

THESIS

VARIATION IN SOIL ORGANIC CARBON ACROSS LOWLAND TROPICAL FOREST  
GRADIENTS: SOIL FERTILITY AND PRECIPITATION EFFECTS ON SOIL CARBON  
ORGANIC CHEMISTRY AND AGE

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## ABSTRACT

### VARIATION IN SOIL ORGANIC CARBON ACROSS LOWLAND TROPICAL FOREST GRADIENTS: SOIL FERTILITY AND PRECIPITATION EFFECTS ON SOIL CARBON ORGANIC CHEMISTRY AND AGE

Tropical forests hold large amounts of carbon (C) in both aboveground biomass and belowground soil organic carbon (SOC) stocks. Climate change is expected to alter tropical forests' precipitation with some forests already showing decreased rainfall. We analyzed SOC molecular composition and age in lowland tropical forests of Panama across fertility gradients, rainfall ranges, and soil order. We hypothesized that H1) rainforests with relatively greater rainfall store larger amounts of proteins (N-alkyl) and lipids (alkyl) in SOC because of greater microbial biomass and H2) subsurface SOC stocks in more strongly weathered, clay-rich soils are older (as indicated by radiocarbon) because of great sorption capacity. We found that overall, carbon decreased and became older with depth across all samples. Solid-state  $^{13}\text{C}$  NMR spectroscopy indicated that soil order and depth were significant predictors of C functional group abundances while phosphorus (P) was a significant predictor of alkyl, aromatic, and carboxyl C. Alkyl/O-Alkyl ratios increased with depth indicating increased degradation of the SOC.  $\Delta 14\text{C}$  values indicated older C with depth and varied significantly with soil order where Oxisols were the oldest and Mollisols the youngest. Soil N % and K % were significant predictors of younger soil C. Additionally, biomolecular composition of SOM from 0-10 cm was a significant predictor of  $\Delta 14\text{C}$  at 25-50 cm. We found that higher abundances of alkyl and O-alkyl C corresponded with

younger C at depth and higher abundances of aromatic and phenolic C contained older C at depth.

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## TABLE OF CONTENTS

ABSTRACT .....	ii
ACKNOWLEDGMENTS .....	iii
LIST OF TABLES .....	vi
LIST OF FIGURES .....	vii
Chapter 1 – Introduction .....	1
Chapter 2 – Methods .....	9
Study Sites .....	9
Isthmus of Panama Lowland Tropical Forests .....	9
A Subset of 13 Forests .....	10
Soil Chemical Analysis.....	11
<sup>13</sup> C Solid State Nuclear Magnetic Resonance .....	11
Sequential Acid Treatment .....	11
Quartz Removal .....	12
<sup>13</sup> C NMR Instrument Acquisition Parameters .....	13
<sup>14</sup> C Radiocarbon .....	13
Statistics .....	14
Biomolecular Chemical Shift Regions from <sup>13</sup> C NMR .....	15
<sup>14</sup> C Radiocarbon Age of SOC .....	15
Chapter 3 – Results .....	17
Soil Carbon Concentrations .....	17
<sup>13</sup> C NMR Chemical Shift Regions .....	17
Molecular Mixing Model .....	19
<sup>14</sup> C Radiocarbon .....	19
<sup>13</sup> C NMR Data as Predictor of $\Delta^{14}\text{C}$ Age .....	20
Chapter 4 – Discussion .....	31
Tropical Forest DOC Biomolecules .....	31
Soil Order Effects .....	31
Soil Depth Effects .....	32
MAP Effects .....	33
Other Ecosystem Drivers .....	34
Molecular Mixing Model in Tropical Forests .....	35
Tropical Soil $\Delta^{14}\text{C}$ Age .....	36
$\Delta^{14}\text{C}$ Among Soil Orders .....	36
$\Delta^{14}\text{C}$ with Depth .....	37
$\Delta^{14}\text{C}$ with MAP .....	38
Other Ecosystem Drivers of $\Delta^{14}\text{C}$ .....	39
SOC Biomolecular Composition and $\Delta^{14}\text{C}$ .....	40
SOC Inputs and Transport .....	40
Cascade of Surface Biomolecule Effect on Deep $\Delta^{14}\text{C}$ .....	40
Conceptual Model .....	41
Chapter 5 – Conclusions .....	42

References .....43

## LIST OF TABLES

Table 1 .....	6
---------------	---

## LIST OF FIGURES

Figure 1 .....	5
Figure 2 .....	6
Figure 3 .....	20
Figure 4 .....	20
Figure 5 .....	21
Figure 6 .....	21
Figure 7 .....	23
Figure 8 .....	23
Figure 9 .....	24
Figure 10 .....	24
Figure 11 .....	25
Figure 12 .....	25
Figure 13 .....	26
Figure 14 .....	26
Figure 15 .....	27
Figure 16 .....	27
Figure 17 .....	28
Figure 18 .....	28
Figure 19 .....	29
Figure 20 .....	29
Figure 21 .....	30
Figure 22 .....	41

## CHAPTER ONE: INTRODUCTION

Tropical forests hold some of the largest carbon (C) stocks globally, containing 30% of aboveground biomass and 20% of belowground soil organic carbon (SOC) (Field et al. 1998; Jobbagy and Jackson 2000). Soils can sequester large quantities of C, storing some fractions of C for millennia (i.e., slowly cycling pools), and other fractions for hours to days (rapidly cycling pools), with a continuum of turnover times in between (Schmidt et al. 2011; Marin-Spiotta et al. 2014; Waring et al 2020). The total stock of SOC is comprised of a variety of organic compounds, or biomolecules, which might be stored for different lengths of time and via different mechanisms across soil depth. For example, numerous studies now indicate that the longest-term storage of SOC occurs in subsoils, via sorption with mineral surfaces and that certain biomolecules might be preferentially sorbed, such as aromatics, lipids, and/or proteins (Cotrufo et al. 2015; Cusack et al. 2018b; Kleber et al. 2007; Koarashi et al. 2012; Kögel-Knabner et al. 2008; Schmidt et al. 2011; Sollins et al. 2006). Still, our understanding of the drivers of variation in tropical forest SOC storage is limited, particularly across gradients in climate and soil characteristics within the tropics. Understanding the mechanisms driving SOC storage across gradients in tropical forest rainfall and soil gradients could improve our prediction of forest-climate feedbacks over space and time.

For example, climate change is causing warming and changing precipitation patterns across tropical forests, such as longer more severe dry seasons and increased drought occurrence, and at the same time rainfall and storm severity is also increasing (Hilbert et al. 2002; Feng et al. 2013; Rodell et al. 2018; Aguirre-Gutierrez et al. 2020). These effects of climate change might have different effects on SOC across tropical forests with different baseline conditions in mean

annual precipitation (MAP), dry season length, and soil characteristics like clay content and fertility.

Characterization of SOC organic chemistry is a first step to understanding what type of C is being stored in different tropical forests and at different depths. SOC is a heterogeneous mixture of organic compounds largely derived from plant litter, root exudates, and microorganism re-processing (Schmidt et al. 2011; Domeignoz-Horta et al. 2021; Heckman et al. 2021; Huang et al. 2021). One way to characterize the organic chemistry of SOC is by using  $^{13}\text{C}$  nuclear magnetic resonance (NMR) spectroscopy.  $^{13}\text{C}$  NMR has been widely used to determine the composition of SOC at a molecular scale, and can be used to detect changes in organic functional groups present in the soil, evidence of C oxidation state (i.e., increases in carbonyl C), and the presence and abundance of organic compound groups such as alkyl C (e.g., lipids), N-alkyl C (e.g., proteins), o-alkyl C (e.g., carbohydrates), and aromatic C (e.g., lignin) (Baldock et al. 1992; Baldock et al. 2004; Hockaday et al. 2009; Simpson and Simpson 2012; Li et al. 2020). These biomolecules can tell us the sources and storage of plant and microbial SOC. For example, microbial inputs, best characterized by proteins, have been shown to vary with depth (Simpson et al. 2007). Assessing which biomolecules are being stored in different soils and relating these SOC age and storage mechanism helps us understand what SOC is being stored over different timespans.

A useful tool for measuring the age of SOC, or the time since it was fixed by plants from the atmosphere, is radiocarbon ( $^{14}\text{C}$ ) content of SOC. During the 1960s, governments began aboveground testing of atomic bombs which released a large amount of  $^{14}\text{C}$  into the atmosphere. This resulted in increased  $^{14}\text{C}$  concentrations throughout the world known as the bomb pulse. Since the ban of nuclear bomb testing in 1963, there has been a steady decline in bomb C which

can be used in radiocarbon dating also known as bomb-pulse dating (Zoppi et al 2004; Johnson-Belford and Blau 2020). The spike of  $^{14}\text{C}$  content of atmospheric carbon dioxide during atomic bomb testing created an atmospheric tracer of C into plants and soils on decadal timescales (Cain and Suess 1976; Enting, 1982; Hua and Barbetti, 2016). And, on longer timescales, the natural degradation of  $^{14}\text{C}$  can help us understand turnover time and ages on the scale of millennia (Eglinton et al 1996; Gierga et al 2016; Druffel et al 2016; French et al 2018). Past studies have used bulk SOC radiocarbon ages to assess the age of C stored in soils at different depths, generally showing increasing C age with depth (Mathieu et al 2015; Shi et al, 2020). Also, compound-specific  $^{14}\text{C}$  analyses have indicated that different biomolecules are stored for different lengths of time in different ecosystems (Eglinton et al. 1996; Eglinton et al 1997; Harrison 1996; Mollenhauer and Rethemeyer 2009; Kusch et al. 2010; Douglas et al. 2014; Mollenhauer et al 2019). Lipids have been most heavily studied using compound-specific radiocarbon with leaf waxes (lipids) have been found to be stored on average between 100-1200 years in river systems (French et al 2018), between 20 and 520 years old in lacustrine environments (Douglas et al 2014), and 820 and 5600 years old in marine sediments (Douglas et al 2014). Understanding the relationships among local climate, soil characteristics, and the age of SOC with depth is vital for characterizing tropical forest SOC.

One factor that can contribute to how long SOC is stored in soils is the mechanism of storage. For example, different fractions of SOC can be stored in soils through different mechanisms, including free, unattached organic litter (resembling plant tissues), organic matter occluded in soil aggregates, and organic matter sorbed to mineral surfaces (i.e., mineral-associated SOC). In general, mineral-associated SOC is the oldest, most stable fraction of SOC in most soils (Kleber et al. 2015; Poeplau et al. 2018; Cotrufo et al. 2019; Abramoff et al. 2021).

Therefore, long-term SOC storage depends on soil characteristics contributing to sorption dynamics, such as clay and silt content, presence of Fe oxides and Al-silicates, soil weathering status (or Order in US soil taxonomy), organic matter surface area, and pH, as well as environmental factors like mean annual temperature (MAT) and mean annual precipitation (MAP) (Mayes et al. 2012; Kleber et al. 2015; Abramoff et al. 2021). Their modes of sorption also vary in strength, such that some sorbed SOC is held in more stable organo-mineral associations, whereas other sorbed C might desorb more dynamically. Sorption modes include ligand exchange, polyvalent cation bridging, electrostatic attraction, H-bonding, and van der Waals forces (Singh et al. 2018). Soils with different types and quantities of clay also have varying sorption mechanisms, related to their specific surface area and surface charge (Saidy et al 2013). Thus, characterizing the capacity for different soil to sorb SOC, and also the relative level of dynamic desorption, can help us understand how SOC storage varies across tropical forests.

Although many climate-land models characterize tropical forests as one functional type, in reality tropical forests occur over large gradients in MAP and soil characteristics. Differences in soil fertility can influence microbial community characteristics, decomposition rates, plant biomass chemistry, and plant productivity (Schulp et al. 2008; Condit et al. 2018; Cusack et al. 2018), all of which influence SOC storage. Similarly, MAP can influence SOC storage through plant species compositions, net primary productivity, nutrient cycling and transformations, and microbial communities (Kindler et al. 2011; Chen et al. 2016).

We assessed the chemical character, age, and sorption dynamics of SOC across 13 distinct humid tropical lowland forests on the Isthmus of Panama, where there is a 1m increase in MAP from the Pacific to the Caribbean, soil fertility varies by orders of magnitude in available

phosphorus (P), nitrogen (N), and base cations, and there is a broad array of soil Orders (fig. 1, fig. 2). Across these sites, MAP and soil fertility do not covary, because soil fertility is related to geological diversity, allowing us to disentangle the effects of MAP and soil properties on SOC storage characteristics. To do this, our 13 forests included groups of sites on fertile and infertile soils within similar rainfall ranges. We hypothesized that: H1) rainforests with relatively greater rainfall store larger amounts of proteins (N-alkyl) and lipids (alkyl) in SOC because of greater microbial biomass; H2) subsurface SOC stocks in more strongly weathered, clay-rich soils are older (as indicated by radiocarbon) because of greater sorption capacity.

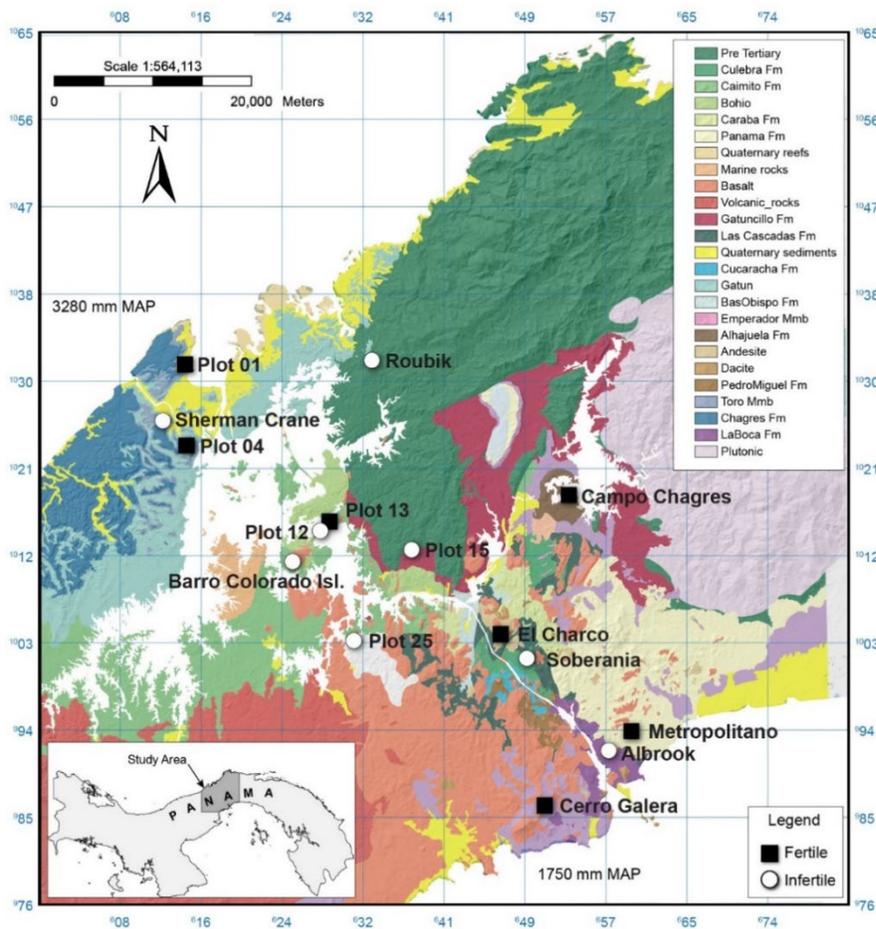


Figure 1. We used a set of 13 distinct lowland tropical forests across the Isthmus of Panama, across gradients in soil fertility (as indicated by geological variation), and over a ~1m increase in rainfall from the Pacific to the Caribbean. Sites were chosen in groups of fertile (black markers) and infertile (white markers) soils within similar rainfall ranges to capture variation in rainfall and fertility, with two to three sites within each rainfall range (Dietterich et al 2021).

## Isthmus-Scale Soil Variation in Lowland Forests of Central Panama



Figure 2. Commonly found soil orders in Panama (photo cred: STRI Soils Lab). Oxisols are the most developed and are a reddish color due to their high Fe content. Inceptisols are a depositional product composed of weathered material from surrounding areas.

Table 1. Site Description: Soil Depths, rainfall (MAP), nutrient content, and clay content.. New data collected from the samples for this study include % C and % N. Other data taken from Cusack et al. (2018) where nutrients were measured at 0-10 cm, 10-20 cm, 20-50 cm, and 50-100cm.

Site	Depth (cm)	Soil Type	MAP (mm)	Fertility Index	C (wt %)	N (wt %)	Total P to 1 m (g/m)	Resin-extractable P to 1 m (gP/m <sup>2</sup> )	% Clay to 1 m	TP (mg/kg)
Albrook	0-10	Ultisol	1860	I	4.83	0.17	316.01	0.25	51.896	339.88
Albrook	10-25	Ultisol	1860	I	2.85	0.03	316.01	0.25	51.896	284.00
Albrook	25-50	Ultisol	1860	I	1.04	0.02	316.01	0.25	51.896	292.88
Albrook	50-75	Ultisol	1860	I	0.62	0.01	316.01	0.25	51.896	335.60
ANAM	0-10	Oxisol	2008	I	3.42	0.25	475.47	0.55	78.217	
ANAM	25-50	Oxisol	2008	I	1.085	0.126	475.47	0.55	78.217	
Casa Roubik	0-10	Oxisol /Ultisol	3053	I	5.78	0.35	85.56	0.10	45.030	
Casa Roubik	10-25	Oxisol /Ultisol	3053	I	2.77	0.19	85.56	0.10	45.030	
Casa Roubik	25-50	Oxisol /Ultisol	3053	I	1.72	0.41	85.56	0.10	45.030	

El Charco	0-10	Inceptisol	2056	F	2.05	0.19	761.15	0.73	45.364	639.75
El Charco	10-25	Inceptisol	2056	F	2.63	0.09	761.15	0.73	45.364	634.59
El Charco	25-50	Inceptisol	2056	F	1.64	0.04	761.15	0.73	45.364	606.24
El Charco	50-75	Inceptisol	2056	F	1.40	0.03	761.15	0.73	45.364	718.78
El Charco	75-100	Inceptisol	2056	F	0.99	0.02	761.15	0.73	45.364	718.78
Met	0-10	Mollisol	1875	F	2.978	0.237	260.42	2.15	31.072	360.37
Met	10-25	Mollisol	1875	F	1.791	0.19	260.42	2.15	31.072	198.52
Met	25-50	Mollisol	1875	F	1.052	0.126	260.42	2.15	31.072	176.57
Met	50-75	Mollisol	1875	F			260.42	2.15	31.072	91.56
Met	75-100	Mollisol	1875	F			260.42	2.15	31.072	91.56
P01	0-10	Alfisol	2864	F	5.41	0.07	283.07	0.80	61.914	652.47
P01	10-25	Alfisol	2864	F	2.21	0.15	283.07	0.80	61.914	381.42
P01	25-50	Alfisol	2864	F	0.87	0.05	283.07	0.80	61.914	144.55
P01	50-75	Alfisol	2864	F	0.26	0.06	283.07	0.80	61.914	77.66
P04	0-10	Mollisol	2829	F	2.336	0.219	298.84	1.22	37.175	633.31
P04	10-25	Mollisol	2829	F	1.756	0.186	298.84	1.22	37.175	736.23
P04	25-50	Mollisol	2829	F			298.84	1.22	37.175	611.38
P04	50-75	Mollisol	2829	F			298.84	1.22	37.175	523.29
P08	0-10	Oxisol	2353	I	3.30	0.20	216.39	0.22	55.273	200.73
P08	10-25	Oxisol	2353	I	1.63	0.14	216.39	0.22	55.273	146.56
P08	25-50	Oxisol	2353	I	1.04	0.07	216.39	0.22	55.273	111.02
P08	50-75	Oxisol	2353	I	0.89	0.05	216.39	0.22	55.273	143.94
P08	75-100	Oxisol	2353	I	0.60	0.03	216.39	0.22	55.273	143.94
P12	0-10	Inceptisol	2595	I	4.212	0.283	241.66	0.33	52.298	307.64
P12	10-25	Inceptisol	2595	I	2.53	0.112	241.66	0.33	52.298	287.90
P12	25-50	Inceptisol	2595	I	1.22	0.134	241.66	0.33	52.298	305.57
P12	50-75	Inceptisol	2595	I			241.66	0.33	52.298	102.02
P12	75-100	Inceptisol	2595	I			241.66	0.33	52.298	102.02
P13	0-10	Alfisol	2591	F	4.299	0.326	204.13	1.58	49.228	717.75
P13	10-25	Alfisol	2591	F			204.13	1.58	49.228	400.36
P13	25-50	Alfisol	2591	F	2.2	0.215	204.13	1.58	49.228	179.16
P13	50-75	Alfisol	2591	F			204.13	1.58	49.228	78.18
P25	0-10	Ultisol	2176	I	2.65	0.33	40.74	0.15	39.413	60.09
P25	10-25	Ultisol	2176	I	2.36	0.21	40.74	0.15	39.413	48.22
P25	25-50	Ultisol	2176	I	0.55	0.08	40.74	0.15	39.413	14.70
P25	50-75	Ultisol	2176	I	0.40	0.03	40.74	0.15	39.413	14.82
P25	75-100	Ultisol	2176	I	0.30	0.03	40.74	0.15	39.413	14.82
Pipeline P15	0-10	Alfisol	2353	I	3.17	0.32	244.37	0.34	59.298	288.09
Pipeline P15	10-25	Alfisol	2353	I	1.60	0.18	244.37	0.34	59.298	235.28

Pipeline P15	25-50	Alfisol	2353	I	0.91	0.13	244.37	0.34	59.298	163.76
SC	0-10	Oxisol	2848	I	4.318	0.276	233.59	0.15	78.217	
SC	25-50	Oxisol	2848	I	2.617	0.183	233.59	0.15	78.217	

## CHAPTER TWO: METHODS

### Study Sites

#### *Isthmus of Panama Lowland Tropical Forests*

The Isthmus of Panama spans a MAP gradient ranging from  $\sim 1750$  mm year<sup>-1</sup> on the Pacific coast to  $\sim 4000$  mm year<sup>-1</sup> on the Caribbean coast (Pyke et al. 2001; Engelbrecht et al. 2007). The forests have a tropical monsoon climate and contain mature humid tropical forests (Pyke et al. 2001; Condit et al. 2013; Turner et al. 2018). The sites are a subset of a larger network of  $\sim 50$  1-ha plots maintained by the Smithsonian Tropical Research Institute (STRI) in the Panama lowlands. The forests have a tropical monsoon climate, and are generally characterized as tropical moist broadleaf forests (Holdridge et al. 1971). The wetter Caribbean coast has a shorter dry season ( $\sim 115$  days), compared with the drier Pacific coast ( $\sim 150$  days). Thus, while all sites are classified as tropical moist broadleaf, the “drier” forest has lower MAP as well as a longer dry season compared with the wetter sites. The mean annual temperature at all sites is  $\sim 26^{\circ}\text{C}$  and mean monthly temperature varies by  $< 1^{\circ}\text{C}$  during the year (Windsor et al. 1990).

Rainfall at each of our study sites was calculated using the nearest long-term rainfall data from Panama Canal Authority (ACP) sites and interpolated, including BCI climate station data, as described previously (Engelbrecht et al. 2017). We note that these calculations tended to underestimate rainfall measured by STRI for Caribbean sites (STRI, 2020), where we instead used STRI rainfall measurements on the Caribbean coast that show  $\sim 3,400$  mm MAP.

Variation in soil fertility across the sites is primarily due to differences in the geologic substrate with parent materials including basalt, andesite, agglomerate, rhyolitic tuff, limestone, calcareous sandstone, siltstone, and mudstone lithologies (Condit et al. 2013; Turner and

Engelbrecht 2011). As a result of the strong relationship between geologic substrate and fertility, there is little to no association between fertility and precipitation in the region (Cusack et al. 2018; Turner et al. 2018; Dietterich et al. 2021). Soil properties and nutrients vary across the isthmus with total phosphorus content ranging from 14.7 mg P/kg soil to 736.5 mg P/kg soil, clay content to 1 m from 31.5% to 78.5%, soil N from 0.12 kg N/m<sup>2</sup> to 2.6 kg N/m<sup>2</sup>, Mehlich extracted aluminum (Meh-Al) from 144.5 mg Al/kg soil to 2206.7 mg Al/kg soil, and Mehlich extracted iron (Meh-Fe) from 7.0 mg Fe/ kg soil to 401.5 mg Fe/kg soil (Cusack et al 2018).

Aboveground biomass does not vary significantly across the 50 1-ha plots nor according to rainfall or soil fertility (Cusack et al. 2018a; Pyle et al. 2001). Tree community composition does vary markedly across the broader rainfall and fertility gradients, driven in large part by species-specific affinities for moisture and soil phosphorus (P) (Condit et al. 2013; Engelbrecht et al. 2007; Turner et al. 2018). There are 200-300 tree species per ha at any given site, and only a few of these species overlap parts of the rainfall gradient, or portions of the range in soil P.

### *A Subset of 13 Forests*

We studied 13 distinct forests across the rainfall gradient in the lowland forests of the Isthmus of Panama (Fig. 1 – map in progress). These sites are a subset of a larger network of over 50 1-ha plots maintained by the Smithsonian Tropical Research Institute (STRI) in the Panama lowlands (table 1). The study sites are on generally flat topography.

One soil pit was dug for each site to at least 1m depth for our sampling, and soil stratigraphy, order, and texture were characterized (table 1) (Cusack et al. 2018). Samples for this study were collected in 2016 from the soil pits at depth increments of 0-10 cm, 10-25 cm, 25-50 cm, 50-75 cm, and 75-100 cm. Site MAP and soil fertility were characterized previously (Cusack

et al. 2019; Dietterich et al. 2021), with this published data used here as covariates. Soils were air-dried until weight stabilized, then ground with a mortar and pestle, removing roots and homogenizing by hand at Colorado State University in 2021. These sites have previously been described for soil carbon density fractions (Dietterich et al. 2021), and seasonal patterns in soil respiration and soil moisture (Cusack et al. 2019). The sites have seasonal trends in some soil properties, including decreased soil moisture and decreased soil respiration during the dry season relative to the late wet season, and accumulation of forest floor litter biomass during the dry season (Cusack et al. 2019). Site characteristics, including aboveground biomass, local MAP, root biomass, soil C stocks, soil nutrients, and other soil properties have been described previously for the 50 1-ha plots (Cusack et al. 2018a, 2018b; Cusack et al. 2019; Cusack and Turner 2020).

### *Soil Chemical Analyses*

Soil carbon concentrations were measured at Baylor University and the Center for Isotope Biogeochemistry, UC Berkeley, in 2021. All samples packed into tin capsules and C and N values were obtained at Baylor University using a Thermo Flash 2000 combustion elemental analyzer and on an IsoPrime 100 elemental analyzer at the Center for Isotope Biogeochemistry. Values were compared to published C and N concentrations from Cusack et al 2018a.

## **<sup>13</sup>C Solid-State Nuclear Magnetic Resonance**

### *Sequential Acid Treatment*

Prior to <sup>13</sup>C NMR analysis, all samples underwent a sequential acid demineralization procedure (Gelinas et al. 2001) to remove paramagnetic metals (Fe and Mn) that quench the magnetic resonance signal, and to concentrate the organic matter by dissolving the mineral

grains. 2 to 3 g of dry, ground, soils were added to a 50 mL polypropylene centrifuge tube. Carbonate minerals were removed first by the stepwise addition of 10 weight % aqueous HCl until effervescence ceased. Samples were then centrifuged at 2800 g for 10 minutes and the pellet was rinsed three times with deionized water. An aqueous mixture of 10 weight % HCl and 10 weight % HF acid was added and placed on an oscillating shaker table at room temperature for 12 hours.

Due to the high Fe content of many samples, the 10% HCl/HF treatment was tested with three, four, and five treatments and then rinsed with deionized water three times. Each test sample's NMR spectra was acquired (using parameters described below) to test the signal to noise ratio and assess the optimal number of HF treatments. Four HF treatments was found to produce the cleanest spectra and was used for all samples. The organic-rich residue was dried over a sand bath at 50 degrees Celsius under a stream of N<sub>2</sub> gas. The residue was then crushed into a fine powder and packed into a four-millimeter zirconia rotor with a Kel-F cap.

### *Quartz Removal*

Some samples contained large quartz crystals that, when packed with soil into the NMR rotor, caused uneven mass distribution resulting in low signal to noise ratios or preventing the rotor from reaching the appropriate spinning speed. A density separation using sodium iodine was attempted but resulted in salts that required removal and did not fully separate the quartz. The optimal method for quartz removal was by-hand removal using tweezers for each high quartz sample.

### *<sup>13</sup>C NMR Instrument and Acquisition Parameters*

The <sup>13</sup>C NMR analyses were performed in the solid-state on a 300 MHz Bruker Advance III spectrometer at Baylor University in Waco, Texas. The NMR signals were generated using a variable-amplitude cross-polarization with 90-degree excitation pulses, a 180-degree refocusing of carbon resonances, composite pulse proton decoupling, and magic angle spinning at 12 kilohertz. This method provides the relative proportion of C in each chemical shift region, indicative of the distribution of different biomolecules in each sample (Johnson and Schmidt-Rohr 2014; Daun and Schmidt-Rohr 2017).

A minimum of 32 k scans were collected with a recycle delay of 2 seconds. The data were Fourier transformed with exponential multiplication, zero-filling, and 60Hz line broadening in the Topspin 3.0 software. Each spectrum was phase corrected, given a linear baseline and signal regions were integrated to determine the relative abundances of C functional groups as follows: Alkyl (0–45 ppm), N-alkyl/methoxyl (45– 60 ppm), O-alkyl (60–95 ppm), di-O-alkyl (95–110 ppm), aromatic (110–145 ppm), phenolic (145–165 ppm), amide/carboxyl (165– 190 ppm), and ketone (190–215 ppm). The signal areas were corrected for spinning sideband artifacts.

The raw data were then input into a Molecular Mixing Model (MMM) described by Baldock et al. (2004) to estimate the relative contributions of four major classes of biomolecules: carbohydrate, protein, lipid, and lignin C. The model calculations were constrained by the carbon-to-nitrogen ratio.

### **<sup>14</sup>C Radiocarbon**

Soil <sup>14</sup>C content was analyzed at the Lawrence Livermore National Laboratory Center for Accelerator Mass Spectrometry. In order to obtain radiocarbon data, the SOC was converted into

a pure graphite form removing all non-carbon components in the SOC, leaving behind solid carbon. To do this, samples underwent sealed-tube combustion to CO<sub>2</sub> (in the presence of CuO and Ag) before being reduced onto iron powder in the presence of H<sub>2</sub> (Vogel et al 1984; Slessarev et al 2020). This process is known as graphitization. Many soils are contaminated with bomb C creating differences in the ratios of <sup>13</sup>C/<sup>14</sup>C creating artificially high <sup>14</sup>C values. Samples with bomb C contamination were unable to be dated and were marked as “modern” for this experiment. Radiocarbon was analyzed using a NE 1.0 MV Tandem accelerator mass spectrometer (AMS) and the <sup>14</sup>C content (expressed as Δ<sup>14</sup>C) was reported in fraction relative to the absolute atmospheric <sup>14</sup>C activity in 1950 (Broek et al 2021). Sample preparation backgrounds were subtracted based on measurements of samples of <sup>14</sup>C-free coal.

## **Statistics**

We used SOC biomolecular composition, age, and sorption/desorption measures and response factors in a series of ANCOA analyses. First, we ran an ANOVA for each response factor, using MAP, soil order, depth, and their interactions as predictors. This simple model was used first to determine if there was any effect of soil weathering/nutrient status on SOC measures, and how these compared in strength to MAP and soil depth effects. Second, we explored individual measures of soil physiochemistry by using a forward, stepwise modeling activity. These included C concentration, N concentration, resin-extractable P to 1 m, clay content to 1 m, Meh-Fe, Meh-Al, Mehlich extractable potassium (meh-K), Mehlich extractable magnesium (meh-Mg), Mehlich extractable zinc (meh-Zn), Mehlich extractable manganese (meh-Mn), Mehlich extractable calcium (meh-Ca), total phosphorus, dissolved organic carbon, and fine roots abundance. Third, for significant factors in these first two statistical activities, we

conducted post-hoc pairwise means comparisons or regressions. Fourth, we assessed correlations among the SOC measures collected, using regression tests to assess covariation of biomolecular composition, age, and sorption/desorption. All statistics were performed in JMP 15.0 (SAS). Means are reported plus or minus standard errors, regression statistics are reported, and significance value was set at  $p < 0.05$  unless otherwise noted (e.g., marginal significance was set at  $p < 0.1$ ). Specific details for each set of dependent factors are given below.

#### *Biomolecular Chemical Shift Regions from $^{13}\text{C}$ NMR*

We ran an initial ANOVA assessing differences between soil orders and depth increment for all spectral regions. We used a forward stepwise approach to build multi-regressions to test continuous factors that were identified in the stepwise process with a minimum Bayesian information criterion (BIC) stopping rule. Significant factors were then run in post hoc pairwise comparisons to assess the strength of the relationship. The response factors included each chemical shift region and molecular mixing model results. Predictors included depth, MAP, % C and % N for corresponding depth increments,  $^{14}\text{C}$ , clay content to 1 m depth (%), Fe content, Al content, and resin P stock to 1 m depth (g P/m<sup>2</sup>). We also compared chemical shift regions against soil orders. The significance level is set at  $p = 0.05$  unless otherwise noted.

#### *$^{14}\text{C}$ Radiocarbon Age of SOC*

We ran an initial ANOVA assessing differences in  $\Delta^{14}\text{C}$  with depth and soil order. Next, we used a forward stepwise approach to build multi-regressions to test continuous factors against  $\Delta^{14}\text{C}$  using a minimum BIC stopping rule. The only response factor included was  $\Delta^{14}\text{C}$  and predictors included depth, MAP, % C, % N, total P to 1 m, resin-extractable P to 1 m, clay

content to 0.5m, clay content to 1 m, Fe content, Al content, and all chemical shift regions.

Predictors that were selected as significant from the model underwent post hoc pairwise comparisons to assess the strength of the relationship.

## CHAPTER THREE: RESULTS

### **Soil Carbon Concentrations**

Soil organic carbon ranged from 0.26 % to 5.78 % among sites, with a significant effect of depth, but no significant effects with soil order or MAP. Alfisols and Ultisols had the highest C concentrations (fig. 2). Soil organic carbon decreased significantly with depth across soil orders ( $p < 0.05$ ) with soil C concentrations declining between 40 and 90% of total SOC from the top to the bottom of the profile. Ultisols had the steepest decline in soil C concentrations with depth, followed by Alfisols and Oxisols while Inceptisols and Mollisols had the most even depth distributions. Soil iron (Fe) content was a significant predictor of soil C concentration, such that soils with larger Fe concentrations had greater C % (fig. 3) ( $p < 0.05$ ). Soil K and Zn concentrations were also found to be predictors of C concentrations where higher abundances of K or Zn had higher C % (fig. 3).

### **Soil $^{13}\text{C}$ NMR Chemical Shift Regions**

Across sites, SOC was predominantly composed of alkyl C (e.g., waxes and other lipids), aromatic C (e.g., lignin) and o-alkyl C (e.g., cellulose and other carbohydrates) (fig. 3) (biomarker examples taken from Li et al 2015). The abundance of these biomolecules was most commonly predicted by soil order, whereas depth and MAP were not often significant predictors.

Soil order was found to be a significant predictor of alkyl, O-alkyl, and phenolic C abundance. Spectra showed differences between soil orders with less weathered soils (Mollisols) showing larger peak areas in the 110-215 ppm range and more weathered soils (Oxisols)

showing larger peak areas in 0-110 ppm ranges (fig. 4, 5, 6). Post-hoc tests revealed the following patterns among soil orders. First, alkyl C varied significantly among soil orders, with the lowest abundance in Inceptisols and the highest abundances in Oxisols and Ultisols (fig. 7). O-Alkyl C also varied with soil order, with the highest abundances in Ultisols and the lowest in Inceptisols. Aromatic C however showed no significant changes in abundance between soil orders. Di-O-alkyls (hemicellulose and other carbohydrates) were more abundant in Ultisols and least abundant in Mollisols and Alfisols. Phenolic C (acids, tannins) showed significant variation among soil orders as well and were more abundant in Mollisols and had lower abundance in Oxisols. The amide/carboxyl (proteins, peptides, hemicellulose) functional group displayed higher amounts in Mollisols and lower amounts in Inceptisols, Oxisol, and Ultisols (fig. 8). Lastly, ketones (degradation product) were not significantly different between soil orders.

Depth was also a significant predictor of some biomolecules (fig.9). Shallower soils had significantly greater abundances of N-alkyl/methoxyl and alkyl C, while deeper soils contained larger amounts of ketone C (fig. 10). Ketone C is likely associated with carbonyl compounds and can be a byproduct of decomposer activity, specifically the microbial degradation of fatty acids (Gillespie et al. 2014; Wang et al 2017; Anokhina et al 2017). The ratio of alkyl: O-alkyl C, which is an index of increasing decomposition with higher values corresponding to more degraded SOC (Baldock and Preston 1995; Almendros et al 2000; Preston et al 2009), significantly increased with depth (fig. 11).

Results from the forward stepwise model building highlighted the importance of soil P as an aspect of soil fertility related to SOC biomolecules. Resin extractible phosphorus (resin P) stocks to 1 m was found to be a significant predictor of O-alkyl C in all soils ( $p < 0.05$ ) with higher resin P concentrations related to lower proportions of alkyl C (fig. 11A). Resin P to 1 m

was also a significant predictor of aromatic C (fig. 11B) and amide/carboxyl C (fig. 11C). The Fe content was also a significant predictor of amide/carboxyl C with amide/carboxyl proportions decreasing with increased Fe concentrations ( $p < 0.05$ ). Di-O-alkyl/alkene C proportions tended to decrease with resin P concentration ( $p = 0.07$ ), but no significant predictors were identified (fig. 10D). Nitrogen content was the only predictor of n-alkyl C ( $p < 0.05$ ).

### **Molecular Mixing Model**

Results from the molecular mixing model were generally less clear than statistical analyses of the chemical shift regions directly. Lignin was the most prominent component of SOC from the mixing model, followed by proteins and carbohydrates, with lipids and carbonyl making up the smallest percentage of most soils (fig. 12). Initial ANOVA tests showed no significant relationships between molecular component abundance and MAP, depth, or soil order. Post-hoc tests showed that carbonyl and carbohydrate abundances declined with depth ( $p = 0.07$ ,  $p = 0.10$ ). A stepwise model building approach identified resin P as a significant predictor of molecular components. Post-hoc tests found that lignin abundance increased with increasing resin P concentrations ( $r^2 = 0.24$ ) while carbohydrates decreased significantly with resin P ( $r^2 = .18$ ) but these relationships were weak. Resin P was also a significant predictor of lipid abundance, which declined with increasing resin P concentrations ( $r^2 = .25$ ). No predictors were identified for protein components.

### **$^{14}\text{C}$ Radiocarbon**

$\Delta^{14}\text{C}$  values became increasingly negative (indicative of older C) with depth, and soil order was also a significant predictor. The largest differences in overall profile  $\Delta^{14}\text{C}$  was

between Mollisols and Inceptisols (fig. 13). MAP was not significant in initial ANOVA tests. The stepwise model identified C %, K %, DOC, and N % as significant predictors of  $\Delta^{14}\text{C}$  across depths (fig.14).

Overall, Mollisols were younger than other orders and Oxisols were oldest (fig. 12). Post-hoc tests showed that  $\Delta^{14}\text{C}$  decreased significantly with increasing depth (fig. 15). Additionally,  $\Delta^{14}\text{C}$  increased significantly with increasing K concentrations (fig. 16) and N concentrations. Dissolved organic carbon was not found to be a significant predictor of  $\Delta^{14}\text{C}$ .

Post hoc tests were conducted to determine predictors of  $\Delta^{14}\text{C}$  at each depth increment. Al concentration was a significant predictor of  $\Delta^{14}\text{C}$  at 0-10 cm (fig. 17). Extractable DOC and Alkyl C concentration were significant predictors of  $\Delta^{14}\text{C}$  at 25-50 cm with increasing DOC corresponding to older C and higher Alkyl abundances displaying younger C (fig. 18).

### **$^{13}\text{C}$ NMR Data as Predictors of $^{14}\text{C}$ Age**

Comparing within depths, there were no significant relationships between  $\Delta^{14}\text{C}$  and biomolecules alkyl/O-alkyl ratios, or Cox. However, surface soil biomolecular composition was related to the radiocarbon age of deeper soils, suggesting an effect of SOC inputs at the soil surface on deeper SOC storage. The biomolecular proportions at 0-10 cm depth were compared using linear regressions to  $\Delta^{14}\text{C}$  values from each depth increment. Both alkyl and O-alkyl abundance at 0-10 cm had a significant positive relationship with  $\Delta^{14}\text{C}$  values at 25-50 cm, while aromatic and phenolic SOC at 0-10 cm had significant negative relationships with  $\Delta^{14}\text{C}$  values at 25-50cm (fig. 19). That is, more aromatic and phenolic C corresponded to older SOC at depth.

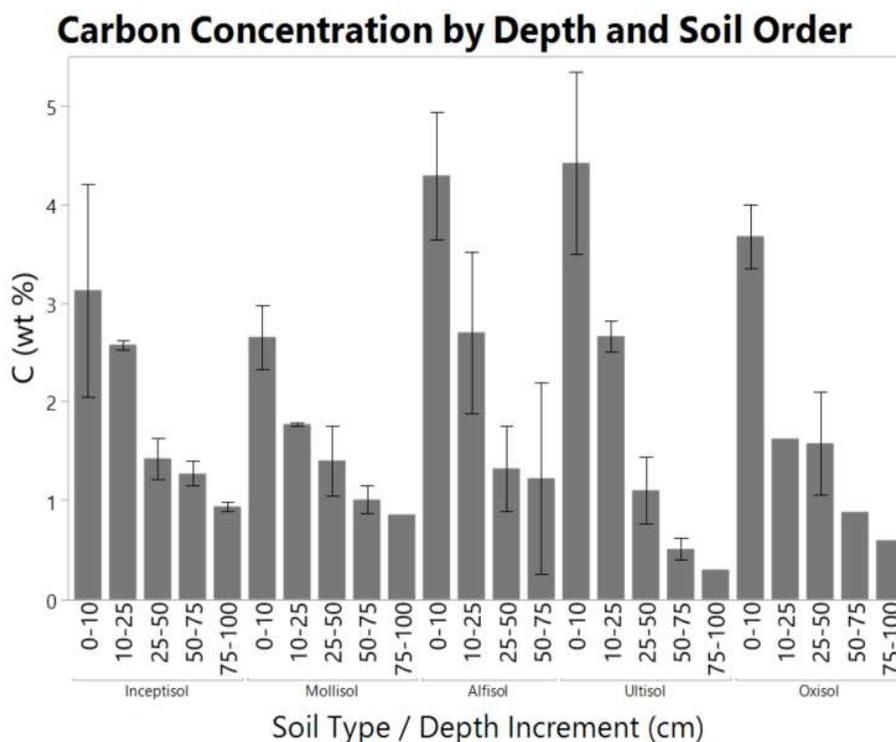


Figure 3. Soil C concentrations by soil order and depth. Soil C concentrations significantly decreased with depth for all soil orders. Soil order so no significant relationship with C concentration. N = 43

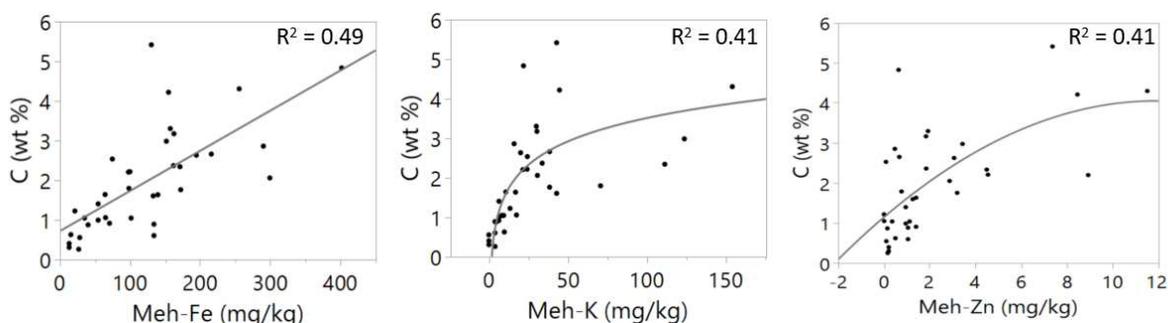


Figure 4. Iron concentration was found to be a significant predictor of carbon concentration at all depths ( $p < 0.05$ ). Carbon concentrations were found to increase as Fe abundance increased linearly where  $C \text{ (wt \%)} = 0.71 + 0.010 * \text{Meh-Fe}$ . Carbon concentration increased with meh-K on a logarithmic scale where  $C \text{ (wt \%)} = -0.42 + 0.85 * \text{Log}(\text{Meh-K})$ . Lastly, C concentration increased with Meh-Zn along a quadratic curve where  $C \text{ (wt \%)} = 1.25 + 0.40 * \text{Meh-Zn} - 0.02 * (\text{Meh-Zn} - 2.18)^2$ . Type of regression was determined by best fit as assessed by the  $r^2$  value. N = 44 for each analysis.

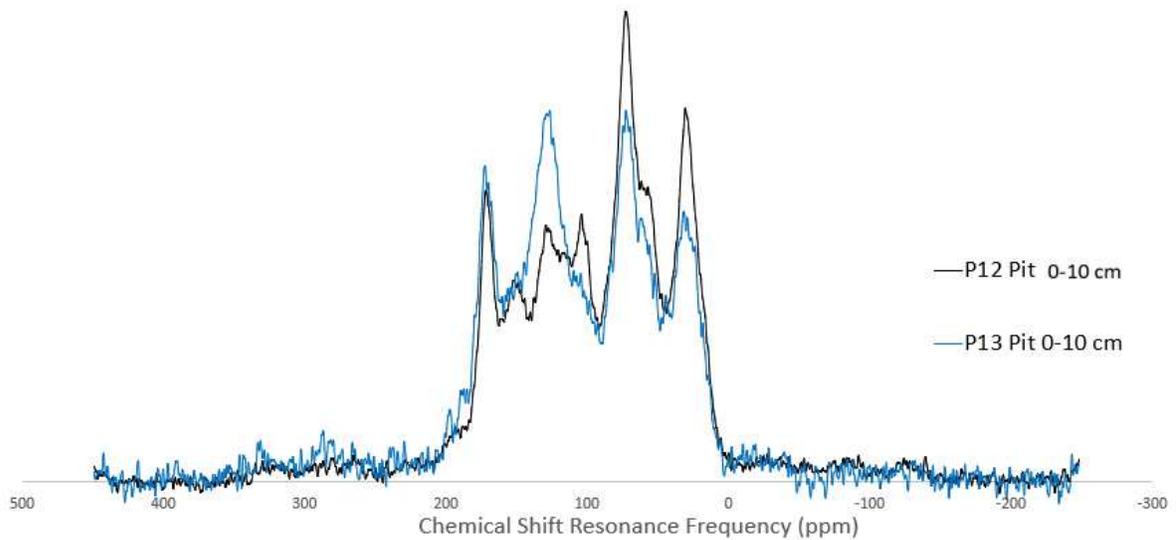


Figure 5.  $^{13}\text{C}$  NMR spectra for 0-10 cm at sites P12 (infertile Inceptisol) and P13 (fertile Alfisol). The infertile spectra show larger peak areas in the 0-110 ppm range (alkyl, N-alkyl, O-alkyl, and di-O-alkyl C) while the fertile soil spectra contain higher peak areas in the 110-215 ppm (aromatic, phenolic, and carboxyl C) range.

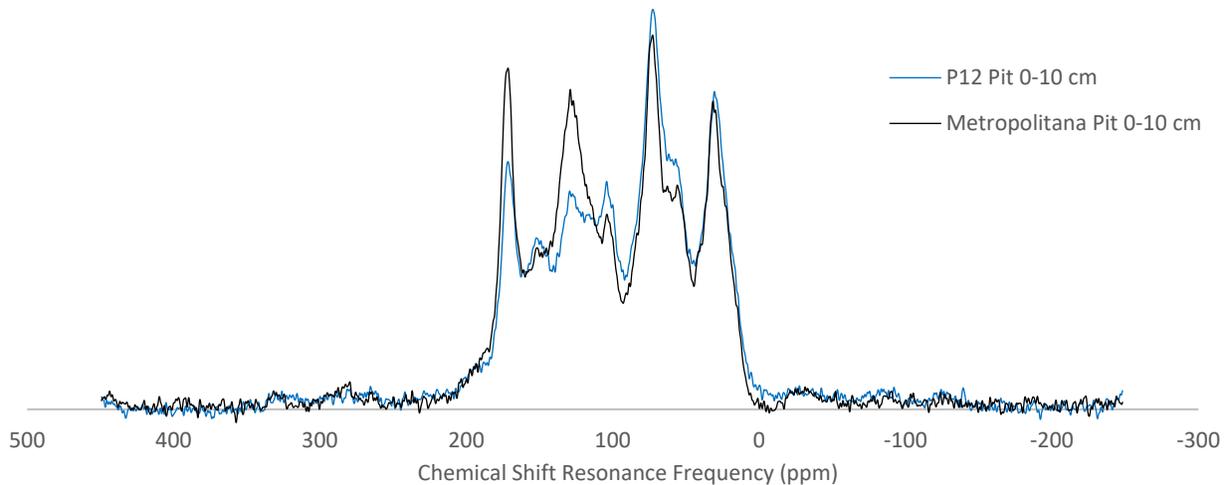


Figure 6.  $^{13}\text{C}$  NMR spectra for 0-10 cm for sites Metropolitana (fertile Mollisol) and P12 (infertile Inceptisol). The Mollisol shows a higher abundance of functional groups in the 95 - 215 ppm (Di-O-alkyl, aromatic, phenolic, carboxyl, and ketone C) range while the Inceptisol shows larger peak areas in the 0 - 95 ppm (Alkyl, N-alkyl, and O-alkyl C) range.

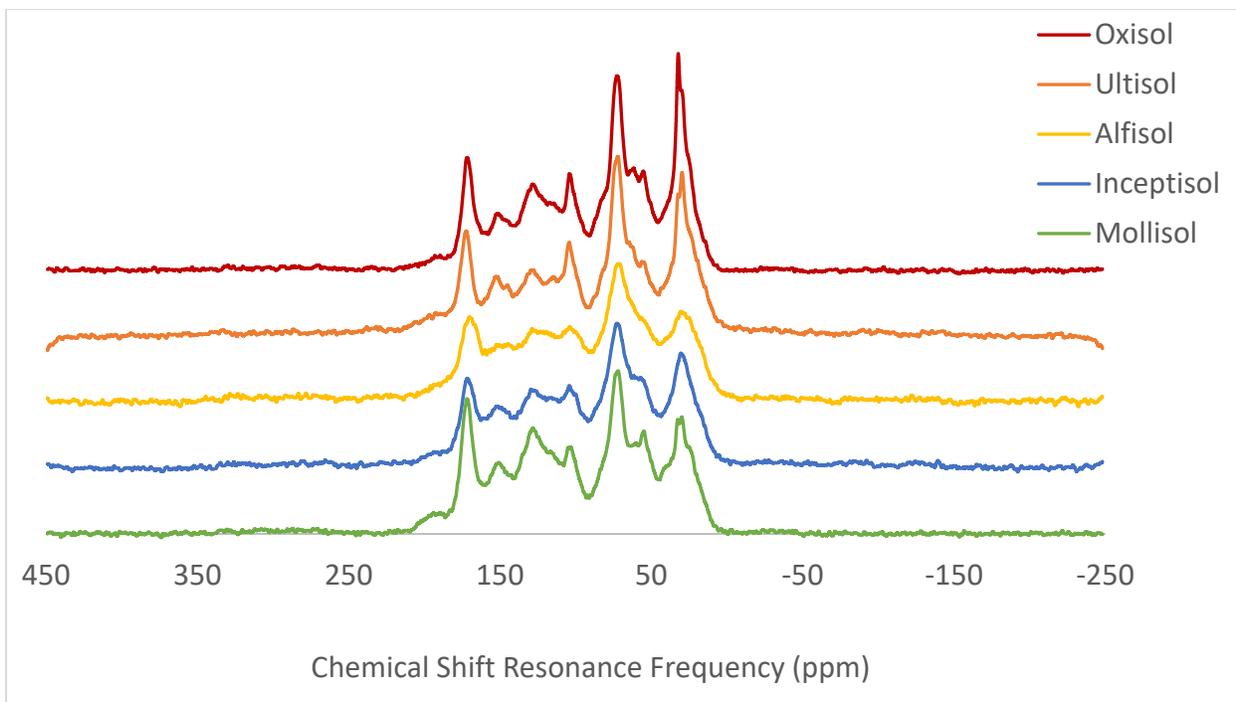


Figure 7. NMR spectra for each soil order. All samples above are taken from depth intervals 0 - 10 cm where the Mollisol is from site Metropolitana, Inceptisol from site P12, Alfisol from site P13, Ultisol from site Albrook, and Oxisol from site ANAM.

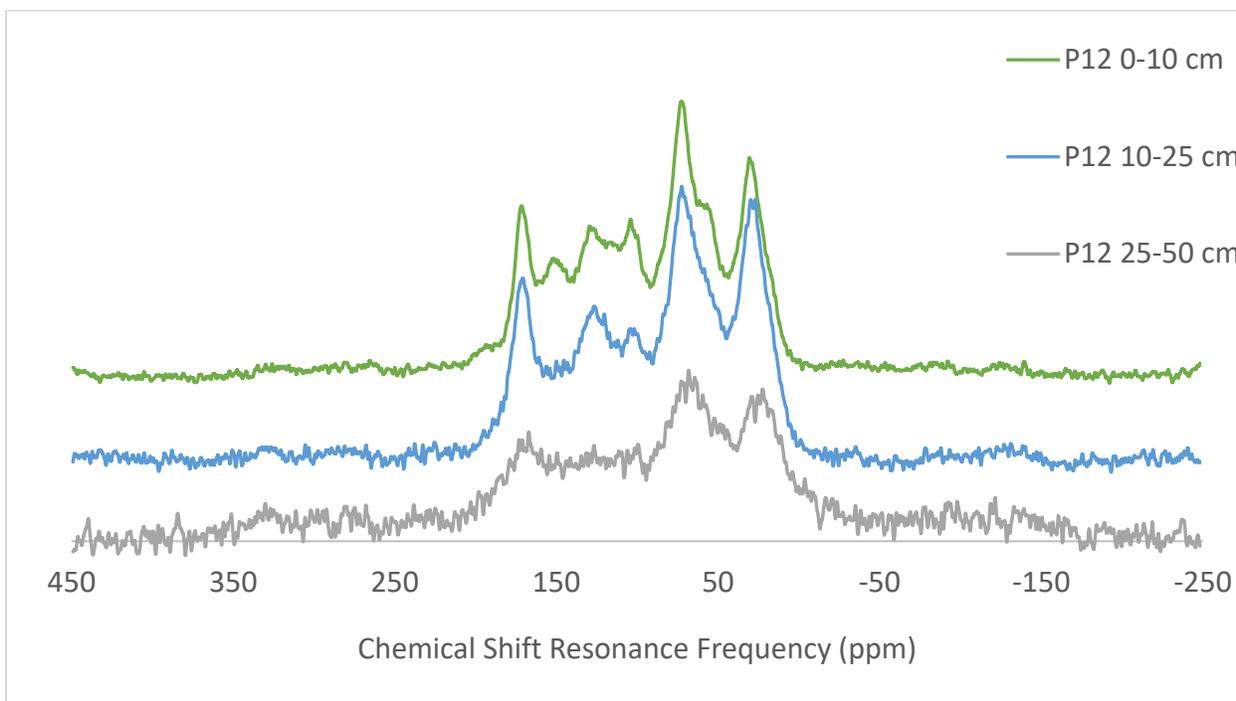


Figure 8. NMR spectra from site P12 with depths 0-10 cm, 10-25 cm, and 25- 50 cm. Ketone region shows a slight increase with depth and alkyl/O-alkyl ratios increase with depth.

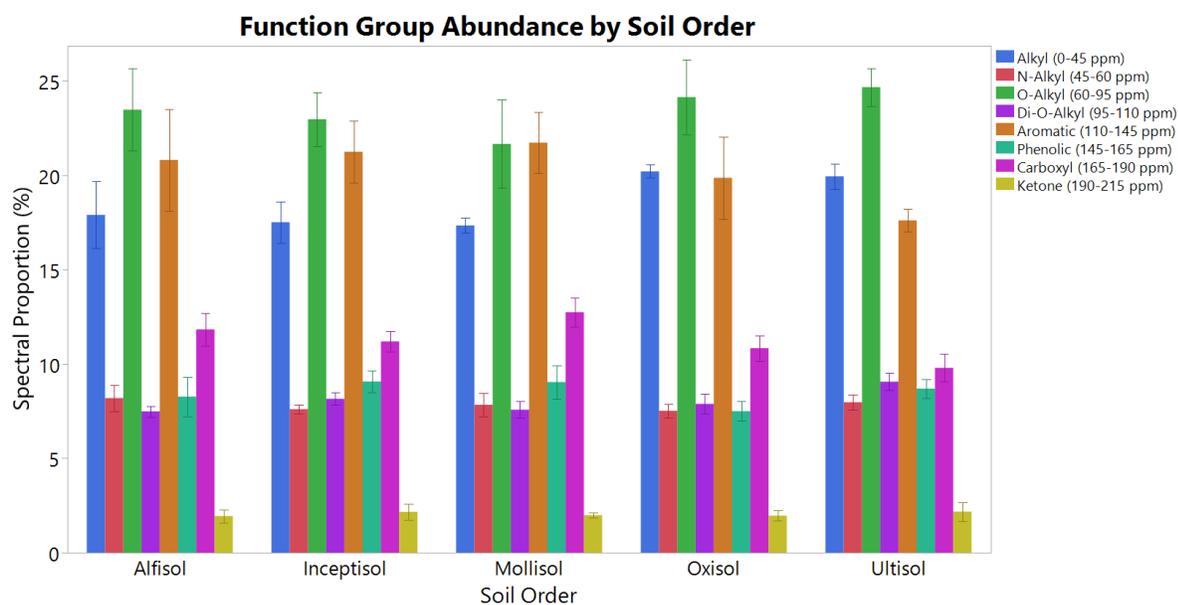


Figure 9. Spectral proportions of chemical shift regions by soil order. Thus, strongly weathered soils (Ultisols and Oxisols) had greater abundances of alkyl C, while less weathered soils (Inceptisols and Mollisols) contained greater quantities of amide/carboxyl C (fig. 7). N = 23

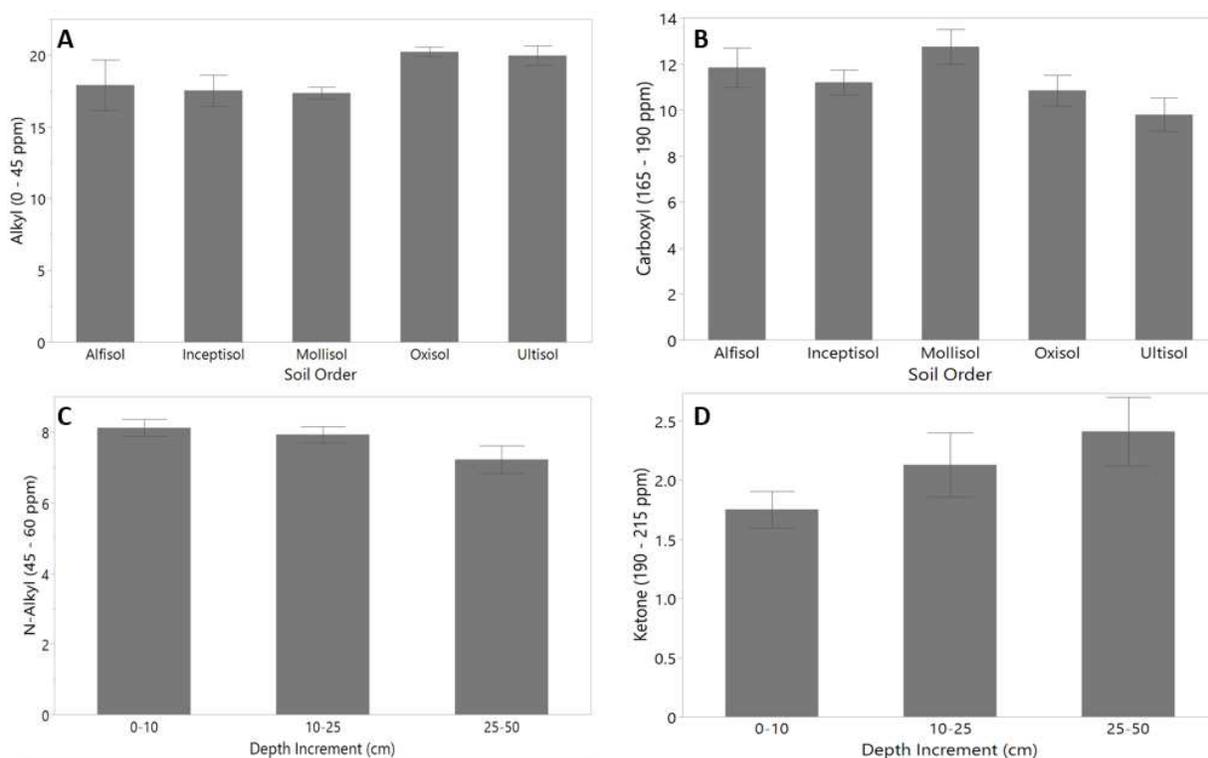


Figure 10. Variation in A) alkyl and B) carboxyl C between soil orders and variation in C) N-Alkyl (protein) and D) Ketone C with depth. N-alkyl/methoxyl groups varied significantly with depth with higher abundances in 0-10 cm compared to the rest of the profile while ketones showed increasing abundance with depth. N = 10 for Inceptisols, n = 9 for Mollisols, n = 11 for Alfisols, n = 12 for Ultisols, and n = 9 for Oxisols.

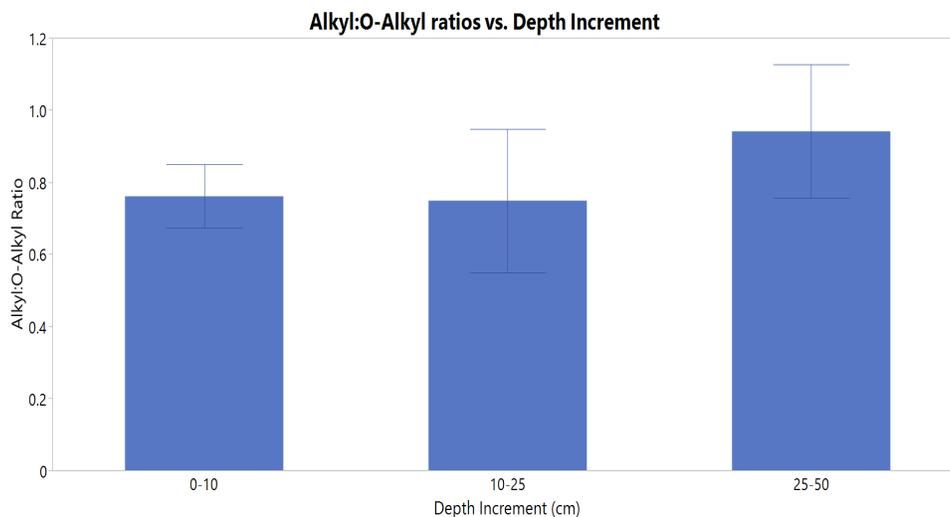


Figure 11. Alkyl/O-Alkyl ratio, a proxy for soil degradation status, with depth. Increasing alkyl to O-alkyl ratios correspond to more heavily degraded organic C. N = 11 for depth 0-10 cm, m = 4 for 10-25 cm, and n = 8 for 25-50 cm.

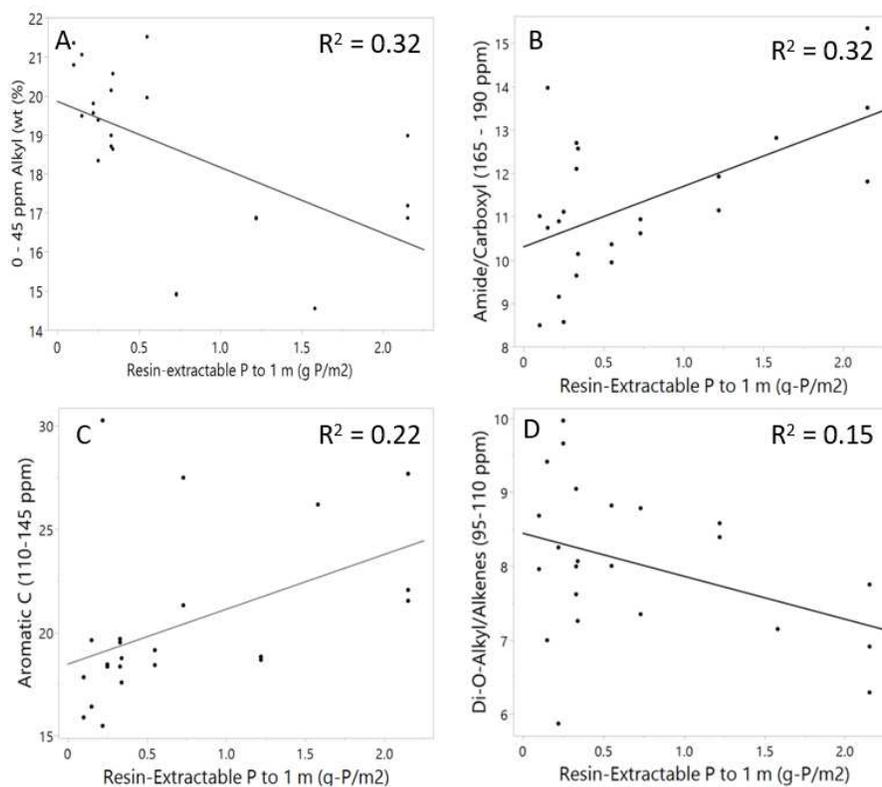


Figure 12. Resin-extractable P was the aspect of soil fertility most commonly related to SOC biomolecular composition. Resin P was a significant predictor of A) alkyl (n = 23), B) amide/carboxyl (n = 23), C) aromatic (n = 23), and D) Di-O-alkyl functional groups (n = 23) (all p < 0.05 except Di-O-alkyl/alkene where p = 0.067).

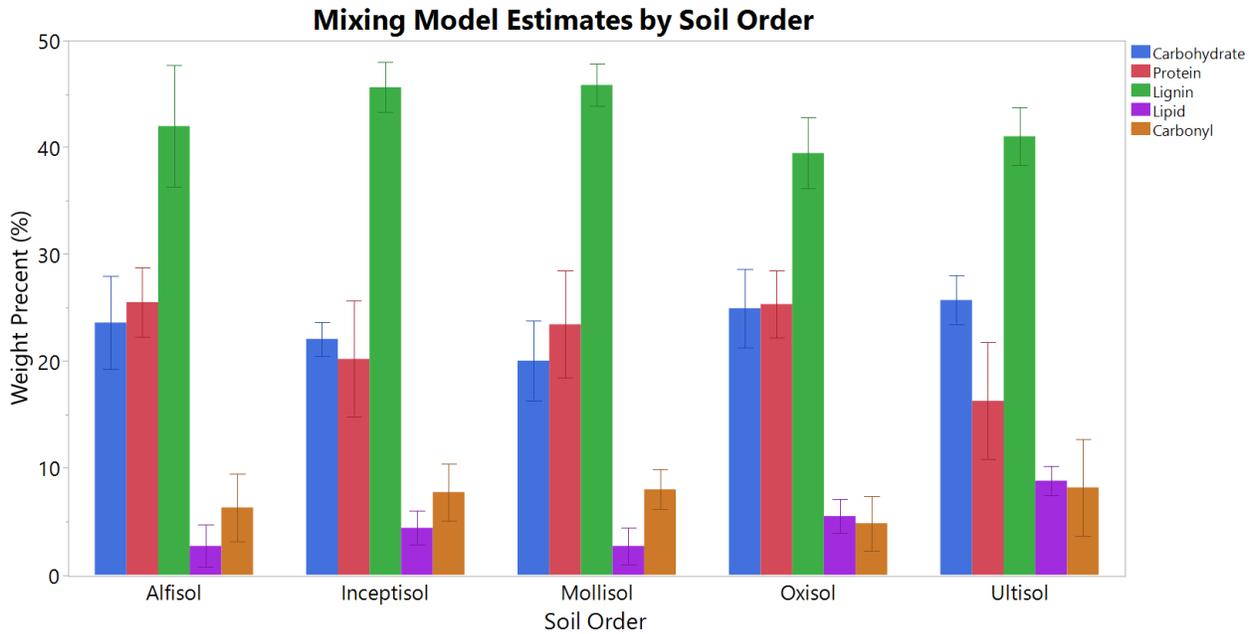


Figure 13. Molecular component composition with soil depth and order. Lignin, protein, and carbohydrates were most abundant across soil order and depth while lipids and carbonyl were estimated to make up a smaller proportion of total biomolecules. N = 23

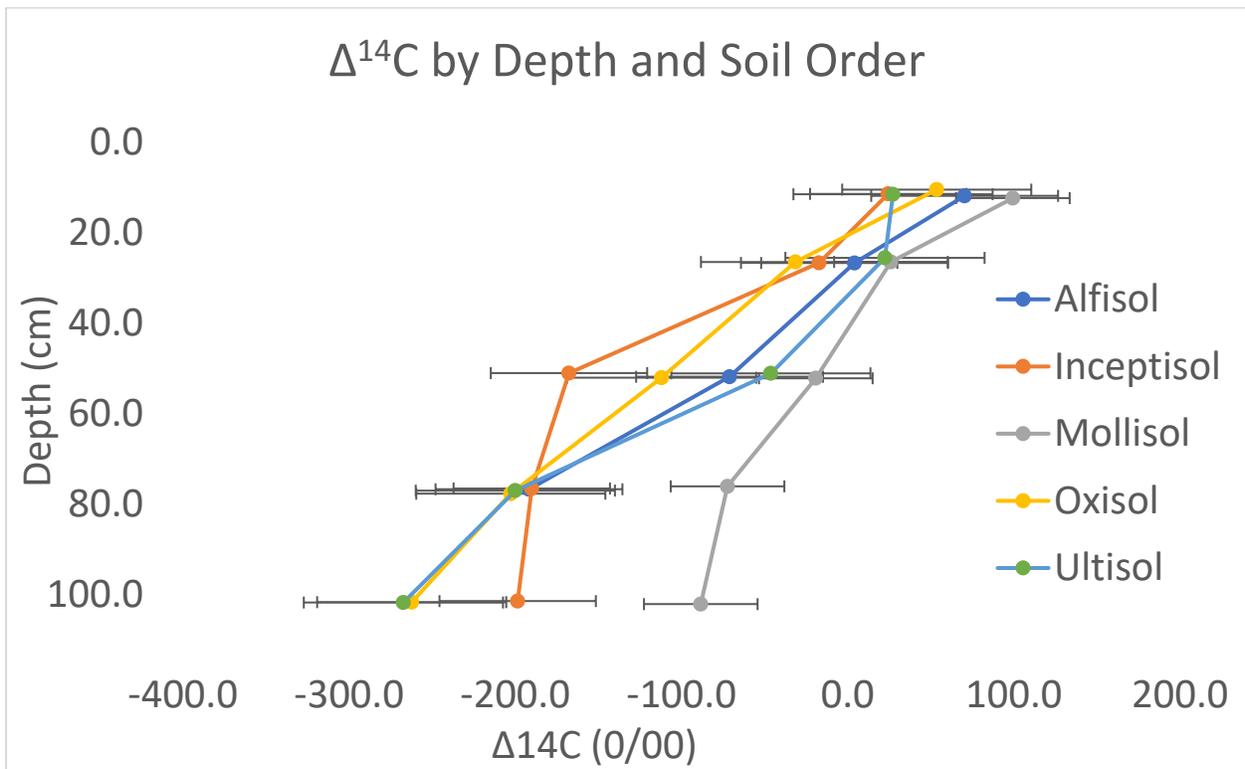


Figure 14.  $\Delta^{14}\text{C}$  by depth for each soil order. Soil order  $\Delta^{14}\text{C}$  values are represented as the mean value of all  $\Delta^{14}\text{C}$  at each depth with error bars showing the standard error. N = 49

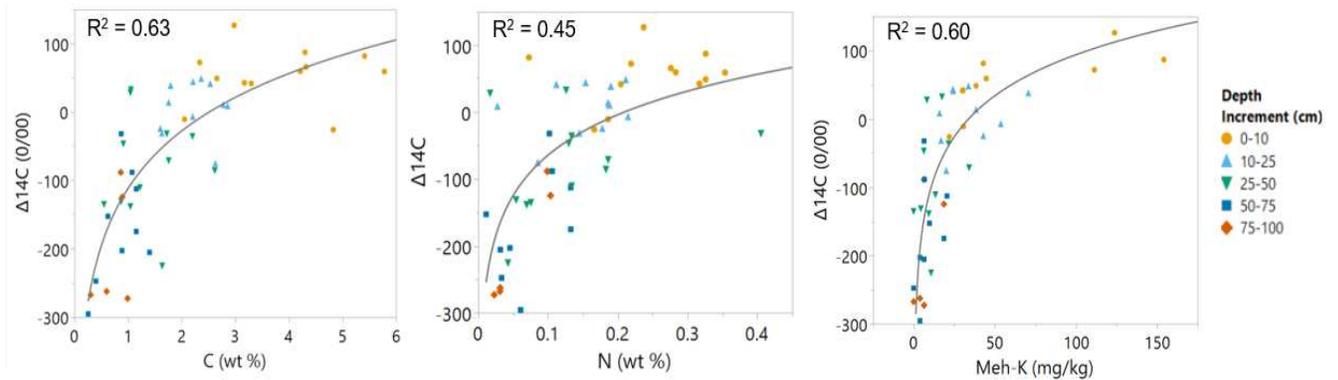


Figure 15.  $\Delta^{14}\text{C}$  with C concentration (left) and nitrogen content (right). The regression line shows a log-linear relationship where  $\Delta^{14}\text{C} = -113.92 + 123.59 \cdot \text{Log}(\text{C (wt \%)})$  and  $\Delta^{14}\text{C} = 150.80 + 89.60 \cdot \text{Log}(\text{N (wt \%)})$  ( $p < 0.05$ ). To the left, regression line shows a quadratic fit between N % and  $\Delta^{14}\text{C}$  where  $\Delta^{14}\text{C} = -157.78 + 972.20 \cdot \text{N (wt \%)} - 3377.26 \cdot (\text{N (wt \%)} - 0.15)^2$ ,  $n = 41$ . Different depths are shown in different marker styles and colors, showing younger C (ages ranging from modern to 140 years old) in more C-rich and N-rich surface soils, and older C in more C-poor and N-poor deep soils (ages ranging from 185 years old to 2740 years old).

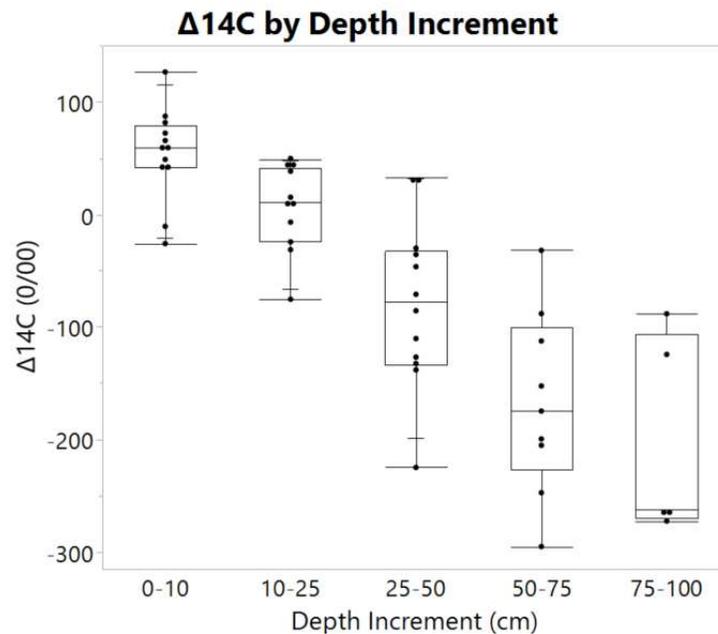


Figure 16. Quantile boxplot with  $\Delta^{14}\text{C}$  by depth increment (0-10 cm  $n = 13$ , 10-25 cm  $n = 11$ , 25-50 cm  $n = 13$ , 50-75 cm  $n = 9$ , and 75-100 cm  $n = 5$ ).  $\Delta^{14}\text{C}$  declined significantly with depth across soil orders.

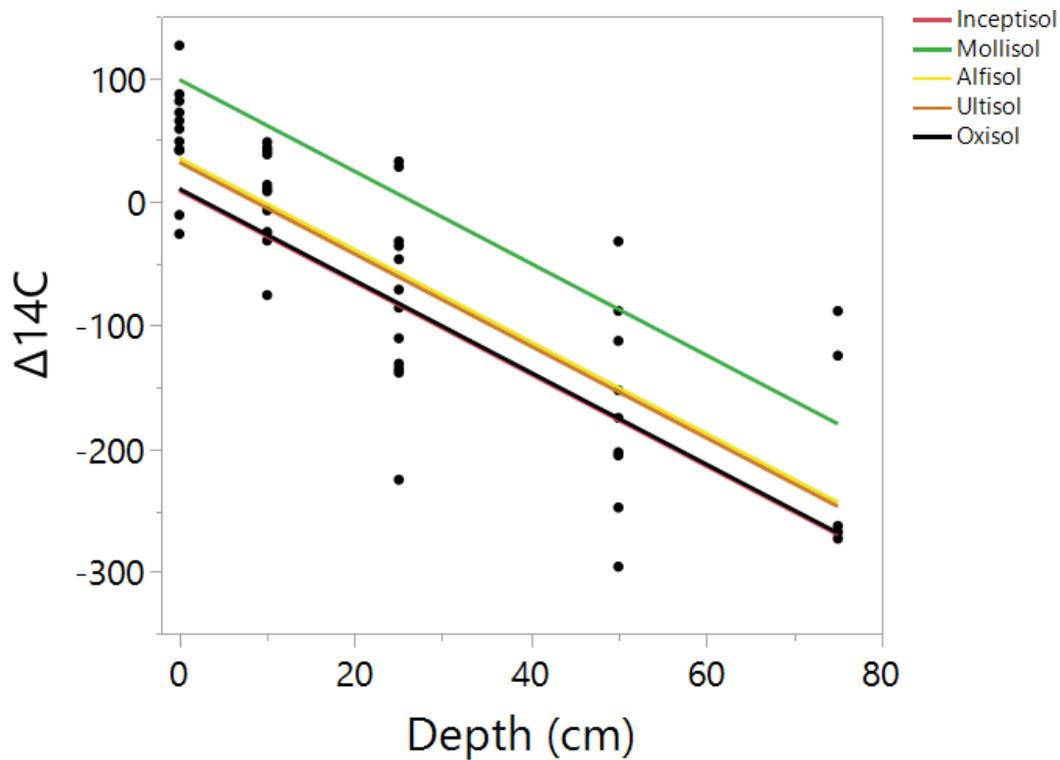


Figure 17. Initial ANOVA indicated that soil order and depth were significant predictors of radiocarbon age, with not interaction. Mollisols were the youngest, while Oxisols were the oldest.

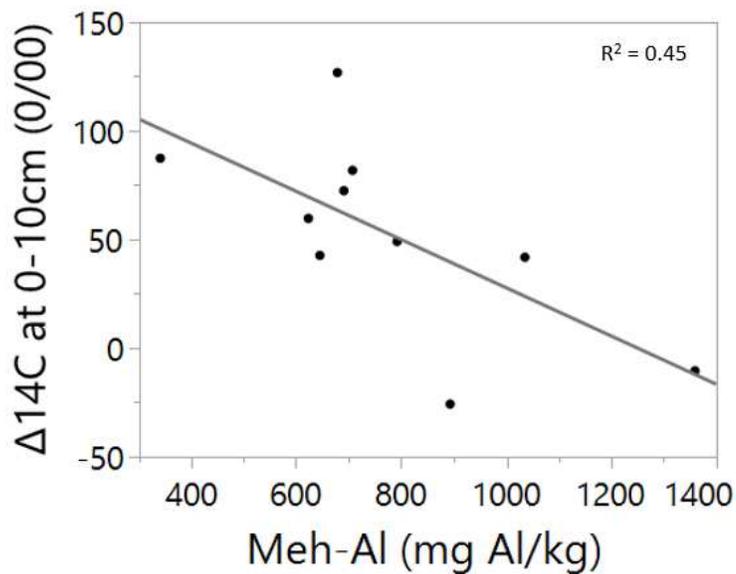


Figure 18.  $\Delta^{14}C$  at depth 0-10 cm was significantly correlated to meh-Al (mg Al/kg). N= 10

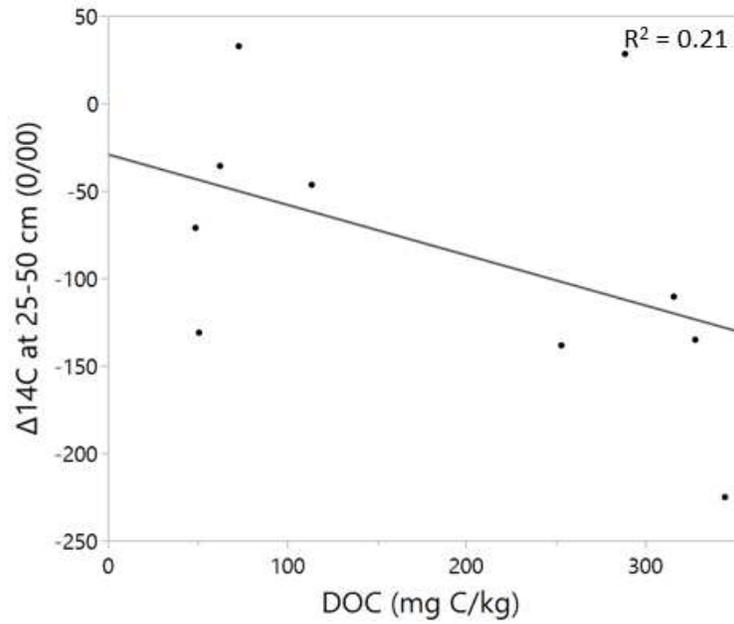


Figure 19. Dissolved organic carbon was significantly correlated with  $\Delta^{14}\text{C}$  at 25-50 cm. N = 10

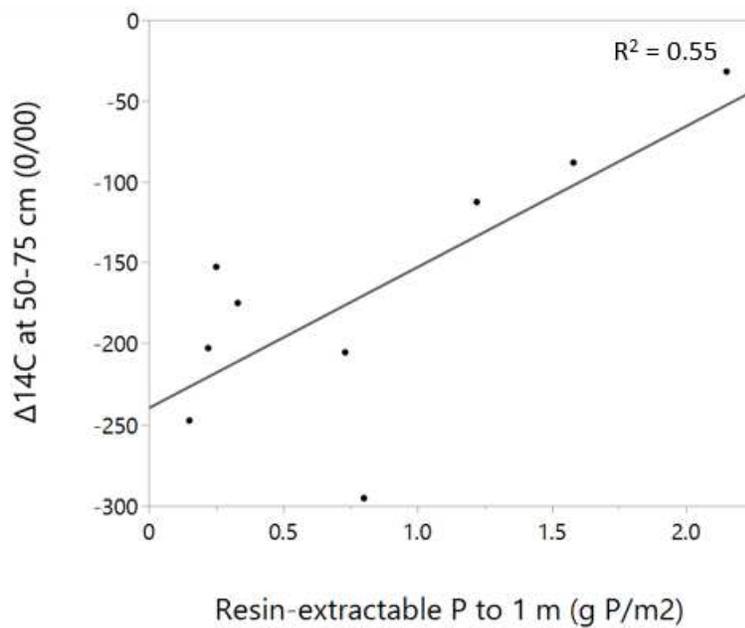


Figure 20. Extractable-resin P was significantly correlated with  $\Delta^{14}\text{C}$  at 50-75 cm. N = 9

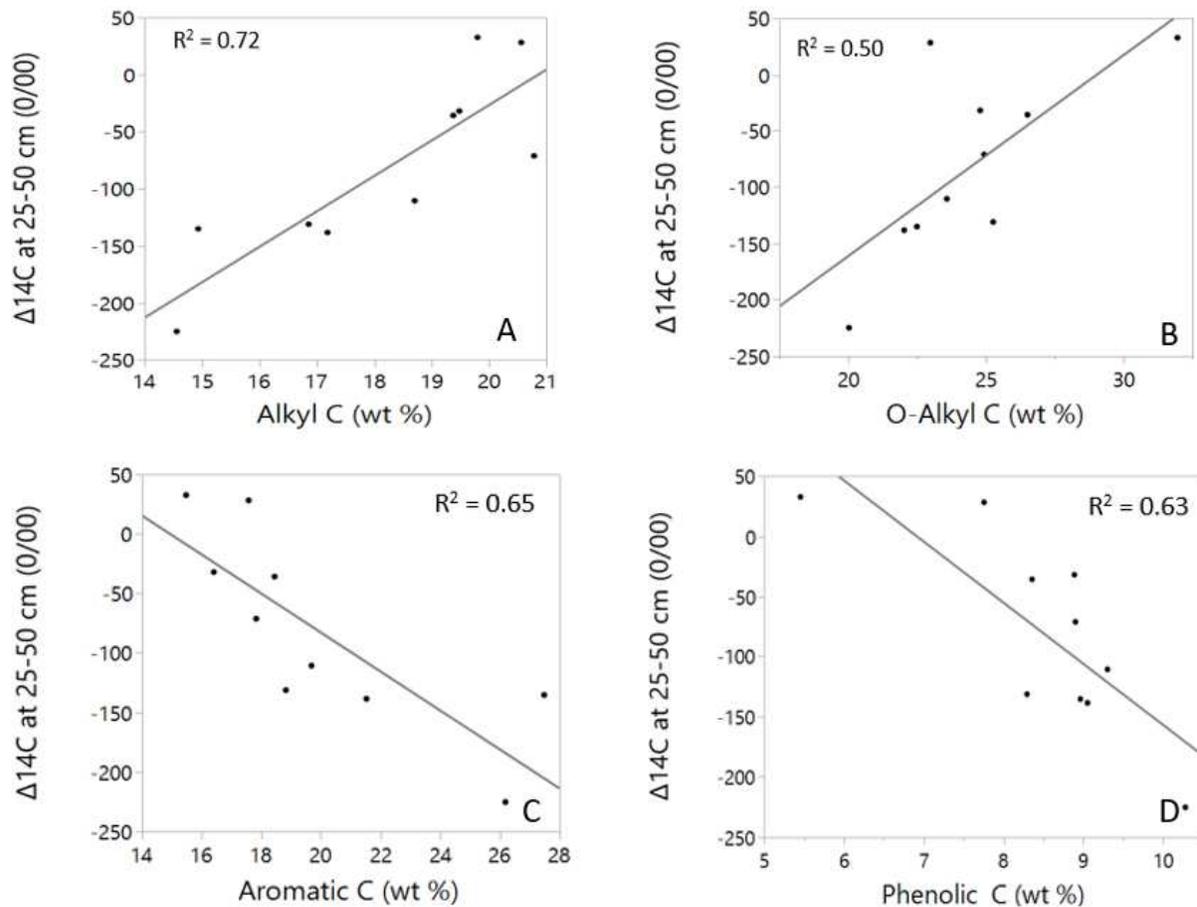


Fig. 21. Significant relationships between surface soil biomolecular composition (0-10 cm) and deeper SOC age (25-50cm).  $\Delta^{14}\text{C}$  values increased representing younger C with increasing A) alkyl C ( $n = 10$ ) and B) O-alkyl C ( $n = 10$ ) while  $\Delta^{14}\text{C}$  values decreased, representing older C with increasing abundance of C) aromatic C ( $n = 10$ ) and D) phenolic C ( $n = 10$ ).

## CHAPTER FOUR: DISCUSSION

### **Tropical Forest $^{13}\text{C}$ NMR Biomolecules**

Our data show clear variation in  $^{13}\text{C}$  NMR spectral regions across soil orders and by depth, but not with rainfall, while using our data in a molecular mixing model did not illuminate these trends.  $^{13}\text{C}$  nuclear magnetic resonance (NMR) has been widely used to describe soils in various ecosystems (Kogel-Knabner and Hatcher 1989; Baldock et al 1992; Kogel-Knabner 1997; Schmidt et al 1997; Baldock et al 2004; Simpson and Simpson 2012; Koarashi et al 2012; Marin-Spiotta et al 2014; vandenEnden et al. 2018). However, few studies focus on the biomolecular composition at depth in tropical soils. Additionally, molecular mixing models have been utilized to predict biomolecular components of environmental tissues from C functional group peak areas given in  $^{13}\text{C}$  NMR (Baldock et al 2004). In our study, however, there was poor fit of the MMM for our spectral areas, suggesting that the traditional MMM used for many temperate ecosystems might not be as adequate in tropical forests.

### *Soil Order Effects on $^{13}\text{C}$ NMR Chemical Regions*

Soil organic carbon molecular composition has been found to vary with soil order in various globally. This is largely found to be a result of differences in the textural composition of the soil with more variation in C composition observed in the clay fraction of soil (Kogel-Knabner and Amelung 2021). Additionally, more developed soil orders such as Oxisols have been found to have higher alkyl C abundance compared to less developed soils such as Mollisols, as shown in a study which investigated temperate, tropical, and Mediterranean climates in Canada and China. (Baldock et al 1992). Differences in molecular composition between soil

order was largely attributed to variation in decomposition of organic material and differences in input chemistry (Baldock et al 1992). However, there have been few studies investigating the differences between soil order molecular composition in the tropics. A comparison two soils with similar parent material, a Terra Preta soil from tropical forests in Brazil with an Oxisol from Mexico, showed that the tropical Terra Preta soil had a higher abundance of carboxyl, amide, and aromatic C compared to the Oxisol (Zech et al 1990). Terra Preta soils are an anthropogenically created soil type specific to the amazon characterized by a dark, thick A horizon that tend to contain high char contents (Kogel-Knabner and Amelung 2021). Variation in the molecular composition between the Terra Preta and Oxisol was attributed to increased “humification” (decomposition) of organic matter in the Terra Preta soils due to an abundance of nutrient rich organic material resulting in the high aromaticity of SOC compared to the Oxisol (Zech et al 1990). Our results showed that the largest variations in SOC molecular composition were between Mollisols and Oxisols, with Mollisols having lower abundances of alkyl and di-O-alkyl C and higher abundances of carboxyl C than Oxisols. The higher abundance of Alkyl C in Oxisols is consistent with studies mentioned above and may be indicative of higher decomposition rates in the Oxisols compared to the Mollisols.

#### *Soil Depth Effects on <sup>13</sup>C NMR*

Our <sup>13</sup>C NMR results suggest increased degradation of SOC with depth. Increasing alkyl/O-alkyl ratios with depth indicating increasingly degraded SOC with depth (Preston et al. 2009). This ratio is based on the observed increase in alkyl C compared to O-alkyl C with decomposition of organic material. Additionally, we saw increases in the proportion of ketone C with depth, which has been used as a proxy for the microbial-transformed SOC, specifically,

ketone C abundance may be interpreted as evidence of the degradation of fatty acids within the soil by microbes (Gillespie et al., 2014). Thus, we appear to have greater microbial processing of C with depth, which influences the organic chemistry of SOC.

More broadly, the vertical distribution of SOC has been widely studied, with C concentrations generally decreasing with depth. Previous studies in Panama have shown trends of decreasing C concentration with depth in more fertile soils across the isthmus, although C stocks increased in subsoils of strongly weathered soils (Cusack et al 2018b). Here, we saw general declines in C concentration decreases with depth.

### *MAP Effects*

Precipitation effects on SOC pools have been widely investigated. One study across a range of tropical forests found that litter decomposition rate increased with MAP (Cusack et al. 2009), indicating while others have reported decreased decomposition with increased MAP (Schoor 2001). While our study did not directly test litter and root tissue, we found no relationship between SOC decomposition and MAP (as determined by alkyl/O-alkyl ratios). Precipitation has been found to have a significant relationship with mineral C storage in tropical forests in the Yucatan Peninsula with lower precipitation study sites showing larger SOC storage (Campo and Merino 2016). <sup>13</sup>C NMR from this study showed that lower MAP sites had larger differences in chemical composition between soil types (specifically Lithosols and Rendzinas) compared to similar soils at moderate or high MAP (Campo and Merino 2016). In our study, MAP was not a significant predictor of any C functional group further suggesting soil nutrients (specifically resin P) dominate C variation in the area.

### *Other Ecosystem Drivers*

Soil organic carbon composition is controlled by litter and root inputs as well as microbial decomposition and. As mentioned, NPP is consistent across t, and vegetation species composition varies with soil P and soil moisture (Cusack et al 2019; Umaña et al 2021). We found that soil P was a strong predictor of biomolecule abundance which may be related to plant species composition changes in the region. Past studies in this region have noted that soil respiration increases with increasing soil P. This may also contribute to the relationship between resin P and biomolecular abundance we observed as molecular components that show decreasing abundance with increasing resin P may be more likely to be degraded by microorganisms whereas biomolecules that display positive relationships with resin P may be more resistant to decomposition.

Root inputs have an impact on SOC composition and have been widely hypothesized to contribute more to SOC and result in more stable C overtime (Rasse et al 2005). Other studies in the region have noted a positive relationship between fine root abundance and C stocks in subsoils where a higher abundance of fine roots at the surface corresponded to larger C stocks at depth (Cusack and Turner 2020). Our results showed no relationship between fine root abundance and biomolecule abundance. This may indicate that root and shoot inputs in this system contain similar C functional groups or that  $^{13}\text{C}$  NMR is not an adequate tool to distinguish differences in these types of inputs.

Microbial activity influences SOC molecular composition through the transformation of C and through stabilization of SOC. One study, with samples from prairie grasses, concluded that microbial biomass contributed >50% of extractable DOC and ~45% of humin fractions (Simpson et al 2007). Another study focused on long-term C stabilization concluded that, in

subtropical long-term organic amendments sites, organic matter accrued on mineral surfaces primarily as microbial biomass (Courtier-Murias). Using ketone abundance as a proxy for microbial altered/stabilized material (Gillespie et al 2014a; Gillespie et al 2014b), we found that SOC showed increased evidence of microbial alteration with depth. This is consistent with other studies that have documented increased microbial residues with depth (Angst et al 2018; Sokol and Bradford 2019).

### *Molecular Mixing Model in Tropical Forests*

We attempted to use the molecular mixing model as described by Baldock et al (2004) to describe the molecular components present in our soils however, we found that it resulted in a high error and that it was likely, not representative of the SOC molecular composition. This model has been widely used in various environments but is rarely used in tropical settings. Two reasons that this model may not have been applicable in our study include the potential over-emphasis of the char component and peak area assignments.

First, this mixing model is traditionally run as a six-component model with lipids, lignin, carbohydrates, proteins, carbonyl, and char. However, there is no fire regime in Panama and therefore we used the five-component model that does not include char. In the 6-component model, char is primarily estimated using the aromatic and phenolic peak areas. These areas are reassigned in the 5-component model which tended to result in higher lignin and lipid estimates and lower carbohydrate, protein, and carbonyl estimates. This increased the sum of squares drastically indicating that the predicted and the observed integrated peak areas were not a good match.

Additionally, this mixing model was designed using a variety of standard samples. Briefly, carbohydrate peak assignments were determined based on cellulose, protein by amino acid distributions in soil from crop lands, grasslands, and pine, spruce forest soils in Germany and the Netherlands (Friedel and Schell 2002), lignin by spruce and red alder lignin (Wilson 1987), lipids by the structure proposed for cutin by Kolattukudy (1980), char by red pine wood which was heated to 300 C (Baldock and Smernik 2002) and carbonyl was added to account for oxidation of organic biomolecules. While these assignments have worked well in most settings, it appears that tropical SOC does not fall into the same categories. For example, using the 6-component model, charcoal was estimated to represent 7-25% of C in our samples which is extremely unlikely due to the lack of fire regime. Further work is needed to determine the best peak assignments for tropical soils.

### **Tropical Soil $\Delta^{14}\text{C}$ Age**

Soil organic carbon in tropical soils has been documented to be younger than SOC in other ecosystems such as boreal forests, temperate forests, tundra, but this may be due to a lack of data for deeper tropical soils (Shi et al 2020). Our results suggest that SOC within tropical forests in Panama could be up to 2,375 +/- 25 years old indicating longer, more stable C stocks than anticipated. Radiocarbon in tropical forests has primarily been studied in surface soils to assess the turnover time of C in the tropics and the effects of land use changes on SOC.

### $\Delta^{14}\text{C}$ Among Soil Orders

In general, mineral-associated SOC is the oldest, most stable fraction of SOC in most soils (Abramoff et al. 2021). Soil orders have varying clay and iron oxide concentrations leading to varying sorption capacities and mineral-associated C abundance (Mayes et al 2012; Abramoff et al 2021). In general, soil orders with higher clay content have been found to have older C (Mathieu et al. 2015). However, our study found no significant trend between clay content and  $\Delta^{14}\text{C}$  but did find significant differences between  $\Delta^{14}\text{C}$  in Oxisols and Mollisols.

Significant differences in  $\Delta^{14}\text{C}$  between soil orders in our study may be due to differences in soil nutrients and inputs. Higher fertility soils (as indicated by resin P) were found to have younger C which may be indicative of higher amounts of fresh C inputs. Additionally, soil K concentration was found to be a significant predictor of  $\Delta^{14}\text{C}$ . Potassium has been documented as a limiting factor for growth in tropical systems (Cuevas and Medina 1988; Tanner et al. 1998; Baribault et al. 2012). Cusack et al. 2018 found that K % was a strong indicator of root biomass. Our results found that soils with higher K concentrations had younger C (as indicated by radiocarbon) which may be due to increased root C input at depth and higher C concentration related to increased productivity.

$\Delta^{14}\text{C}$  was also not significantly different between Inceptisols, Alfisols, and Ultisols. Inceptisols have been characterized as less developed and thought to contain more primary minerals as defined by the USDA classification (USDA, 1999). However, our results suggest older C inputs and more evidence of weathering. This is likely due to differences between the USGS classification system and soil order characteristics in the tropics.

### *$\Delta^{14}\text{C}$ with Depth*

Depth is a strong predictor of  $\Delta^{14}\text{C}$  globally with deeper soils having older SOC or more negative  $\Delta^{14}\text{C}$  values (Mathieu et al 2015; Shi et al 2020). While most tropical studies have focused on shallower soils, some have noted the relationship between depth and  $\Delta^{14}\text{C}$  (Trumbore et al 1995; Trumbore 2000). Our results are consistent with these findings and found that depth was the best predictor of  $\Delta^{14}\text{C}$  throughout all soil orders. This increase of age with depth may be driven by changes in microbial activity, a decrease in plant inputs, or SOC protection and stabilization through sorption processes (Kramer and Chadwick 2018; Hemingway 2019; Shi 2020).

### *$\Delta^{14}\text{C}$ with MAP*

Some studies have documented a negative relationship between MAP and  $\Delta^{14}\text{C}$  while others noted a positive relationship. It has been hypothesized that MAP is a stronger control on carbon concentration and stock size than C turnover with other factors dominating C age and turnover variation. However, this relationship has not been well documented in tropical systems. One study noted a slight (but insignificant) positive relationship between soil C turnover rates (as determined by  $\Delta^{14}\text{C}$ ) and MAP in seven Neotropical lowland rainforest sites with a range of 2,650 mm to 9,510 mm in MAP (Posada and Schuur 2011) and other studies have noted a positive relationship between soil moisture and  $\Delta^{14}\text{C}$  indicating more moist soils contained younger C (Shi et al 2020). Our results showed that MAP is not a significant predictor of  $\Delta^{14}\text{C}$  or C concentration. This indicates that MAP is not a dominant factor in C storage processes within this environment. This may be because nutrient availability or soil order has a more direct effect on C storage and stability.

### *Other Ecosystem Drivers of $\Delta^{14}\text{C}$*

$\Delta^{14}\text{C}$  can be affected by a variety of other factors such as NPP, root growth, soil respiration, and land use changes (Trumbore 2000; Mathieu et al 2015; Shi et al 2020). Net primary productivity controls soil C inputs and can influence  $\Delta^{14}\text{C}$  values. Increased NPP has resulted in higher  $\Delta^{14}\text{C}$  values due to increased input of modern (post-1950) C (Trumbore 2000). Root growth also provides inputs into the system in the form of root exudates but has also been linked to increased SOC sorption (Rasse et al., 2005; Katterer et al 2011; Poirier et al 2018). Root respiration has been found to contain higher  $\Delta^{14}\text{C}$  values with one study indicating  $\Delta^{14}\text{C}$  values higher than atmospheric values (Schuur and Trumbore 2006). Soil respiration is the measure of C outputs with increasing respiration indicating higher amounts of C being oxidized and released as  $\text{CO}_2$  (Trumbore 2000). Studies have linked higher respiration rates to SOC pools with faster turnover times and higher  $\Delta^{14}\text{C}$  (Trumbore 2000; Nagy et al 2017). Lastly, land use change can disturb the soil and introduce modern more modern C at depth while also exposing older C to increased decomposition and oxidation (Finstad et al 2020; Shi et al 2020).

Other studies have characterized NPP, root growth, and respiration throughout our study region (Turner and Engelbrecht 2011; Condit et al 2013; Cusack et al 2017; Cusack et al 2018a; Cusack et al 2018b; Cusack et al 2019; Cusack and Turner 2020; Dietterich et al 2021). Overall, NPP shows little variation across the isthmus however there are changes in soil respiration with changes in soil moisture and soil resin P (Cusack et al 2018). We found that resin P was only a significant predictor of  $\Delta^{14}\text{C}$  at the 50-75 cm depth increment with increasing resin P resulting in younger C with depth. This may be a result of increased soil respiration in soils with higher resin P where more C is being oxidized compared to lower respiration soils. While root biomass was

not identified as significant in the stepwise model building approach, this may be because depth, C concentration, and soil order accounted for the majority of  $\Delta^{14}\text{C}$  variability.

## **SOC Biomolecular Composition and $\Delta^{14}\text{C}$ Age**

### *SOC Inputs and Transport*

Soil organic matter inputs have been hypothesized to play a large role in SOC composition and age with depth (Rasse et al 2005; Simpson et al 2007; Schmidt et al 2011; Kindler et al 2011; Lajtha et al 2014). Some argue this is due to the chemical recalcitrance of the organic matter while others emphasize the microbial ability to access the SOC (Waring et al 2020).

### *Cascade of Surface Biomolecule Effect on Deep $\Delta^{14}\text{C}$*

Our results suggest there is a relationship between surface SOC composition and age at depth 25-50 cm. We found that four functional groups had significant relationships with  $\Delta^{14}\text{C}$ , where alkyl and O-alkyl C abundance corresponded to younger C while phenolic and aromatic abundance C corresponded to older C. Preston et al 2009 documented similar results with decomposition of litter, where phenolic and aromatic C proportions increased with decomposition indicating selective preservation while O-alkyl decreased. However, they found that alkyl C generally increased with decomposition. Their study used fresh litter that decomposed in polypropene bags which may create variations in decomposition when compared to SOC. Overall, these trends indicate that input chemistry does have some impact on C storage and age with depth.

## Conceptual Model

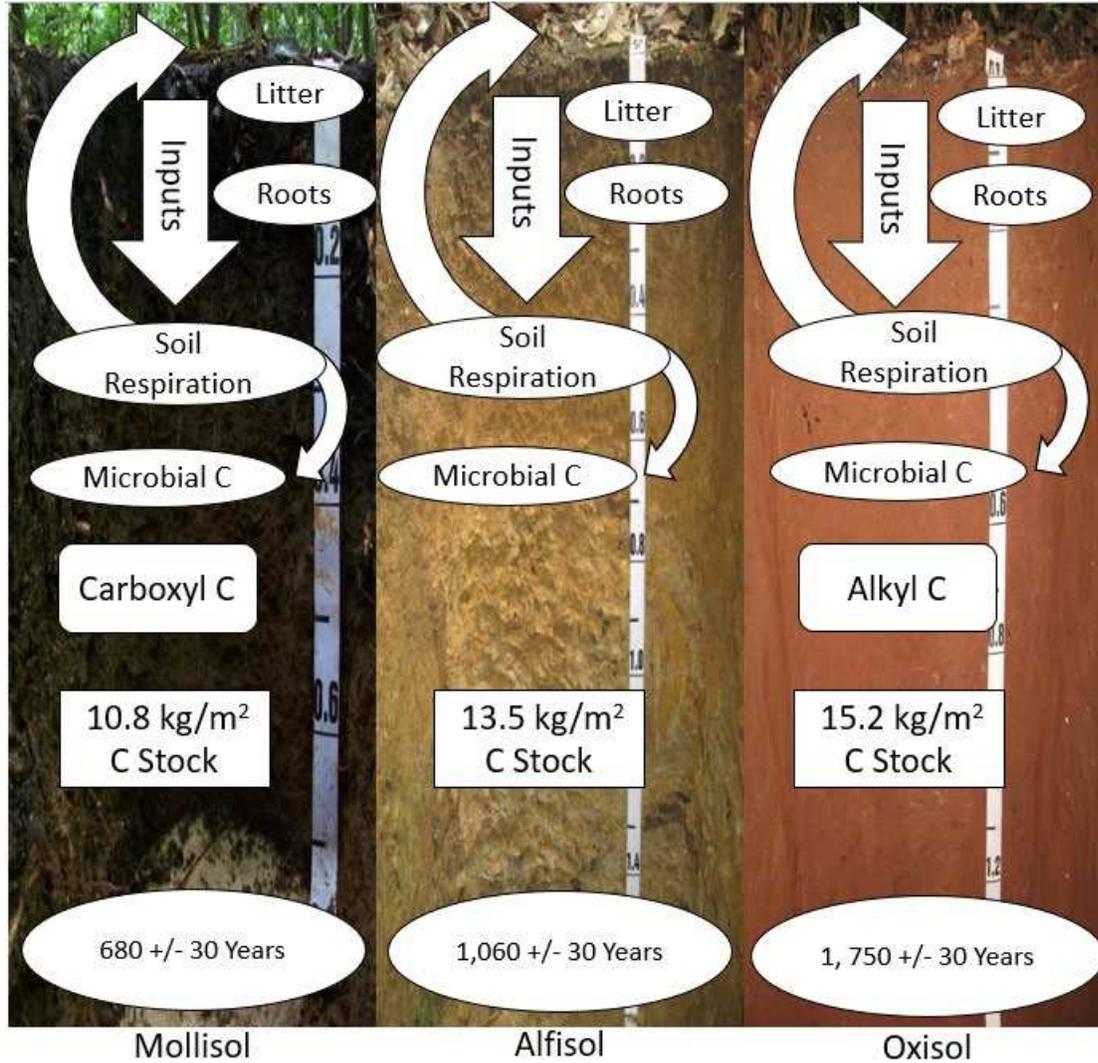


Figure 22. Conceptual model describing C cycling, composition, and age as reported in this study. Mollisols were the youngest soils overall with higher abundance of carboxyl C and smaller C stocks with depth. Oxisols were the oldest soils with higher abundance of Alkyl C and larger C stocks. Alfisols were not significantly different in age or chemical composition from Inceptisols or Ultisols and contained moderate C stock sized and were older than Mollisols but younger than Oxisols. Carbon stock estimates from Cusack et al. (2019) were used to demonstrate variation in overall C stock size. Ages shown are from depths 25-50 cm.

## CHAPTER FIVE: CONCLUSION

Climate change threatens to alter global C cycling through alterations in temperature and precipitation globally. Tropical forests hold large quantities of C in soils, but little is known about how these stocks will be impacted by global change. Our study investigated the impacts of precipitation, fertility, and depth on soil organic carbon molecular composition and age to better characterize the drivers of SOC storage and decomposition in tropical soils. We found that there were no significant relationships between MAP and SOC age or composition but rather, that soil fertility (as indicated by soil P) was a stronger predictor of SOC biomolecules and  $\Delta^{14}\text{C}$ . This suggests that C stock in this region may be resistant to changes in precipitation and possibly more resilient as MAP decreases and dry seasons lengthen in tropical regions. Soil C age was most closely related to depth and C concentration which is consistent with findings in other regions. Soil organic C composition at 0-10 cm was found to be a significant predictor of  $\Delta^{14}\text{C}$  at 25-50 cm. This indicates that input chemistry plays a key role in the long-term storage of C in this region. This could mean that SOC stocks are susceptible to changes with shifts in aboveground vegetation that may occur as the climate in the region changes. Overall, we found that Oxisols had older C stocks indicating that they have the potential to store C longer than less developed soils in the same region and may be less vulnerable to changes in climate. Oxisols make up nearly 60% of soils in this region meaning a large portion of belowground C may be more resilient to changes at the surface. More work is still needed to better characterize the controls on long-term C storage in this region as well as to characterize the molecular composition of SOC.

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