom appropriately reduced based on the initial ANOVA model. We used MAP range as a categorical factor because of the clumped distribution of MAP values (Table 1; Cusack et al. 2019). Post-hoc Fisher's Least Significant Difference (LSD) means separation tests were used to explore differences among seasons, soil fertility levels, and MAP ranges. Residual distributions were examined for all response variables, and the following variables were log+1 transformed to ensure that the assumptions of linear models were met: debris fraction proportions of soil mass and C and N content, and fraction soluble C and N for all fractions (mg C soluble / g C in fraction, or mg N soluble / g N in fraction).

As a parallel exploratory exercise, we used a forward stepwise model selection approach to investigate relationships between season, MAP, and ecosystem characteristics with SOC fractions, using previously published data for these sites  (Cusack et al. 2018b; Cusack et al. 2019; Cusack and Turner 2020). Site-level predictors included season, MAP, resin-extractable P to 10 cm depth, aboveground biomass of trees greater than 10 cm diameter at breast height, root biomass to 1 m depth, soil pH in water to 10 cm depth, total extractable soil bases to 10 cm depth, total inorganic soil N to 10 cm depth, soil clay content to 50 cm depth, and forest floor biomass. The minimum adequate model was produced using a $P < 0.1$ cutoff and seeking to minimize the Bayesian Information Criterion (BIC). This approach identifies significant predictors and penalizes the addition of parameters to avoid overparametrization (Schwarz 1978), yielding the minimum adequate general linear model. We then performed multiple regression analysis of the terms in the minimum adequate model on the transect-level data to see whether any of these more specific predictors refined our understanding from the above ANOVA results. The predictors most commonly identified in the minimum adequate models were season and the following measures of soil fertility: resin P, total extractable bases, and soil pH (Table S3), supporting the importance of season and fertility to SOC in these sites. Model selection was performed in JMP (SAS Institute, 2018 JMP Pro 14.0.0). All other analyses were performed in R (CRAN R Project, version 4.0.0 "Arbor Day"). Data are presented as averages ± 1 standard error of the mean (SE), using $P < 0.05$ for statistical significance.

## Results

*Distribution of SOC among density fractions*

The free-debris and occluded-debris SOC fractions accounted for a small proportion of soil mass overall (free-debris SOC 2.5 ± 0.3%, occluded-debris SOC 1.4 ± 0.2%, n = 84, Fig. 2A), but because of their relatively high C concentrations (free-debris SOC 32.2 ± 0.53%, occluded-debris SOC 30.6 ± 1.01%, n = 84, Table 2, Fig 4A-B), they contributed a significant proportion of bulk soil C (free-debris SOC 16.5 ± 1.1%, n = 84, occluded-debris SOC 8.3 ± 0.7%, n = 84, Fig. 2B-C, Fig. 3A-D). Conversely, although the mineral-associated SOC fraction had a much lower C concentration than the debris fractions (3.2 ± 0.12%, n = 84, Table 2, Fig. 4E), it accounted for such a large majority of the soil mass (96.1 ± 0.4%, n = 84, Fig. 2A) that it still contributed a majority of bulk soil C (75.1 ± 1.5%, n = 84, Fig. 2B-C).

*Patterns of free-debris SOC*

In our initial ANOVAs, season and site significantly affected all response variables related to the free-debris SOC fraction, so we focus here on the results of the contrasts analysis, which allowed us to disentangle effects of season, fertility, MAP, and their interactions. Season and fertility were significantly related to the free-debris SOC fraction as a proportion of soil mass (Table S1), which was greater in the dry season than the wet season, and greater in fertile sites than infertile sites (Fig. 2A). The C content of the free-debris SOC fraction was best predicted by season, fertility, MAP, and a fertility×MAP interaction. Specifically, the free-debris C content more than doubled from the wet season (4.24 ± 0.41 mg C / g soil, n = 42) to the dry season (10.84 ± 1.77 mg C / g soil, n = 42), and increased with greater MAP on fertile soils but not on infertile soils (Fig. 3A-B). The free-debris fraction as a proportion of bulk soil C followed a similar pattern (Fig. 2B).

The C concentration of the free-debris SOC fraction varied significantly with season and there was a fertility×MAP interaction, in which free-debris %C increased slightly in the wet season (Table 2, S5), and was greatest in infertile sites at our highest MAP range (Table S5, Fig. 4A). In contrast with the patterns for C, only soil fertility affected free-debris SOC N concentrations, which were greater in fertile sites than infertile sites (Table 2, S5). Free-debris C and N concentrations were positively correlated with each other (r = 0.34, n = 84), and C and N generally behaved similarly throughout this dataset (Table S1). Season, fertility, and a fertility×MAP interaction predicted the C:N ratio of the free-debris SOC fraction, which was greater in the wet season than the dry season, and greater in infertile than fertile soils (Table 2, S4, S5, Fig. 4B).

For soluble C extracted while separating and rinsing this SOC fraction, season, MAP, and a fertility×MAP interaction were significant predictors. Free-debris fraction soluble C (mg C soluble / g C in fraction) was 147.2% greater in the wet season (170.4 ± 17.9 mg/g, n = 42) than the dry season (68.9 ± 6.4 mg/g, n = 42), and decreased with MAP (Fig. 5A, S2A, S3A, Table 2, S4, S5). Only MAP significantly affected free-debris total soluble C (mg C soluble / g bulk soil) (Fig. 1D, 5B, S2B, S3B). Total soluble C extracted during the separation and rinsing of the free-debris fraction was 0.579 ± 0.034 mg C / g bulk soil, representing 11.9 ± 1.1% of the C retained in the free-debris fraction.

In summary, the strongest patterns for the free-debris SOC fraction were an increase in mass and thus accumulation in the dry season, and greater potential solubility in the wet season.

*Patterns of occluded-debris SOC*

Site was a significant predictor in initial ANOVAs of all response variables related to the occluded-debris SOC fraction, but season was never a significant predictor for this fraction. In the contrast analysis, fertility, MAP, and a fertility×MAP interaction were the significant effects on the occluded-debris SOC fraction as a proportion of soil mass (Table S1). This pattern was driven by increases in the mass of this fraction in some fertile sites but not all across the MAP gradient (Table 2, Fig. 2A). The occluded-debris C content and proportion of soil C followed similar patterns to its proportion of soil mass (Fig. 2B-C, 3C-D, Table 2, S4, S5).

For the C concentration of the occluded-debris fraction, fertility, MAP, and a fertility×MAP interaction were significant effects. The C concentration of the occluded-debris SOC fraction was greater in infertile sites (33.1 ± 1.3 %, n = 44) than fertile sites (27.7 ± 1.5 %, n = 40) and was lower in wetter sites than drier sites (Fig. 4C). In contrast, occluded debris N concentrations did not vary with soil fertility, but were also lowest in the wettest sites. Occluded-debris C and N concentrations were strongly correlated (r = 0.71, n = 84), and C and N produced similar statistical results for this fraction (Table S1). The C:N ratio of the occluded-debris fraction was greater in infertile than fertile soils (Fig. 4D).

Fraction soluble C from the occluded-debris SOC fraction (mg C soluble / g C in fraction) varied among sites but was not related to season, fertility, or MAP (Fig. 5C, S2C, S3C). However, for total soluble C from this fraction (mg C soluble / g bulk soil), there were was a significant main effect of fertility and significant fertility×MAP, season×MAP, and season×fertility×MAP interactions. Total occluded-debris soluble C was greater in fertile soils than infertile soils, especially so in the dry season, and varied widely but not directionally among MAP ranges (Fig. 5D, S2D, S3D). Total soluble C associated with the occluded-debris fraction was 0.981 ± 0.063 mg C / g bulk soil, representing 42.9 ± 4.3% of the C retained in the occluded-debris fraction.

In summary, the strongest patterns for the occluded-debris SOC fraction were that mass, C content, and total soluble C associated with this fraction increased in some but not all fertile sites, but the C concentration of this fraction was greater in infertile, drier sites.

*Patterns of mineral-associated SOC*

In the initial ANOVAs, site was a significant predictor for all response variables relating to the mineral-associated SOC fraction, most of which also responded significantly to season or a season×site interaction. In the contrasts analysis, a main effect of MAP and the fertility×MAP and season×MAP interactions were significant predictors of the C content of the mineral-associated SOC fraction (Table S1), which increased at higher MAP in infertile sites, primarily in the wet season (Fig. 3E-F, Table 2).

For the C concentration of the mineral-associated SOC fraction, there were significant effects of fertility, MAP, and fertility×MAP and season×MAP interactions (Table S1). The C concentration of this fraction, like its C content, increased with MAP in infertile sites primarily in the wet season (Fig. 4E). There was a strong correlation between the C and N concentrations of the mineral-associated SOC fraction ($r = 0.945$, $n = 84$), and the C and N results for this fraction were largely similar (Table S1). Season, fertility, MAP, and the fertility×MAP interaction affected the C:N ratio of the mineral-associated SOC fraction, which was greater in the wet season than the dry season, tended to decrease with increasing MAP in fertile sites, and reached its lowest values at the 2500 mm MAP range for both fertility levels (Fig. 4F). The mineral-associated SOC fraction's proportions of soil mass and soil C offset concurrent changes in the debris fractions described above (Fig. 2A-B, 3E-F), so they were not analyzed separately.

For soluble C released during rinsing of the mineral-associated SOC fraction, both fraction soluble C (mg C soluble / g C in fraction) and total soluble C (mg C soluble / g bulk soil) showed a significant main effect of fertility and a fertility×MAP interaction; total soluble C also showed a significant main effect of MAP. Both of these measures of potentially soluble C associated with this fraction were greater in fertile soils, especially at our wettest and driest sites (Fig. 1D, 5E-F, S2E-F, S3E-F, Table 2, S4, S5). On average, total soluble C associated with the mineral-associated fraction was 1.54 ± 0.090 mg C soluble / g bulk soil, representing 4.96 ± 0.28% of the C retained in the mineral-associated fraction. This is a much smaller proportion than for either of the debris fractions (Fig. 5F, S2E, S3E, Table 2, S4, S5), consistent with the idea that C in mineral-associated SOC is more stable than in the debris fractions.

*Patterns in bulk soil C*

Fertility, MAP, and a fertility×MAP interaction were the significant predictors of bulk soil C content, C:N ratios, and total soluble C (mg C soluble / g bulk soil) which followed similar patterns to the mineral-associated SOC fraction. Bulk soil C content increased with MAP in infertile soils, but did not vary directionally with MAP in fertile soils (Fig. 4G). Bulk soil C:N ratios were higher in infertile than fertile soils overall but with occasional exceptions (Fig. 4H). Total soluble C tended to be greater in fertile than infertile soils, though again with substantial variation among sites (Fig. 5G, S2G, S3G). On average, bulk soil total soluble C comprised 3.09 ± 0.153 mg C / g bulk soil, representing 8.29 ± 0.41% of the bulk soil C retained in the solid fractions, and indicating that 7.57 ± 0.34% of the original bulk soil C was potentially soluble.

Season, fertility, MAP, and a season×fertility interaction were all significant predictors of site-level bulk density. Bulk density was greater in the dry season (0.903 ± 0.039 g/cm$^3$, n = 14) than the wet season (0.749 ± 0.033 g/cm$^3$, n = 14), tended to decrease with increasing MAP, and was greater in fertile (0.857 ± 0.043 g/cm$^3$, n = 14) than infertile sites (0.796 ± 0.038 g/cm$^3$, n = 14) driven by a difference in the dry season (Table 1).

## Discussion

*Spatial patterns in mineral-associated SOC*

Perhaps our most striking result was that in infertile soils, the C content of the mineral-associated SOC fraction increased strongly across the MAP gradient, approximately doubling from our lowest to highest MAP sites. This result is consistent with a modeling study parametrized from a drought-deciduous Brazilian caatinga which predicted increases in SOC storage with greater wet season length because in longer wet seasons, productivity was increased more than respiration overall (Rohr et al. 2013). In general, the infertile Panama soils are more strongly weathered than the fertile soils according to US Soil Taxonomy (Cusack et al. 2019; Cusack and Turner 2020), so they likely have greater binding site density on clay surfaces (Wiesmeier et al. 2019). This could give rise to greater formation of organo-mineral associations, especially in wetter sites in which leaf litter may be enriched in complex compounds such as lignin (Santiago and Mulkey 2005; Santiago 2009; Dale et al. 2015), whose aromatic and phenolic residues can form strong organo-mineral associations under certain conditions (Kaiser and Zech 1999; Kaiser 2003). The mineral-associated SOC fraction is commonly thought to represent a relatively stable C pool with slower turnover times, suggesting that wetter, infertile tropical forest soils may contain more of this stable C. However, components of the mineral-associated SOC fraction have also been shown to be dynamic on decadal timescales in a forest in Panama (Cusack et al. 2018a), so greater investigation is required to understand the long-term stability of mineral-associated SOC in wet, infertile tropical forests.

Soil clay content can also influence the size of the mineral-associated SOC fraction via differential availability of sites for SOC to sorb to clay mineral surfaces (e.g. Baldock and Skjemstad 2000; Eusterhues et al. 2003; Mora et al. 2014; Angst et al. 2018). However, in our data set there was no relationship between clay content and the C content of any density fraction in these sites. Alternatively, variation in soil cations could affect the mineral-associated SOC fraction via cation bridging (Roychand and Marschner 2014; Singh et al. 2016), although we also found no relationship between clay content and total bases in this sites, nor between clay content and resin P or fertility index. Sesquioxide formation (Oh and Richter 2005), specific surface area, and concentrations of sesquioxides and electrolytes in soils could also influence organo-mineral associations (Singh et al. 2016). These factors require further investigation as drivers of mineral-associated SOC at these sites.

The mineral-associated SOC fraction had slightly greater C content during the wet season than the dry season, supporting previous results from a mid-rainfall site in this region (Cusack et al. 2018a). However, this pattern was much stronger for the mineral-associated C:N ratio, which increased markedly in the wet season and in infertile soils. This is consistent with greater mineral-associated SOC storage in wet, infertile soils, and may also indicate faster N cycling or reduced N accumulation under these conditions. We did see strong seasonal changes in bulk density for our surface soils, and future work could investigate how bulk density and soil fractions vary below the top 10 cm to get a deeper understanding of overall SOC stocks. We also note that, while our 14 study sites encompass substantial variation in MAP and soil fertility, the global tropics vary more widely in both of these factors, so examining the behavior of SOC fractions in a greater diversity of sites could also provide useful insights.

*Seasonal shifts in free-debris SOC*

Our data support the conceptual model that free-debris SOC is derived from plant litter that accumulates in the dry season due to greater litterfall and reduced decomposition, which has been suggested in these sites (Turner et al. 2015; Cusack et al. 2018a). This pattern held despite the C concentration and C:N ratio of the free-debris SOC increasing slightly during the wet season. The higher C:N ratio and lower fraction mass we observed in infertile soils at high rainfall is consistent with plants in these sites producing more complex C compounds for defense and structural tissues, as has been shown in this region of Panama (Santiago and Mulkey 2005; Santiago 2009; Fyllas et al. 2009; Dale et al. 2015) and in a survey of plants in the Amazon Basin (Fyllas et al. 2009). Tree community composition varies significantly among our study sites (Condit et al. 2013; Turner et al. 2018; Umaña et al. 2020), so patterns in leaf and root tissue chemistry could be driven by community shifts as well as by intraspecific variation. Limitation of soil metabolism by nutrients other than C (Nottingham et al. 2018; Camenzind et al. 2018) could also contribute to higher C:N ratios in wet, infertile soils. We conclude that the quantity of free-debris SOC is driven primarily by seasonal trends in litterfall, and its chemical characteristics are driven by effects of fertility and MAP on litter chemistry and soil metabolism.

*Spatial patterns in occluded-debris SOC*

For the occluded-debris SOC fraction, soil fertility and MAP were the strongest predictors, with greater C content in this fraction at fertile sites at some rainfall ranges. This pattern was particularly strong in our Metropolitano and Campo Chagres sites, both of which are on Mollisols with large seasonal changes in bulk density, suggesting that some properties of this soil order or seasonal shrink/swell dynamics may affect occlusion of SOC in aggregates, though we note that site P04 shares these characteristics but had relatively low occluded-debris SOC. Broadly, fertility and moisture effects on SOC dynamics appear to be more important than seasonal patterns in litterfall for driving accumulation of occluded-debris SOC.

We expected that the occluded-debris SOC fraction would accumulate in the wet season, as found in a mid-rainfall site in Panama (Cusack et al. 2018a) and a tropical dry forest in

Mexico (García-Oliva et al. 2003). In the latter study, the proportion of macro-aggregates increased over the course of the wet season, as did C and nutrient concentrations within micro-aggregates. This suggests that aggregate formation during the wet season might promote accumulation of occluded-debris SOC in some sites. Another study in the same site found that bulk SOC was greater in the dry season, which the authors attributed to variation in the microbial community composition and C and N availability of soil micro- and macroaggregates (Noguez et al. 2008). In a study of aggregate dynamics in agricultural systems in Brazil, Reis Ferreira et al. (2020) suggested that root and hyphal growth and organic matter decomposition support soil aggregation. In our study sites, these factors are likely greater in fertile soils and in the wet season. On the other hand, physical factors such as raindrop impacts and wet-dry shrink-swell cycles could disrupt aggregates in the wet season, as found in a series of agricultural soils in Greece (Dimoyiannis 2009). Such conflicting effects of rainfall seasonality on aggregate dynamics could explain why fertility and MAP, but not season, were consistently related to accumulation of occluded-debris SOC in our analysis.

If occluded-debris SOC is derived primarily from free-debris SOC, then processes that increase the mass of the free-debris SOC fraction in fertile sites could in turn increase the mass of the occluded-debris SOC fraction. Such processes could include increased root turnover (Ostertag 2001; Jourdan et al. 2008) or faster initial decomposition of less complex or more nutrient-rich leaf litter in fertile sites. Forests in both Panama (Umaña et al. 2020) and the Brazilian Amazon (Fyllas et al. 2009) have been shown to have leaves with traits favoring faster decomposition in more fertile sites. Another study in Panama found that early leaf litter decomposition rates varied widely among species (Dale et al. 2015), so a fertility-induced community shift to tree species with faster-decomposing litter is plausible, although Dale et al.

(2015) interestingly did not find direct effects of leaf nutrient concentrations on decomposition rate.

*Soluble C associated with SOC fractions*

Fraction soluble C (mg C soluble / g C in fraction) released during the isolation of free-debris SOC was much greater in the wet season than in the dry season, suggesting a more advanced stage of decomposition in the wet season for this fraction and perhaps for the bulk soil overall (Gmach et al. 2020). This is supported by much higher rates of soil respiration during the wet season at these sites (Cusack et al. 2019). Free-debris SOC is considered the most mobile of these fractions (Swanston et al. 2005), and previous work in Panama has also shown that soluble C released while isolating this fraction is greater than that for the mineral-associated SOC fraction (Cusack et al. 2018a). More soluble C was released during the isolation of occluded-debris SOC than for free-debris SOC, suggesting that occluded-debris SOC is more highly decomposed than free-debris SOC, and that occlusion within aggregates can allow for accumulation of this more mobile SOC fraction. This supports findings from a review of temperate soil C stabilization (Lützow et al. 2006) and an incubation experiment using soils from a temperate forest in Germany (Angst et al. 2017).

Total soluble C (mg C soluble / g bulk soil) from the mineral-associated SOC fraction and from bulk soil was greater in fertile sites than infertile sites, especially at low MAP. This pattern was apparently driven by increased solubility of mineral-associated SOC in fertile sites. We suggest that factors related to soil fertility, such as soil mineralogy, might influence the nature of organo-mineral associations, leading to a more dynamic mineral-associated SOC fraction in fertile soils. In particular, in our study sites, changes in soil fertility are associated with soil weathering status, thus, the less weathered, more fertile soils examined in this study may have lower availability of secondary clays and sesquioxides (Cusack and Turner 2020). These chemical factors can accumulate with weathering and strongly bind organic matter, as found by (Oh and Richter 2005) in the southeastern United States, perhaps accounting for the decreased soluble C we observed in infertile soils.

Fraction soluble C (mg C soluble / g C in fraction) was lowest for the mineral-associated SOC fraction, consistent with the idea that this SOC fraction is relatively stable. At the same time, the solubility of some mineral-associated SOC indicates that a portion of this fraction is dynamic. This is consistent with previous studies showing rapid accumulation of SOC in this fraction with N addition in Puerto Rico (Cusack et al. 2011) losses of mineral-associated SOC on decadal timescales with litter removal in Panama (Cusack et al. 2018a), and rapid cycling of ~7% of this fraction in a California grassland (Torn et al. 2013). Together, these studies support a shifting paradigm in which the mineral-associated SOC fraction has a much more dynamic component than previously thought.

*Conclusions*

We examined spatial and temporal patterns of four SOC fractions across landscape-scale variation in seasonality, soil fertility, and MAP for 14 distinct lowland tropical moist forests in Panama. Mineral-associated SOC increased with MAP in infertile sites and in the wet season, suggesting more stable sorption dynamics in wetter and infertile tropical soils. This could be related to soil mineralogy, decomposition patterns, and/or differences in plant tissue chemistry across our sites. The mechanisms driving this pattern merit further investigation. We also observed strong seasonal effects on the free-debris SOC fraction, which accumulated during the dry season, reflecting large increases in litterfall. The occluded-debris SOC fraction varied primarily with soil fertility and MAP, showing large increases in a subset of fertile sites. The lack of a seasonal effect on the occluded-debris SOC fraction was surprising, given prior studies and the large seasonal shift in the free-debris SOC fraction. Overall, these results highlight the importance of MAP and seasonality in driving the physical and chemical nature of tropical forest SOC, and the interacting influence of soil fertility. These results improve our understanding of the variation in SOC across tropical forests, and could be used to advance prediction of how changes in rainfall might alter SOC storage in tropical forest soils.

## Tables

**Table 1**. Baseline characteristics of study sites, compiled from (Turner and Engelbrecht 2011; Condit et al. 2013; Cusack et al. 2018b; Turner et al. 2018; Cusack et al. 2019). Fertility index is abbreviated as follows: F, fertile; I, infertile.

**Table 2.** Effects of fertility and MAP on fraction C and N concentrations, C:N ratios, contributions to soil C and N stocks, and proportional soluble C and N. For each response variable, values within a SOC fraction marked with the same letter are not significantly different at the $P < 0.05$ level according to Fisher's LSD test.

## Supplementary Tables

**Table S1**. Results from ANOVA and contrasts analysis of the effects of season, soil fertility index ("Fertility"), and MAP range ("MAP") and their interactions on response variables relating to density fraction contributions to (A) soil C and (B) soil N. Each row corresponds to one response variable. Mineral-associated SOC proportions of soil mass, C, and N are omitted because they are not independent of the corresponding debris fraction proportions, which are considered to drive these patterns. For bulk density, we had just one measurement per site and season, so we analyzed it by three-way ANOVA with season, fertility, and MAP as fixed effects. Statistically significant terms are marked as follows in the cells to the right of the described response; non-significant terms are left blank. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. For season and fertility, directions of significant effects are shown and abbreviated D, dry season; W, wet season; F, fertile soils; I, infertile soils.

**Table S2.** Statistical results as in Table S1, using raw data not standardized for seasonal changes in bulk density.

**Table S3.** Results from model selection and multiple regression procedure to identify important predictors of density fraction contributions to soil C. Each row corresponds to one response variable. Predictors included in the minimum adequate model are marked with an X, as well as + or – to indicate a positive or negative relationship, except for the effect of season which is marked D>W for variables greater in the dry season than the wet season, and W>D for variables greater in the wet season than the dry season. Significant results from multiple regression of each response against the selected predictors are appended to the X as follows: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

**Table S4.** Effects of season and MAP on fraction carbon and nitrogen concentrations (%), C:N ratio, contributions to soil C and N stocks, and proportional soluble C and N. For each response variable, values within a fraction marked with the same letter are not significantly different at the $P < 0.05$ level according to Fisher's LSD test.

**Table S5.** Effects of season and fertility on fraction carbon and nitrogen concentrations (%), C:N ratio, contributions to soil C and N stocks, and proportional soluble C and N. For each response variable, values within a fraction marked with the same letter are not significantly different at the $P < 0.05$ level according to Fisher's LSD test.

**Figures**

**Figure 1.** A map showing the 14 study sites across rainfall and geological gradients in central Panama, extending from the drier Pacific coast in the south to the wetter Caribbean coast in the north. Colors represent different geological substrates and formations (Fm). Soil fertility index is shown as fertile (black squares) or infertile (white circles). Site names match Table 1 except for Roubik, which was not included in this study. Reprinted by permission from Springer Nature Customer Service Centre GmbH: Springer *Biogeochemistry* "Seasonal changes in soil respiration linked to soil moisture and phosphorus availability along a tropical rainfall gradient" Daniela F. Cusack et al., copyright 2019.

**Figure 2.** Effects of season and fertility on soil density fractions as (A) proportions of soil mass, (B) proportions of soil C, (C) C contents, and (D) total soluble C (mg C soluble / g bulk soil). Within each panel and fraction, lowercase letters inside bars show Fisher LSD means separations for each fraction. In (C-D), uppercase letters above bars show Fisher LSD means separations for total bulk soil C content and soluble C, respectively. Season and fertility are abbreviated as follows: Dry-Fert, dry season in fertile sites; Dry-Inf, dry season in infertile sites; Wet-Fert, wet season in fertile sites; Wet-Inf, wet season in infertile sites.

**Figure 3.** Effects of season and MAP on C contents of (A, B) free-debris SOC fraction, (C, D) occluded-debris SOC fraction, and (E, F) mineral-associated SOC fraction, for (A, C, E) low fertility sites and (B, D, F) high fertility sites. Within each panel, lowercase letters above bars show Fisher LSD means separations among MAP ranges.

**Figure 4.** Soil fertility and MAP interactively affected (A, C, E, G) C concentrations and (B, D, F, H) C:N ratios of (A, B) free-debris SOC fractions, (C, D) occluded-debris SOC fractions, (E, F) mineral-associated SOC fractions, and (G, H) bulk soil. Within each panel, lowercase letters inside bars show Fisher LSD means separations for each combination of fertility and MAP range, and uppercase letters above pairs of bars show Fisher LSD means separations among MAP ranges.

**Figure 5.** Effects of fertility and MAP on soluble C extracted while isolating (A, B) free-debris SOC fractions, (C, D) occluded-debris SOC fractions, (E, F) mineral-associated SOC fractions, and calculated for (G,H) bulk soil. These are expressed as (A, C, E, G) (mg C soluble / g C in

fraction) and (B, D, F, H) total soluble C (mg C soluble / g bulk soil). Within each panel, lowercase letters inside bars show Fisher LSD means separations for each combination of fertility and MAP range, and uppercase letters above pairs of bars show Fisher LSD means separations among MAP ranges.

**Supplementary Figures**

**Figure S1.** Depth profile data from Cusack et al. (2011), Cusack and Turner (2020), and Finstad et al. (in prep), and logarithmic fits used to standardize data for seasonal changes in bulk density. (A, C, E) Fraction proportions of soil mass and (B, D, F) fraction C concentrations are plotted against soil depth for (A, B) free-debris SOC, (C, D) occluded-debris SOC, and (E, F) mineral-associated SOC fractions. Because each measurement of fraction mass or C concentration was measured for a depth interval (e.g. 0-10 cm) rather than a specific depth, we used the midpoints of each depth interval (e.g. 5 cm) for plotting and fitting.

**Figure S2.** Season and MAP interactively affected soluble C extracted while isolating (A, B) free-debris SOC, (C, D) occluded-debris SOC, (E, F) mineral-associated SOC, and (G,H) bulk soil. These are expressed as (A, C, E, G) (mg C soluble / g C in fraction) and (B, D, F, H) total soluble C (mg C soluble / g bulk soil). Within each panel, lowercase letters inside bars show Fisher LSD means separations for each combination of season and MAP range, and uppercase letters above pairs of bars show Fisher LSD means separations among MAP ranges.

**Figure S3.** Season and soil fertility interactively affected soluble C extracted while isolating (A, B) free-debris SOC, (C, D) occluded-debris SOC, (E, F) mineral-associated SOC, and (G) bulk soil. These are expressed as (A, C, E, G) (mg C soluble / g C in fraction) and (B, D, F, H) total soluble C (mg C soluble / g bulk soil). Within each panel, lowercase letters inside bars show Fisher LSD means separations for each combination of fertility and MAP range, and uppercase letters above pairs of bars show Fisher LSD means separations among MAP ranges.

**Online Resource 1:** Raw data produced and analyzed in this study.

**Online Resource 2:** R code used for calculations and data analysis in this study.

**Acknowledgments**

Funding was provided by NSF GSS grant #BCS-1437591 and DOE grant DE-SC0015898 to D. F. Cusack. We thank B. L. Turner for help selecting and characterizing sites and for providing constructive comments on the manuscript, and we thank Lucas Lu, Emma Friedl, Yun Choi, Amanda Lai, Shirley Lin, Dayana Agudo, and Aleksandra Bielnicka for laboratory support.

**References**

Angst G, Messinger J, Greiner M, et al (2018) Soil organic carbon stocks in topsoil and subsoil controlled by parent material, carbon input in the rhizosphere, and microbial-derived compounds. Soil Biology and Biochemistry 122:19–30. doi: 10.1016/j.soilbio.2018.03.026

Angst G, Mueller KE, Kögel-Knabner I, et al (2017) Aggregation controls the stability of lignin and lipids in clay- sized particulate and mineral associated organic matter. Biogeochemistry 132:307–324. doi: 10.1007/s10533-017-0304-2

Baldock JA, Skjemstad JO (2000) Role of the soil matrix and minerals in protecting natural organic materials against biological attack. Organic Geochemistry 31:697–710.

Boisier JP, Ciais P, Ducharne A, Guimberteau M (2015) Projected strengthening of Amazonian dry season by constrained climate model simulations. Nature Climate Change 5:656–660. doi: 10.1038/nclimate2658

Camenzind T, Hättenschwiler S, Treseder KK, et al (2018) Nutrient limitation of soil microbial processes in tropical forests. Ecol Monogr 88:4–21. doi: 10.1002/ecm.1279

Ciais P, Sabine C, Govindasamy B, et al (2013) Chapter 6: Carbon and Other Biogeochemical Cycles. In: Stocker T, Qin D, Platner G-K (eds) Climate Change 2013 The Physical Science Basis. Cambridge, UK,

Condit R, Engelbrecht BMJ, Pino D, et al (2013) Species distributions in response to individual soil nutrients and seasonal drought across a community of tropical trees. Proceedings of the National Academy of Sciences 110:5064–5068. doi: 10.1073/pnas.1218042110

Cusack DF, Ashdown D, Dietterich LH, et al (2019) Seasonal changes in soil respiration linked to soil moisture and phosphorus availability along a tropical rainfall gradient. Biogeochemistry 10:9–22. doi: 10.1007/s10533-019-00602-4

Cusack DF, Halterman SM, Tanner EVJ, et al (2018a) Decadal-scale litter manipulation alters the biochemical and physical character of tropical forest soil carbon. Soil Biology and Biochemistry 124:199–209. doi: 10.1016/j.soilbio.2018.06.005

Cusack DF, Karpman J, Ashdown D (2016) Global change effects on humid tropical forests: Evidence for biogeochemical and biodiversity shifts at an ecosystem scale. Reviews of …. doi: 10.1002/(ISSN)1944-9208

Cusack DF, Markesteijn L, Condit R, et al (2018b) Soil carbon stocks across tropical forests of Panama regulated by base cation effects on fine roots. Biogeochemistry 137:253–266. doi: 10.1007/s10533-017-0416-8

Cusack DF, Silver WL, Torn MS, McDowell WH (2011) Effects of nitrogen additions on above- and belowground carbon dynamics in two tropical forests. 104:203–225. doi: 10.1007/s10533-010-9496-4

Cusack DF, Turner BL (2020) Fine Root and Soil Organic Carbon Depth Distributions are Inversely Related Across Fertility and Rainfall Gradients in Lowland Tropical Forests. Ecosystems 1–18. doi: 10.1007/s10021-020-00569-6

Dale SE, Turner BL, Bardgett RD (2015) Isolating the effects of precipitation, soil conditions, and litter quality on leaf litter decomposition in lowland tropical forests. Plant Soil 394:225–238. doi: 10.1007/s11104-015-2511-8

Dimoyiannis D (2009) Seasonal soil aggregate stability variation in relation to rainfall and temperature under Mediterranean conditions. Earth Surf Process Landforms 34:860–866. doi: 10.1002/esp.1785

Engelbrecht BMJ, Comita LS, Condit R, et al (2007) Drought sensitivity shapes species distribution patterns in tropical forests. Nature 447:80–82. doi: 10.1038/nature05747

Eusterhues K, Rumpel C, Kleber M, Kögel-Knabner I (2003) Stabilisation of soil organic matter by interactions with minerals as revealed by mineral dissolution and oxidative degradation. Organic Geochemistry 34:1591–1600. doi: 10.1016/j.orggeochem.2003.08.007

Feng X, Porporato A, Rodriguez-Iturbe I (2013) Changes in rainfall seasonality in the tropics. Nature Climate Change 3:811–815. doi: 10.1038/nclimate1907

Field C, Behrenfeld M, Randerson J, Falkowski P (1998) Primary production of the biosphere: integrating terrestrial and oceanic components. Science 281:237–240.

Fyllas NM, Patiño S, Baker TR, et al (2009) Basin-wide variations in foliar properties of Amazonian forest: phylogeny, soils and climate. Biogeosciences 6:2677–2708.

García-Oliva F, Sveshtarova B, Oliva M (2003) Seasonal effects on soil organic carbon dynamics in a tropical deciduous forest ecosystem in western Mexico. Soil Biology & Biochemistry 19:179–188. doi: 10.1017/S0266467403003201

Glaser B, Balashov E, Haumaier L, et al (2000) Black carbon in density fractions of anthropogenic soils of the Brazilian Amazon region. Organic Geochemistry 31:669–678.

Gmach MR, Cherubin MR, Kaiser K, Cerri CEP (2020) Processes that influence dissolved organic matter in the soil: a review. Sci agric (Piracicaba, Braz) 77:127–10. doi: 10.1590/1678-992x-2018-0164

Holdridge L, Grenke W, Hatheway W, et al (1971) Forest environments in tropical life zones. Pergamon Press, New York

Jandl R, Lindner M, Vesterdal L, et al (2007) How strongly can forest management influence soil carbon sequestration? Geoderma 137:253–268. doi: 10.1016/j.geoderma.2006.09.003

Jobbágy EG, Jackson RB (2000) The vertical distribution of soil organic carbon and its relation to climate and vegetation.

Joetzjer E, Douville H, Delire C, Ciais P (2013) Present-day and future Amazonian precipitation in global climate models: CMIP5 versus CMIP3. Clim Dyn 41:2921–2936. doi: 10.1007/s00382-012-1644-1

Johnson NC, Wilson GWT, Bowker MA, et al (2010) Resource limitation is a driver of local adaptation in mycorrhizal symbioses. Proceedings of the National Academy of Sciences 107:2093–2098. doi: 10.1073/pnas.0906710107

Jourdan C, Silva EV, Gonçalves JLM, et al (2008) Fine root production and turnover in Brazilian Eucalyptus plantations under contrasting nitrogen fertilization regimes. Forest Ecology and Management 256:396–404. doi: 10.1016/j.foreco.2008.04.034

Kaiser K (2003) Sorption of natural organic matter fractions to goethite (α-FeOOH): effect of chemical composition as revealed by liquid-state 13C NMR and wet-chemical analysis. Organic Geochemistry 34:1569–1579. doi: 10.1016/S0146-6380(03)00120-7

Kaiser M, Berhe AA (2014) How does sonication affect the mineral and organic constituents of soil aggregates?-A review. J Plant Nutr Soil Sci 177:479–495. doi: 10.1002/jpln.201300339

Kaiser K, Zech W (1999) Release of natural oragnic matter sorbed to oxides and a subsoil. Soil Science Society of America Journal 63:1157-1166. doi: 10.2136/sssaj1999.6351157x

Kharin VV, Zwiers FW, Zhang X (2007) Changes in temperature and precipitation extremes in the IPCC ensemble of global coupled model simulations. J Climate. doi: 10.1175/JCLI4066.1

Kleber M, Eusterhues K, Keiluweit M, et al (2015) Mineral-Organic Associations: Formation, Properties, and Relevance in Soil Environments. Elsevier Ltd

Lützow von M, Kögel-Knabner I, Ekschmitt K, et al (2006) Stabilization of organic matter in temperate soils: mechanisms and their relevance under different soil conditions - a review. European Journal of Soil Science 57:426–445. doi: 10.1111/j.1365-2389.2006.00809.x

Magrin GO, Marengo JA, Boulanger J-P, et al (2014) Central and South America. In: Barros VR, Field CB, Dokken DJ, et al. (eds) Climate Change Impacts, Adaptation, and Vulnerability. Part B Regional Aspects, Contribution of Working Group II to the Fifth Assessment Report on the Intergovernmental Panel on Climate Change. Cambridge, UK and New York, NY, USA, pp 1499–1566

Marin-Spiotta E, Silver WL, Swanston CW, Ostertag R (2009) Soil organic matter dynamics during 80 years of reforestation of tropical pastures. Glob Change Biol 15:1584–1597. doi: 10.1111/j.1365-2486.2008.01805.x

Marín-Spiotta E, Swanston CW, Torn MS, et al (2008) Chemical and mineral control of soil carbon turnover in abandoned tropical pastures. Geoderma 143:49–62. doi: 10.1016/j.geoderma.2007.10.001

Mora JL, Guerra JA, Armas-Herrera CM, et al (2014) Storage and depth distribution of organic carbon in volcanic soils as affected by environmental and pedological factors. Catena 123:163–175. doi: 10.1016/j.catena.2014.08.004

Noguez AM, Escalante AE, Forney LJ, et al (2008) Soil aggregates in a tropical deciduous forest: effects on C and N dynamics, and microbial communities as determined by t-RFLPs. Biogeochemistry 89:209–220. doi: 10.1007/s10533-008-9214-7

Nottingham AT, Hicks LC, Ccahuana AJQ, et al (2018) Nutrient limitations to bacterial and fungal growth during cellulose decomposition in tropical forest soils. 54:219–228. doi: 10.1007/s00374-017-1247-4

Nottingham AT, Turner BL, Stott AW, Tanner EVJ (2015) Nitrogen and phosphorus constrain labile and stable carbon turnover in lowland tropical forest soils. 80:26–33. doi: 10.1016/j.soilbio.2014.09.012

Nottingham AT, Turner BL, Winter K, et al (2013) Root and arbuscular mycorrhizal mycelial interactions with soil microorganisms in lowland tropical forest. FEMS Microbiol Ecol 85:37–50. doi: 10.1111/1574-6941.12096

Oh N-H, Richter DD (2005) Elemental translocation and loss from three highly weathered soil–bedrock profiles in the southeastern United States. Geoderma 126:5–25. doi: 10.1016/j.geoderma.2004.11.005

Ostertag R (2001) Effects of nitrogen and phosphorus availability on fine-root dynamics in Hawaiian montane forests. Ecology 82:485–499.

Phillips RP, Brzostek E, Midgley MG (2013) The mycorrhizal-associated nutrient economy: a new framework for predicting carbon-nutrient couplings in temperate forests. New Phytologist 199:41–51. doi: 10.1111/nph.12221

Pyke CR, Condit R, Aguilar S, Lao S (2001) Floristic composition across a climatic gradient in a neotropical lowland forest. Journal of Vegetation Science 12:553–566.

Quesada CA, Lloyd J, Anderson LO, et al (2011) Soils of Amazonia with particular reference to the RAINFOR sites. Biogeosciences 8:1415–1440. doi: 10.5194/bg-8-1415-2011

Reis Ferreira dos C, da Silva Neto EC, Pereira MG, et al (2020) Dynamics of soil aggregation and organic carbon fractions over 23 years of no-till management. Soil & Tillage Research 198:104533. doi: 10.1016/j.still.2019.104533

Rodell M, Famiglietti JS, Wiese DN, et al (2018) Emerging trends in global freshwater availability. Nature Publishing Group 557:651–659. doi: 10.1038/s41586-018-0123-1

Rohr T, Manzoni S, Feng X, et al (2013) Effect of rainfall seasonality on carbon storage in tropical dry ecosystems. J Geophys Res Biogeosci 118:1156–1167. doi: 10.1002/jgrg.20091

Roychand P, Marschner P (2014) Respiration and sorption of water-extractable organic carbon as affected by addition of Ca2+, isolated clay or clay-rich subsoil to sand. Pedosphere 24:98–

106.

Santiago LS (2009) Can growth form classification predict litter nutrient dynamics and decomposition rates in lowland wet forest? Biotropica 42:72-79. doi: 10.1111/j.1744-7429.2009.00604.x

Santiago LS, Mulkey SS (2005) Leaf productivity along a precipitation gradient in lowland Panama: patterns from leaf to ecosystem. Trees 19:349-356. doi: 10.1007/s00468-004-0389-9

Schmidt MWI, Torn MS, Abiven S, et al (2011) Persistence of soil organic matter as an ecosystem property. Nature 478:49–56. doi: 10.1038/nature10386

Schulp CJE, Nabuurs G-J, Verburg PH, de Waal RW (2008) Effect of tree species on carbon stocks in forest floor and mineral soil and implications for soil carbon inventories. Forest Ecology and Management 256:482–490. doi: 10.1016/j.foreco.2008.05.007

Schwarz G (1978) Estimating the dimension of a model. The Annals of Statistics 6:461–464.

Seidel DJ, Fu Q, Randel WJ, Reichler TJ (2008) Widening of the tropical belt in a changing climate. Nature Geoscience 1:21–24.

Sheldrake M, Rosenstock NP, Revillini D, et al (2017) A phosphorus threshold for mycoheterotrophic plants in tropical forests. 284:20162093–20162099. doi: 10.1098/rspb.2016.2093

Singh M, Sarkar B, Biswas B, et al (2016) Adsorption-desorption behavior of dissolved organic carbon by soil clay fractions of varying mineralogy. Geoderma 280:47–56. doi: 10.1016/j.geoderma.2016.06.005

Sollins P, Glassman CA, Paul EA, et al (1999) Soil carbon and nitrogen: pools and fractions. In: Sollins P (ed) Standard Soil Methods for Long-term Ecological Research. New York, NY,

Sollins P, Swanston C, Kleber M, et al (2006) Organic C and N stabilization in a forest soil: Evidence from sequential density fractionation. Soil Biology and Biochemistry 38:3313–3324. doi: 10.1016/j.soilbio.2006.04.014

Stewart R, Stewart J, Woodring W (1980) Geologic map of the Panama Canal and vicinity, Republic of Panama.

Swanston CW, Torn MS, Hanson PJ, et al (2005) Initial characterization of processes of soil carbon stabilization using forest stand-level radiocarbon enrichment. Geoderma 128:52–62. doi: 10.1016/j.geoderma.2004.12.015

Swift RS (1996) Organic matter characterization. In: Sparks DL (ed) Methods of Soil Analysis: Part 3: Chemical Methods. Soil Science Society of America, Madison, WI, pp 1011–1069

Tanner EVJ, Sheldrake MWA, Turner BL (2016) Changes in soil carbon and nutrients following

6 years of litter removal and addition in a tropical semi-evergreen rain forest. Biogeosciences 13:6183-6190. doi: 10.5194/bg-13-6183-2016

Tedersoo L, Bahram M (2019) Mycorrhizal types differ in ecophysiology and alter plant nutrition and soil processes. Biol Rev 94:1857–1880. doi: 10.1111/brv.12538

Todd-Brown KEO, Randerson JT, Hopkins F, et al (2014) Changes in soil organic carbon storage predicted by Earth system models during the 21st century. Biogeosciences 11:2341–2356. doi: 10.5194/bg-11-2341-2014

Torn MS, Kleber M, Zavaleta ES, et al (2013) A dual isotope approach to isolate soil carbon pools of different turnover times. Biogeosciences 10:8067–8081. doi: 10.5194/bg-10-8067-2013

Torn MS, Trumbore SE, Chadwick OA, Vitousek PM (1997) Mineral control of soil organic carbon storage and turnover. Nature 389:170–173.

Trumbore SE (1993) Comparison of carbon dynamics in tropical and temperate soils using radiocarbon measurements. Global Biogeochem Cycles 7:275–290.

Trumbore SE, Zheng S (1996) Comparison of fractionation methods for soil organic matter 14 C analysis.

Turner BL, Brenes-Arguedas T, Condit R (2018) Pervasive phosphorus limitation of tree species but not communities in tropical forests. 555:367–370. doi: 10.1038/nature25789

Turner BL, Engelbrecht BMJ (2011) Soil organic phosphorus in lowland tropical rain forests. Biogeochemistry 103:297–315. doi: 10.1007/s10533-010-9466-x

Turner BL, Yavitt JB, Harms KE, Garcia MN, Wright SJ (2015) Seasonal changes in soil organic matter after a decade of nutrient addition in a lowland tropical forest. Biogeochemistry 123:221-235. doi: 10.1007/s10533-014-0064-1

Umaña MN, Condit R, Pérez R, et al (2020) Shifts in taxonomic and functional composition of trees along rainfall and phosphorus gradients in central Panama. Journal of Ecology 1:260–11. doi: 10.1111/1365-2745.13442

Wiesmeier M, Urbanski L, Hobley E, et al (2019) Soil organic carbon storage as a key function of soils - A review of drivers and indicators at various scales. Geoderma 333:149–162. doi: 10.1016/j.geoderma.2018.07.026

Windsor DM, Rand AS, Rand WM (1990) Características de la Precipitación de la Isla de Barro Colorado. In: Leigh EG Jr, Rand AS, Windsor DM (eds) *Ecología de un bosque tropical: ciclos estacionales y cambios a largo plazo*. Balboa, Rep. of Panama, pp 53–71

Xie S-P, Deser C, Vecchi GA, et al (2010) Global Warming Pattern Formation: Sea Surface Temperature and Rainfall. J Climate 23:966–986. doi: 10.1175/2009JCLI3329.1