1 *Title:* **Decadal-Scale Litter Manipulation Alters the Biochemical and Physical Character of**

2 **Tropical Forest Soil Carbon**

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Abstract

Climate change and rising atmospheric carbon dioxide (CO_2) concentrations are likely to alter tropical forest net primary productivity (NPP), potentially affecting soil C storage. We examined biochemical and physical changes in soil C fractions in a humid tropical forest where litter manipulation changed total soil C stocks. We hypothesized that: (1.) low-density soil organic C (SOC) fractions are more responsive to altered litter inputs than mineral-associated SOC, because they cycle relatively rapidly. (2.) Any accumulation of mineral-associated SOC with litter addition is relatively stable (i.e. low leaching potential). (3.) Certain biomolecules, such as waxes (alkyl) and proteins (*N*-alkyl), form more stable mineral-associations than other biomolecules in strongly weathered soils. A decade of litter addition and removal affected bulk soil C content in the upper 5 cm by +32% and −31% in total quantity, respectively. Most notably, C concentration in the mineral-associated SOC fraction was greater in litter addition plots relative to controls by 18% and 28% in the dry and wet seasons, respectively, accounting for the majority of increased bulk soil C stock. Radiocarbon and leaching analyses demonstrated that the elevated mineral-associated SOC consisted of new and relatively stable C, with only 3% of mineral-associated SOC leachable in salt solution. ^{13}C NMR spectroscopy indicated decadal-scale stability of waxes (alkyl C) and microbial biomass compounds (*O*-alkyl and *N*-alkyl C) in mineral-associated SOC with litter removal, and losses plant-derived compounds (aromatic and phenolic C). We conclude that shifts in tropical forest NPP, as will likely occur with global change, are likely to alter the quantity, biochemistry, and stability of C stored in strongly weathered tropical soils.

1. Introduction

Soil organic carbon (SOC) storage in humid tropical forests is likely to be affected by shifting net primary productivity (NPP) in response to global change. Remote sensing data show increased NPP for much of the tropics during recent decades in response to warming, increased rainfall (Nemani et al., 2003), and elevated atmospheric carbon dioxide (CO_2) (Lewis et al., 2009). At the same time, field studies have observed that warming (Clark et al., 2013) and drying (Tan et al., 2013) can suppresses NPP in tropical forests. This spatial variation may result from increased dry season duration in some areas (Boisier et al., 2015), versus increased wet season rainfall in other areas with climate change (Feng et al., 2013). Rainfall seasonality, in turn, is related to intra-annual shifts in SOC stocks, as shown for a humid forest in Panama (Turner et al., 2015). Since ~30% of global SOC stocks are in tropical forests (Jobbagy and Jackson, 2000), potential interacting effects of altered NPP and seasonality are of broad significance.

Changes in NPP are most likely to affect SOC storage via altered litterfall and/or root turnover. A global meta-analysis of 70 litter addition experiments showed a 31% overall increase in SOC content to 5 cm depths, with the largest changes in six tropical or sub-tropical studies included in the study (Xu et al., 2013b). More recently, litter manipulation in a humid Panamanian forest also resulted in significant changes in SOC stocks to 20 cm depths (Tanner et al., 2016). However, we know very little about how altered litterfall might affect the biochemistry, physical character, and stability of tropical SOC.

A useful conceptual model divides SOC into three fractions (Sollins et al., 1999): (1) free-debris SOC, which visually resembles decomposing litter and roots, is unattached to mineral particles, and tends to have younger C than other fractions in tropical soils (Marin-Spiotta et al., 2008); (2) occluded-debris SOC, which is litter that has been incorporated into aggregate

structures, or which is otherwise easily dispersible from soil; (3) mineral-associated SOC, often referred to as the "heavy fraction," which consists of organic compounds sorbed to mineral surfaces via physiochemical mechanisms (Kleber et al., 2015). Studies of clay-rich tropical soils have found that the majority of SOC resides in the mineral-associated fraction (Trumbore, 1993; Glaser et al., 2000; Marin-Spiotta et al., 2009), which is generally thought to be the most stable, long-term SOC stock (Trumbore and Zheng, 1996; Torn et al., 1997; von Lutzow et al., 2006). Recent evidence, however, suggests that the mineral-associated SOC fraction can contain heterogeneous sub-fractions that turn over on different timescales (Sollins et al., 2006; Cusack et al., 2011b), with certain compounds like proteins comprising the most stable portion of mineral-associated SOC (Kleber et al., 2007). In humid tropical forests in particular waxy substances and other lipids may also contribute to more stable soil fractions (Cusack et al., 2011a).

Here, we investigate the biochemistry, physical character, and stability of SOC after a decade of litter manipulation in a humid Panamanian forest, following up on documented shifts in SOC stocks with litter addition or removal at these sites (Tanner et al., 2016). We measured three SOC fractions in surface soils, where the greatest changes in SOC stocks were documented, during a wet and a dry season. We hypothesized that: (1) changes in bulk SOC concentrations with litter manipulation are explained by shifts in rapidly cycling free-debris and occluded-debris SOC fractions, because these are likely to change over short timescales; (2) the quantity and chemical character of mineral-associated SOC is relatively insensitive to decadal-scale changes in litter inputs; (3) any new SOC accumulation in mineral-associated soil fraction with litter addition is relatively stable (i.e., low solubility); (4) waxy (alkyl) and protein-rich (*N*-alkyl) C groups represent the most stable portion of mineral-associated SOC, persisting on decadal

81 timescales even in the absence of new litter inputs. Finally, (5) seasonal shifts in SOC result

82 primarily from changes in the free-debris and occluded-debris SOC fractions.

83

84 **2. Materials and Methods**

85 *2.1 Site and Soil Collection*

86   Research was conducted in a lowland semi-deciduous tropical forests on the Gigante

87 Peninsula in the Barro Colorado Nature Monument, Panama (9°06'N, 79°54'W) (Windsor,

88 1990). The forest is a tropical moist forest *sensu* Holdridge et al. (1971). The site has a tropical

89 monsoon seasonal climate, mean annual rainfall is 2600 mm, and mean annual temperature is

90 26°C (Leigh, 1999). High tree species richness and the stature (canopy heights > 35 m) of this

91 forest are characteristic of an old (> 200 years) seasonal primary forest (Leigh et al., 1996).

92 Litterfall peaks during the dry season from December to April, and decreases by 50% during the

93 wet season (Turner et al., 2015) Soils are moderately acidic (pH 4.8 − 5.4) kaolinitic Oxisols

94 developed on basalt (Yavitt et al., 2009). The soils lack a distinct O horizon other than a

95 continuous cover of leaf litter that builds up during the dry season and is virtually fully

96 decomposed by the end of the wet season. Soils were classified in profile pits outside of plots

97 according to Soil Taxonomy (SoilSurveyStaff, 1999). Soil were well-structured, with medium

98 and coarse subangular blocky structure in the upper parts of the profile, breaking to strong fine

99 subangular blocky. Some shrink-swell clays are evident in surface soils at these sites, possibly

100 resulting from African dust inputs (Gross et al., 2016).

101   Fifteen 45 × 45 m litter manipulation plots were established in 2000 and trenched to a

102 depth of 50 cm. The sides of the trenches were lined with heavy plastic. This depth was chosen

103 to contain fine roots and deter growth into or out of plots, since >92% of fine root biomass is in

104   the top 50 cm of soil in forests in this region (Yavitt and Wright, 2001; Cusack et al., 2018).

105   Monthly transfer of litter from five removal plots to five addition plots began in January 2003,

106   and five control plots were included (n = 5). Litter was removed by rake and by hand monthly

107   and evenly distributed onto the litter addition plots. A study to 1-m depth after 6 years of litter

108   manipulation at these sites showed that litter addition and removal significantly increased and

109   decreased bulk soil C stocks to 20 cm depth, respectively, with the greatest effect in the upper 5

110   cm of the profile (Tanner et al., 2016). This was consistent with an earlier study at 3 years

111   showing significant treatment effects on organic matter concentrations in the top 2 cm of soils at

112   the site (Vincent et al., 2010). Our goal was to better understand the physiochemical nature of the

113   changing SOC stocks documented in these previous studies.

114   Soils for this study were collected during the dry season in February 2013, and the wet

115   season in July 2013. Soils were sampled from the surface 5 cm using a 2.5 cm diameter constant-

116   volume soil corer, thus sampling equivalent depths and volumes in all treatments. Surface litter

117   was gently removed and coring began at the surface of the mineral soil. In litter addition plots a

118   spatially heterogeneous duff layer had developed, and this was included in sampling where it

119   was present. Three soil cores were sampled from 10 random stratified points within the 30 x 30

120   m core area of each plot, then pooled and homogenized to give one representative sample per

121   plot. The 0 − 5 cm depth was selected because it showed the strongest changes in bulk soil C

122   with litter manipulation at 6 years (Tanner et al., 2016), and this depth has shown the strongest

123   changes in litter manipulation experiments more broadly (Xu et al., 2013a).

124   Bulk density cores were collected using a 5 cm diameter corer in each plot. Gravimetric

125   soil moisture, pH, bulk density, and SOC fractions (see below) were measured on fresh soils.

126   Loss on ignition (LOI) was also measured for dry season samples to assess changes in the

127 mineral content of soils across treatments to the same depth. Carbon content normalized to

128 mineral mass was assessed as an alternative to the strategy used here of collecting samples from

129 a constant depth and volume across plots.

130   Given the previously documented change in bulk density with litter removal at these sites

131 (Tanner et al., 2016), an alternative sampling approach could have been to adjust sampling depth

132 in an attempt to collect equivalent mass. Soil volume increases and bulk density declines as

133 organic matter is added. Thus, we would have had to sample to shallower depths in litter removal

134 plots and deeper depths in litter addition plots to collect equivalent mass. This would pose

135 problems because the composition of SOC changes with depth. For example, the proportion of

136 dense, mineral-associated SOC increases with depth. We therefore determined that sampling

137 equivalent mass would have masked any increased importance of low-density organic matter in

138 surface soils, as reviewed by Sollins and Gregg (2017). Since our goal was to characterize the

139 physiochemical characteristics of the change in surface SOC including chemical shifts related to

140 changes in bulk density, we decided to sample to constant depth and volume rather than correct

141 for changes related to bulk density.

142

143 *2.2 Soil Fractionation*

144   Density fractionation was used to separate soils into three soil SOC pools using a solution

145 of C-free sodium polytungstate (SPT) following Swanston et al. (2005), with modifications as

146 noted. This method was chosen over particle size fractionation because it puts greater emphasis

147 on adsorption and aggregation processes, rather than the fate of particulate organic matter (Moni

148 et al., 2012). The density fractions included: (**1**) free-debris SOC, which was collected as the

149 fraction of the soil that easily floated in dense liquid with minimal disturbance (sometimes

150   referred to as the "free light fraction" in the literature). (**2**) Occluded-debris SOC, which was

151   collected as floatable SOC that was released from soil via mixing and dispersed via sonication at

152   450 J/mL (sometimes referred to as the "occluded light fraction"). This SOC fraction is generally

153   considered to turnover more slowly that the free-debris SOC fraction because of its physical

154   protection with aggregate structures, as shown in an Australian Oxisol (Golchin et al., 1995).

155   This fraction may also represent SOC at more advanced stages of decomposition than the free-

156   debris, as suggested by elevated alkyl:O-alkyl ratios (Golchin et al., 1994). However, the

157   quantity and chemistry of SOC released via sonication can be highly variable across soils (Wagai

158   et al., 2009), and may include debris buried inside macro-aggregates, debris entrained in the

159   organic coatings that have built up at the surface of micro-aggregates, and/or individual organic

160   particles released from mineral grains by sonication. Iron-oxide particles in Oxisols are often

161   bound up in exceptionally stable micro-aggregates (<5 um), and these were not likely broken

162   down by the method used here. (**3**) Mineral-associated SOC, which was collected as the dense

163   pellet of soil remaining after removing the above two fractions (sometimes referred to as the

164   "dense" or "heavy" fraction). This fraction generally represents the most stable and oldest soil C.

165   Separation of fractions was done in sodium polytungstate (SPT) mixed to a density of 1.7 g/cm^3,

166   following initial tests to identify optimal density and dispersal parameters (Appendix S1).

167   Bulk soils and SOC fractions were analyzed for C and N concentrations on a Costech

168   Elemental Analyzer (Valencia, CA) using acetanilide as a standard. While the focus of this paper

169   is on SOC, we included parallel N analyses to understand broader effects of litter manipulation

170   on soil organic matter (SOM) N content and C:N ratios, particularly in relation to SOM protein

171   content versus other C functional groups.

172

2.3 Δ14C and 13C NMR of Mineral-Associated SOC

Because the mineral-associated SOC fraction commonly contains the longest-term soil C sink (Sollins et al., 1999), we conducted extensive chemical analysis of this C fraction to determine the potential effects of changing NPP on long-term tropical soil C storage.

First, we measured the ^{14}C content of the mineral-associated SOC fraction to assess relative changes in the overall age of this C fraction with litter addition and removal. The spike in atmospheric $^{14}CO_2$ from nuclear bomb testing in the 1960s and subsequent declines make it possible to assess relative ages of plant organic matter inputs to ecosystems on decadal timescales (Trumbore, 2000). The mineral-associated SOC fraction was measured for $\Delta^{14}C$ at the WM Keck Carbon Cycle Accelerator Mass Spectrometry (AMS) Laboratory at UC Irvine following sample graphitization (Vogel et al., 1984). AMS analytical precision was ± 2.2 ‰ for these samples, and results are reported as the per mil (‰) deviation from a standard normalized for ^{13}C.

We also assessed changes in the organic chemistry of the mineral-associated SOC fraction to understand how litter addition and removal altered this important C fraction. The organic chemistry of the mineral-associated fraction was assessed using ^{13}C nuclear magnetic resonance (NMR) spectroscopy following hydrofluoric acid treatment (details in Appendix S1), which removes mineral and glass fractions to reduce magnet interference, and concentrates SOC to reduce noise in the spectra (Schmidt et al., 1997). This method also removes a portion of acid-soluble C as DOC from mineral associations (Gelinas et al., 2001), leaving a sample of mineral-free, concentrated organic matter that is not soluble in hydrofluoric acid. Here, the hydrofluoric-acid-insoluble portion of mineral-associated SOC was 60.5% of the C contained in the mineral-associated fraction (Table S6), which is within the range found for a comparison of

196   different soil types (Hockaday et al., 2009). The mineral-associated SOC remaining after

197   hydrofluoric acid treatment is the least dynamic, least soluble component of this pool.

198   Cross polarization ^{13}C NMR was conducted on the concentrated SOC after hydrofluoric

199   acid treatment using a 300 MHz Bruker Avance III NMR spectrometer (Bruker BioSpin,

200   Billerica, MA) at Baylor University (Waco, TX). The 4mm sample probe was operated at a

201   magic angle spinning (MAS) frequency of 12 kHz (details in Appendix S1). Resulting spectra

202   were assessed for the relative contributions of seven C functional groups by integrating the signal

203   magnitude on the δ–scale. Carbon functional groups are followed in parentheses by some of the

204   many common C compounds in which they occur: (1.) alkyl 0 to 45 ppm (e.g., waxes, other

205   lipids); (2.) *N*-alkyl+methoxyl (hereafter *N*-alkyl) 45 to 60 ppm (e.g., proteins, peptides,); (3.) O-

206   alkyl 60 to 95 ppm (e.g., cellulose, other carbohydrates); (4.) di-O-alkyl 95 to 110 ppm (e.g.

207   hemicellulose); (5.) aromatic 110 to 145 ppm (e.g., lignin, tannin); (6.) phenolic 145 to 165 ppm

208   (e.g., acids, tannin (Min et al., 2015)); and (7.) amide+carboxyl 165 to 215 ppm (e.g., chitin,

209   proteins, peptides, and hemicellulose) (Li et al., 2015). The alkyl:*O*-alkyl ratio was calculated as

210   an index of decomposition stage, with greater ratios generally indicative of later stages of litter

211   decomposition (Preston et al., 2009).

212

213   *2.4 Soil C Stability and DOC Leaching*

214   The stability of C was assessed in each SOC density fraction by measuring the solubility

215   of organic matter as DOC and TDN leached in the SPT supernatant plus approximately 1-L of

216   deionized water (DI). This approach specifically mobilized DOC from cation and anion

217   exchange sites, in an analogous approach to the commonly used potassium chloride (KCl) salt

218   extractions for collecting DOC from soil (Swift, 1996). Floatable debris fractions were rinsed

219   with DI in filter holders through 0.4 μm membrane filters until approximately 1-L was reached,

220   which both cleaned the fraction of SPT, and allowed us to conduct the leaching experiment. The

221   dense fraction was rinsed with DI in centrifuge tubes by shaking, centrifuging, decanting of

222   supernatant. Decanted supernatant was then filtered as above, and any residue on the filter was

223   added back to the dense fraction. Initial rinses included removal of the SPT from the fraction.

224   Thus, all C in initial bulk soils was accounted for either in SOC solid fractions, or in leachate

225   from the fractionation procedure and subsequent leaching (details in Appendix S1). Leachate

226   samples were analyzed on a Shimadzu TOC-L with TDN analyzer (Columbia, MD).

227

228   *2.5 Statistical Analyses*

229   Data from the litter manipulation plots were analyzed using analysis of variance

230   (ANOVA) and analysis of covariance (ANCOVA). The effects of season, treatment, and their

231   interaction were tested for bulk soil C and N content, C and N in each density fraction, ^{14}C

232   content of the mineral-associated fraction, and for ^{13}C NMR chemical regions of the mineral-

233   associated fraction. Effects of season and treatment were also tested for DOC and TDN leached

234   from each SOC fraction. We used post-hoc Tukey HSD means separation tests to assess

235   differences among litter treatments and seasons (n = 5 per season and litter treatment). Post-hoc

236   regressions were used to determine relationships between continuous variables across seasons (n

237   = 30 for bulk soils, 30 for each density fraction). We used JMP 11 statistical software (SAS

238   Institute). Data are presented as means ± 1 S.E., using $p < 0.05$ for statistical significance.

239

240   **3. Results**

241   After 10 years of litter manipulation, bulk soil C concentrations from 0 − 5 cm depths

242 were significantly greater with litter addition (+32%), and significantly lesser with litter removal

243 (-31%) relative to control plots (Figure 1a, Table 1). Similar significant effects were observed for

244 soil N concentrations, but the magnitude of the differences were not as large, so soil C:N values

245 were significantly affected by treatment, with the lowest levels in litter removal plots (Table 1).

246   Bulk density was also significantly greater in litter removal plot (0.97 ± 0.05 g/cm^3)

247 relative to the other treatments, while there was no significant difference between control ($0.73 \pm$

248 0.03 g/cm^3) and litter addition plots (0.79 ± 0.05 g/cm^3, n = 5), and no seasonal effect (Table 1).

249 This change represents a relatively large (+28%) increase in bulk density with litter removal

250 relative to the other two treatments. Our approach of sampling equivalent depths and volumes

251 across treatments explicitly did not correct for changes in bulk density, since these changes were

252 due to addition of low-density organic matter, which we did not want to dilute by sampling to

253 greater depth. Nonetheless, we can evaluate the effect of the change in bulk density on our bulk

254 soil C content values. Using published data from our sites for C concentration versus depth to

255 200 cm at six years (Tanner et al., 2016), we can calculate the depths we would have had to

256 sample, and the resulting effect on bulk soil C content. The 28% greater bulk density that we

257 observed with litter removal relative to controls is equivalent to a 1.4 cm increase in effective

258 soil depth. Using the published soil depth profiles, we calculate that this greater effective soil

259 depth would lead to a 2.4 % increase in C for litter removal plots relative to control plots, if our

260 goal had been to calculate C stocks on a per-mass of soil basis. This is a small proportion of our

261 observed 31% decline in bulk soil C concentration with litter removal.

262   Another approach is to normalize soil C content to mineral content. Loss on ignition

263 values were significantly lower in litter removal plots (17.5 ± 0.3 %) relative to other treatments,

264 indicating greater content of minerals/ash, and there was no significant difference between

265   control and litter addition plots (19.7 ± 0.3 % and 20.6 ± 0.8, respectively, n = 5). Using these

266   LOI values and bulk density to standardize soil C content to mineral content for $0 - 5$ cm depth,

267   there was significantly greater soil C content per mineral content in litter addition plots (98.5 ±

268   4.5 mg-C/g-mineral-content) versus control plots (75.3 ± 3.0 mg-C/g-mineral-content), with the

269   lowest values in litter removal plots (49.5 ± 2.5 mg-C/g-mineral-content, $p < 0.05$). Thus,

270   normalizing soil C to mineral content gave similar differences between addition vs. control

271   (+31%), and removal vs. control (-34%) as did C concentration and C stock data sampling

272   equivalent volume (Table 1).

273

274   *3.1 Soil Fraction C Contents, Relative Masses, and C Concentrations*

275   Soil organic C content was greater with litter addition versus controls and litter removal

276   for all three soil fractions (Figure 1), with accompanying changes in C concentrations within

277   each fraction (Table S2), and changes in the relative masses of the three fractions (Figure S2a),

278   such that the contribution of each fraction to total soil C shifted significantly with litter

279   manipulation (Figure S2b). There were also seasonal effects on all fractions, with interactions

280   between season and treatment effects. The most important effect of litter manipulation on the

281   mineral-associated SOC fraction, in terms of the net effect on bulk soil C content, was a change

282   in C concentration within this fraction. In contrast, the most important effect on the free-debris

283   and occluded-debris SOC fractions was an increase in the relative mass of these fractions as a

284   proportion of total soil mass with litter addition. Results for the mineral-associated fraction are

285   presented first, followed by the two floatable fractions together.

286

287   *3.1.1. Mineral-Associated SOC Fraction*

288   The mineral-associated SOC fraction comprised the largest portion of greater bulk soil C

289   with litter addition (Figure 1a). The changes in mineral-associated soil C content resulted from

290   greater C concentrations in this soil fraction with litter addition, and lesser C concentrations with

291   litter removal (Table S2). Even though average C concentrations for the mineral-association

292   SOC fraction were low relative to the other two fraction (3 – 5% C in mineral-association SOC,

293   versus 20 – 40% C in the floatable fractions, Table S2), the large relative mass of this soil

294   fraction (91 – 97 % of soil mass, Figure S2a) meant that it dominated net changes in soil C

295   content. So, even with the relatively low average C concentrations, the mineral-association SOC

296   fraction contributed the largest overall proportion of soil C content, containing 64 – 82 % of total

297   soil C (Figure S2b).

298   There was also a seasonal effect on C concentrations within the mineral-associated

299   fraction, with greater C concentrations overall and larger differences among treatments during

300   the wet season (Figure 1a). Specifically, mineral-associated C concentrations were 4.0 ± 0.2 % in

301   control plots versus 4.7 ± 0.2 % in litter addition plots during the dry season (Table S2),

302   representing an 18% increase. During the wet season, mineral-associated C concentrations were

303   4.3 ± 0.1 % in control plots, versus 5.5 ± 0.2 % in litter addition plots (Table S2), representing an

304   28% increase in C concentration. Mineral-associated C concentrations in litter removal plots

305   were 25% lower than control plots the dry season, and 28% lower than control plots during the

306   wet season (Table S2). Nitrogen concentrations in the mineral-associated soil fraction showed

307   similar significant shifts with litter removal, but the magnitude of changes in N concentration

308   were not as large, so C:N ratios in the mineral-associated SOC fraction were lower in litter

309   removal plots than in litter addition plots, indicating a relative enrichment in N with litter

310   removal (Table S2).

14

311   Assessing the total C content within the mineral-associated fraction, using C

312   concentrations and the mass of this fraction as a proportion of soil mass, there was 43.5 ± 2.1

313   mg-C/g-bulk-soil in mineral-associated SOC in litter addition plots, 38.7 ± 1.7 mg-C/g-bulk-soil

314   in control plots, and 28.9 ± 1.6 mg-C/g-bulk-soil in litter removal plots in the dry season (Figure

315   1a). In the wet season there was 50.7 ± 2.0 mg-C/g-bulk-soil in the mineral-associated SOC

316   fraction in litter addition plots, 41.0 ± 1.1 mg-C/g-bulk-soil in control plots, and 30.2 ± 1.5 mg-

317   C/g-bulk-soil in litter removal plots (Figure 1a). Thus, litter addition plots had 12% greater

318   mineral-associated SOC content than controls during the dry season, and 24% greater during the

319   wet season relative to controls. Litter removal plots showed less of a seasonal effect on mineral-

320   associated SOC content, with removal plots 25% lesser than controls during the dry season, and

321   26% lesser during the wet season relative to controls. Comparing shifts from the dry season to

322   the wet season, mineral-associated SOC content increased 17% in the litter addition plots from

323   the dry to the wet season, 6% in control plots, and only 4% in litter removal plots. These

324   seasonal shifts illustrate the interaction between litter treatment and season, with wet season

325   increases in mineral-associated SOC content much greater in litter addition versus litter removal

326   plots.

327

328   *3.1.2. Low-Density SOC Fractions*

329   Unlike the mineral-associated SOC fraction, the change in soil C content attributable to

330   the two floatable fractions (Figures 1b and 1c) was related primarily to shifts in their relative

331   masses within soil (Figure S2a), rather than large changes in their C concentrations (Table S2).

332   Nonetheless, there were significant but small effects of litter manipulation and season on C

15

333 concentration in the free-debris SOC fraction (Table S2). The occluded-debris fraction had no

334 significant effect of litter manipulation on C concentrations.

335 The shift in the relative masses of these fractions with litter manipulation was quite large.

336 For the free-debris SOC fraction, relative mass was 89% and 37% greater in litter addition plots

337 relative to controls during the dry and wet seasons, respectively (Table S2, Figure S2a), and -

338 63% and -58% lesser in litter removal plots relative to controls during the dry and wet seasons,

339 respectively. The occluded-debris SOC fraction did not show strong treatment effects during the

340 dry season, but during the wet season its mass was 74% greater in litter addition plots relative to

341 controls, and -18% lower in litter removal plots relative to controls (Table S2, Figure S2a).

342 Given the sizeable shifts in the proportional masses of the two low-density fractions with litter

343 manipulation, and their large C concentrations, their overall contributions to total soil C content

344 was significant, even though their proportional masses were small, contributing only 1-6% of

345 total soil mass (Table S2).

346 Using C concentration values and soil mass proportions to calculate the C content in the

347 two low-density SOC fractions, the free-debris SOC fraction had 17.7 ± 3.4 mg-C/g-bulk-soil in

348 litter addition plots, 9.2 ± 1.0 mg-C/g-bulk-soil in control plots, and 3.4 ± 0.5 mg-C/g-bulk-soil

349 in litter removal plots in the dry season (Figure 1b). In the wet season, there was 10.3 ± 2.4 mg-

350 C/g-bulk-soil in litter addition plots, 7.2 ± 1.0 mg-C/g-bulk-soil in control plots, and 2.6 ± 0.3

351 mg-C/g-bulk-soil in litter removal plots for the free-debris SOC fraction (Figure 1b). This

352 represents 92% greater free-debris C content in litter addition plots versus controls in the dry

353 season, and 43% greater free-debris C content in litter addition plots versus controls during the

354 wet season, as well as 63% less in litter removal plots versus controls during the dry season, and

355 64% less in litter removal plots versus controls during the wet season.

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356   In the occluded-debris SOC fraction, there was 6.1 ± 1.1 mg-C/g-bulk-soil in litter

357   addition plots, 3.6 ± 1.1 mg-C/g-bulk-soil in control plots, and 3.8 ± 0.5 mg-C/g-bulk-soil in

358   litter removal plots in the dry season (Figure 1a). In the wet season, there was 12.1 ± 3.5 mg-C/g-

359   bulk-soil in litter addition plots, 5.8 ± 1.8 mg-C/g-bulk-soil in control plots, and 3.9 ± 1.0 mg-

360   C/g-bulk-soil in litter removal plots for the free-debris SOC fraction (Figure 1c). This represents

361   69% more occluded-debris C in litter addition plots versus controls during the dry season, and

362   108% more occluded-debris C in litter addition plots versus controls during the wet season.

363   There was no significant decrease in occluded-debris C content with litter removal in the dry

364   season, but there was 33% less occluded-debris C content in litter removal plots versus controls

365   during the wet season.

366   There were also overall seasonal effects on the proportion of soil mass and the proportion

367   of C content for the two low-density SOC fractions. The free-debris SOC fraction was a larger

368   proportion of total soil C (Figure S2b) and of total soil mass (Figure S2a, Table S2) during the

369   dry versus wet season. In contrast, the occluded-debris fraction represented a significantly larger

370   proportion of total soil C (Figure S2b) and of total soil mass (Table S2) during the wet versus dry

371   season. Thus, seasonal patterns for C content in the occluded-debris fraction mirrored patterns

372   for the mineral-associated SOC fraction, and the occluded-debris fraction had opposite

373   seasonality (Figure 1).

374

375   *3.2. Organic Chemistry and $\Delta\,^{14}C$ Age of Mineral-Associated SOC*

376   The $\Delta\,^{14}C$ of the mineral-associated SOC fraction was significantly greater with litter

377   removal versus addition, with no seasonal effect (Figure 2). Because the $\Delta\,^{14}C$ signal in surface

378   soil is dominated by recent C inputs from plant litter, the greater values in litter removal plots

17

379   correspond to an older overall age of the mineral-associated SOC fraction, versus a younger

380   overall mineral-associated SOC fraction in litter addition plots with greater dominance by recent

381   litter inputs.

382   Solid-state ^{13}C NMR spectroscopy revealed decadal-scale effects of litter manipulation

383   on the organic chemistry of mineral-associated SOC, with no seasonal effects. All C groups

384   responded to litter manipulation except amide+carboxyl C. Overall, alkyl C, *N*-alkyl C, and *O*-

385   alkyl C were proportionally larger components of mineral-associated SOC in litter removal

386   versus addition plots, while di-*O*-alkyl C, aromatic C, and phenolic C showed the opposite

387   pattern (Table 2). These changes resulted in an overall shift in the chemical nature of mineral-

388   associated SOC, as illustrated by comparing representative spectra from litter addition and

389   removal plots (Figure 3). Thus, there was a significant treatment effect on the ratio of (alkyl + *O*-

390   alkyl + *N*- alkyl):(phenolic + aromatic) C (Table 2). There was also a significant interaction

391   between season and treatment only for the alkyl:*O*-alkyl ratio in mineral-associated SOC, with

392   significantly greater ratios in litter addition plots (1.5 ± 0.04) versus control (1.4 ± 0.03) and

393   removal plots (1.3 ± 0.02) during the dry season only, and no effect during the wet season. This

394   dry season pattern is the opposite pattern of what would be expected for more advanced stages of

395   decomposition in litter removal plots. Overall, these alkyl:*O*-alkyl ratio for mineral-associated

396   SOC were all significantly greater than values obtained for occluded-debris SOC at varying

397   sonication energy levels, suggesting that our method appropriately separated these two fractions

398   (Appendix S1).

399   Two C chemical groups were significantly related to the Δ ^{14}C content of mineral-

400   associated SOC. Mineral-associated Δ ^{14}C was positively significantly correlated to *O*-alkyl C

401   (R^2 = 0.18, n = 30), indicating older C present when more *O*-alkyl C was present. In contrast,

18

402   mineral-associated Δ ^{14}C was negatively significantly correlated to phenolic C (R^2 = 0.27, n =

403   30), indicating younger C present when more phenolic C was present.

404

3.3. SOC Stability: DOC Leaching

406   The proportion of salt-extractable C (i.e., leachable DOC) in each soil fraction, expressed

407   as mg-C-leached/g-C retained, varied across SOC fractions, with no effect of season. Overall,

408   DOC leaching was 5–14 % of the stable C in the free-debris ("free floatable") fraction, 15–30 %

409   of the stable C in the occluded-debris ("dispersible floatable") fraction, and only 2–3 % of the

410   stable C in the mineral-associated ("dense") fraction (Figure 4, Table S3). Thus, all SOC

411   fractions had a component that was salt-extractable, but this proportion was smallest in the

412   mineral-associated fraction, indicating that more of the C in this fraction was relatively stable.

413   The effect of litter manipulation on the proportion of C that was salt-extractable varied

414   across SOC fractions. In the free-debris fraction, a significantly greater proportion of DOC was

415   leachable from litter removal plots (12.7 ± 0.8 %) relative to control (6.2 ± 0.6 %) or litter

416   addition plots (4.9 ± 0.4%), suggesting a high level of instability and solubility for the small

417   stock of free-debris organic matter remaining in litter removal plots after a decade (Figure 4a). In

418   contrast, the mineral-associated SOC fraction had a significantly greater proportion of leachable

419   DOC in litter addition plots (2.9 ± 0.3 %), relative to control (2.3 ± 0.2 %) or litter removal plots

420   (1.8 ± 0.1 %, Figure 4b), indicating that C remaining in mineral associations in litter removal

421   plots after a decade was strongly sorbed to mineral surfaces and stable. Significant treatment

422   effects on proportional losses of total dissolved N (TDN) were similar to those for DOC,

423   although N was much more mobile overall than C (Table S3).

424   Overall, the proportion of C that was leachable as DOC from the mineral-associated

425 fraction was positively correlated with the total quantity of C in that fraction ($R^2 = 0.66$, p <

426 0.05, n = 30). Accordingly, bulk quantities of leachable DOC per g soil showed similar treatment

427 effects as did soil C concentrations across fractions (Figures S4 versus Figure 1, Table S2), and

428 so did trends for leachable TDN per g soil (Tables 2, S5). Total DOC leaching was 1.1 ± 0.2 mg-

429 C/g-bulk-soil in litter addition plots, 0.9 ± 0.1 mg-C/g-bulk-soil in control plots, and 0.5 ± 0.04

430 mg-C/g-bulk-soil in litter removal plots in the dry season (Figure 1a). In the wet season DOC

431 leaching was 1.6 ± 0.3 mg-C/g-bulk-soil in litter addition plots, 0.9 ± 0.1 mg-C/g-bulk-soil in

432 control plots, and 0.6 ± 0.1 mg-C/g-bulk-soil in litter removal plots (Figure S4). These quantities

433 represent very small fractions of total soil C content (Figure 1), ranging from just $1.7 - 3.1$ % of

434 total soil C leachable as DOC.

435

436 **4. Discussion**

437   The effects of litter manipulation on bulk soil C concentrations after 10 years in these

438 Panamanian forests indicate a sustained effect, with similar effects observed from $0 - 2$ cm depth

439 after three years (Vincent et al., 2010), and from $0 - 20$ cm depth after six years (Tanner et al.,

440 2016). The increase in soil C content with litter addition occurred despite an apparent priming

441 effect of added litter on soil CO_2 fluxes at these sites (Sayer et al., 2011). Similar to our $\pm30\%$

442 change in bulk soil C content with litter addition or removal, two years of litter manipulation in a

443 Costa Rican forest on Ultisols also changed bulk soil C concentrations by approximately $\pm30\%$

444 (Leff et al., 2012). In contrast, a review of litter manipulations in temperate forests showed

445 generally non-significant or small effects on bulk soil C content (Sayer, 2006). Our results, in

446 conjunction with Leff et al. (2012). and the few tropical studies in the meta-analysis (Xu et al.,

447 2013b), suggest that soil C stocks in strongly weathered tropical forest soils may be more

20

448   sensitive to changes in NPP over shorter time periods than soils in temperate sites. Such a trend

449   may reflect faster C cycling, greater overall C inputs to soils per year, and/or sorptive

450   characteristics of the strongly weathered clay soils common in humid tropical forests.

451

452   *4.1. Elevated SOC across Soil Fractions with Litter Addition*

453   The substantial accumulation of organic matter that we observed in the mineral-

454   associated SOC fraction was somewhat surprising, since this soil fraction is commonly a

455   relatively stable pool of long-term soil C storage (Schmidt et al., 2011). Elevated mineral-

456   associated SOC may have resulted in part from the elevated soil pH observed in litter addition

457   plots (Tanner et al., 2016), since increased pH can mobilize DOC from organic matter

458   (Whitehead et al., 1981), transporting C from litter down into mineral soils. Mobilized DOC may

459   then have sorbed to the relatively large number of binding sites common on clay surfaces in

460   highly weathered tropical forest soils (Chorover and Sposito, 1995). Or, DOC may have

461   associated with other organic compounds already sorbed to mineral surfaces (Kleber et al.,

462   2007), or to surfaces of very stable microaggregates (< 1 µm diameter) (Torrent et al., 1990).

463   The low-density SOC fractions also accumulated in soils with litter addition, as expected,

464   indicating that decomposition rates and other C losses did not accelerate to balance new litter

465   inputs. The greater levels of occluded-debris SOC in litter addition plots could have resulted

466   from organic matter incorporation into aggregate structures, particularly during formation and

467   dissolution of aggregates over the course of rainfall seasonality (Dimoyiannis, 2009). In contrast

468   to our results, a 50-year experiment in two temperate forests on silt-loam soils with lower clay

469   content found that litter addition increased only the low-density SOC fractions, while litter

470   removal reduced both low-density and mineral-associated SOC fractions (Lajtha et al., 2014). A

471   better understanding of the ecosystem and soil characteristics that promote rapid accumulation of

472   organic matter into stable mineral associations could help us better predict C cycle responses to

473   global change across biomes.

474

475   *4.2. Stability of C in SOC Fractions: Potential DOC Leaching*

476   As hypothesized, C incorporated into the mineral-associated soil fraction was

477   subsequently relatively stable, as demonstrated by its low solubility and leaching potential.

478   Similarly, a range of soil orders from North and Central American sites showed rapid

479   incorporation of added ^{15}N into the mineral-associated fraction in a laboratory study, with

480   subsequent low levels of solubility (Strickland et al., 1992). A desorption study using goethite

481   and a subsoil similarly showed <3% desorption of organic matter when leaching solution was

482   similar to sorption solution, and elevated desorption only under elevated ion concentrations or

483   elevated pH (Kaiser and Zech, 1999). Thus, it appears that in one direction organo-mineral

484   associations can be highly dynamic (e.g., rapid mineral sorption of organic compounds), but in

485   the other direction, losses from this pool are more limited (i.e., not leachable as DOC),

486   illustrating how this fraction can be a reservoir of long-term soil C storage. The longer-term

487   stability of elevated mineral-associated SOC in litter addition plots merits further investigation.

488   Interestingly, the litter manipulation effect on DOC leaching as a proportion of extant

489   SOC was different for the two low-density SOC fractions compared with the mineral-associated

490   fraction. Litter removal versus addition plots had greater DOC leaching from low-density SOC

491   fractions, but lesser DOC leaching from mineral-associated SOC fractions. This opposite

492   treatment effect likely reflects the different physiochemical nature of organic matter in these

493   different SOC fractions. Organic matter in the low-density fractions is comprised primarily plant

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494   or microbial macro tissues at some stage of decomposition, whereas the mineral-associated SOC

495   fraction contains small organic compounds sorbed to mineral surfaces out of DOC. Thus, high

496   levels of DOC leaching from low-density SOC fractions in litter removal plots likely resulted

497   from the rapid dissolution of very decomposed macro tissues. In contrast, low levels of DOC

498   leaching from the mineral-associated DOC fraction in litter removal plots likely resulted because

499   the organo-mineral associations remaining after a decade of *in situ* leaching with rainwater were

500   the most stably sorbed portion of mineral-associations.

501

4.3. Changes in the $\Delta^{14}C$ of Mineral-Associated SOC

503   Our radiocarbon data support growing evidence that only a portion of the mineral-

504   associated SOC fraction is stable over the long term, whereas another portion is dynamic on

505   decadal timescales. The relatively greater $\Delta^{14}C$ values of mineral-associated SOC in litter

506   removal plots suggest decadal-scale retention of relatively older C, assuming that the majority of

507   C in surface soils originated from NPP during the past 60 years (Trumbore, 2000). In contrast,

508   the lesser $\Delta^{14}C$ values in mineral-associated SOC in litter addition plots suggest an accumulation

509   of relatively recent C inputs. Because $\Delta^{14}C$ is an integrated measure of all C included in the SOC

510   fraction, the "modern" overall ages measured here do not preclude the presence of some much

511   older C. For example, mineral-associated SOC fractions of surface soils in Puerto Rico had $\Delta^{14}C$

512   values of 74–81 ‰, similar to values here, and turnover times calculated using archived samples

513   and a two-pool model showed that > 30% of the C had turnover times on the scale of millennia

514   (Hall et al., 2015). The mineral-associated SOC fraction in general may be more accurately

515   represented using a two-pool model (Torn et al., 2013). Thus, a portion of our mineral-associated

516   SOC fraction is likely very old, and the treatment effect we observed suggests that this older

517   portion was the most stable component of mineral-associated SOC.

518

519   *4.4. Changes in the Organic Chemistry of Mineral-Associated SOC*

520   Mineral-associated SOC chemistry changed after a decade of litter removal, with

521   increased dominance of alkyl, *O*-alkyl, and *N*-alkyl C, which is likely indicative of the relative

522   stability of these C functional groups on mineral surfaces. The increased dominance of *N*-alkyl C

523   groups in litter removal plot mineral associations points to the importance of proteins. Proteins

524   have been put forth as a central component of stable mineral-associated SOC, because they sorb

525   strongly to mineral surfaces and have hydrophobic outward-facing protons, leading to further

526   associations with a wide range of organic compounds (Kleber et al., 2007). Several recent

527   laboratory and field studies also provide support for the central role of microbial proteins in the

528   formation of stable organo-mineral associations. One set of lab experiments showed rapid and

529   preferential sorption of microbial proteins to mineral surfaces (Swenson et al., 2015), and

530   another set showed that microbial biomass products dominated SOC accumulation in model soils

531   (Kallenbach et al., 2016). In the field, a European forest litter decomposition experiment

532   demonstrated that C from added litter became stably associated with mineral surfaces after

533   incorporation into microbial biomass (Hatton et al., 2012), and an isotope labeling experiment in

534   temperate grassland soils showed that soluble C compounds from litter were stored in SOC after

535   microbial uptake (Cotrufo et al., 2015). In our study, the lower C:N ratios we observed in

536   mineral-associated SOC with litter removal also suggest more advanced stage of microbial

537   processing (Baisden et al., 2002), and/or greater protein content. Together, these results supply

538   growing evidence that microbial biomass products like proteins play a dominant role in the

539   formation of stable, mineral-associated SOC.

540   Other C groups have also been posited as playing a role in long-term, stable C retention

541   in soils. In particular, aromatic and phenolic compounds like lignin, as well as alkyl C,

542   representative of waxes and other lipids, have been seen to be preferentially preserved in soils

543   (Baldock et al., 1997; De Marco et al., 2012) (Baldock et al., 1992). However, there is

544   conflicting data about the long-term stability of aromatic C in particular. For example, field data

545   from cultivated soils demonstrated that added aromatic compounds (lignin) were lost relatively

546   rapidly from soils, with no preferential retention (Gleixner and Poirier, 2001). More generally,

547   there is growing consensus in the literature that few, if any, organic compounds are retained in

548   soils over the long-term based solely on their chemical structure (i.e. without some form of

549   physical protection like sorption to minerals) (Schmidt et al., 2011) (Lehmann and Kleber,

550   2015). For aromatic-rich compounds, desorption experiments suggest that the sometimes

551   observed preferential retention of compounds like lignin may occur because aromatics can bind

552   more strongly to mineral surfaces than other compounds in some cases (Kaiser and Zech, 1999;

553   Kaiser, 2003). In our data, we saw no evidence for long-term retention of aromatic C in the

554   mineral-associated SOC fraction. Also, the negative relationship between Δ ^{14}C and phenolic C

555   in mineral-associated SOC indicated that phenolics contributed a relatively younger, more

556   dynamic portion of this SOC fraction. We did see increased dominance of alkyl C with litter

557   removal, as expected. The waxy, tough leaves characteristic of these Panamanian forests (Coley,

558   1983) likely had an influence on SOC chemistry (Kögel-Knabner, 2002). Alternately, alkyl C in

559   soils may be more strongly related to soil microbial biomass rather than litter chemistry, as

560   observed in subtropical plantations in China (Wang et al., 2015). Here, the increased dominance

561   of alkyl C in litter removal plots was in mineral-associated SOC, suggesting that even this

562   relatively recalcitrant C group depended on mineral associations for protection from

563   decomposition.

564   In contrast to alkyl C, O-alkyl C, representative of carbohydrates like cellulose, is

565   generally lost from litter quickly during microbial decomposition (Preston et al., 2009). Thus, the

566   ratio of alkyl:O-alkyl C has been used to measure the extent of microbial processing of organic

567   matter in litter and soil (Baldock et al., 1997; LaRowe et al., 2012; Keiluweit et al., 2016). It was

568   therefore surprising that the Panama litter removal plots had elevated proportions of both alkyl

569   and O-alkyl C in mineral-associated SOC, and significantly lower soil alkyl:O-alkyl ratios

570   relative to control plots. Also, the positive relationship between Δ^{14}C and O-alkyl C indicated

571   that O-alkyls contributed a relatively older, more stable portion of mineral-associated SOC.

572   Although unusual, preferential retention of O-alkyl C has been observed in some other forests,

573   particularly for plant litters with high alkyl:O-alkyl starting ratios (e.g. waxy leaves), and

574   resistant O-alkyl C compounds (e.g. from tannins) (Almendros et al., 2000). Also, cell wall

575   polymers in fungal and bacterial biomass (e.g., chitin and peptidoglycan) are comprised largely

576   of O-alkyl C plus proteins. Thus, the preferential retention of O-alkyl C that we observed with

577   litter removal may indicate a particularly resistant carbohydrate group from plant litter, but it

578   seems more likely, together with the N-alkyl data (above), that increased O-alkyl C indicated

579   dominance of microbial biomass products in mineral-associated SOC following litter removal.

580   Overall, our data indicate that most plant-derived compounds, of which phenolic and

581   aromatic C are indicative, are not retained in mineral associations over the long-term in humid

582   tropical forest soils. Rather, microbial biomass compounds like proteins and cell wall polymers

583   (containing O-alkyl and N-alkyl C groups), as well as lipids (alkyl C), appear to be preferentially

584   retained on mineral surfaces in these soils on a decadal timescale. Our ^{13}C NMR data also

585   suggest that a revised ratio of (alkyl C + O-alkyl C + N-alkyl):(aromatic + phenolic), may be a

586   useful indicator of the biochemical stability of SOC in strongly weathered tropical forest soils.

587

588   *4.5. Different Seasonal Effects Across SOC Fractions*

589   Our data indicate that dry-season declines in mineral-associated and occluded-debris

590   SOC explain an observed overall decrease in bulk soil C stocks during the dry season at these

591   sites (Turner et al., 2015). The wet season increase in mineral-associated SOC, particularly with

592   litter addition, may indicate the importance of rainfall leaching DOC from litter downward and

593   subsequent DOC sorption to mineral surfaces (Qualls et al., 2002). The wet-season increase in

594   occluded-debris SOC could be related to elevated formation and dispersal of macro aggregates (2

595   – 4.75 mm diameter), which is common during wet periods in seasonally dry sites (Bach and

596   Hofmockel, 2016). In contrast, the wet-season decline in free-debris SOC likely reflects a two-

597   fold increase in forest floor decomposition rates during the wet season in these Panamanian

598   forests relative to the dry season (Wieder and Wright, 1995). Overall, our data suggest a seasonal

599   transfer of SOC between fractions, with added litter incorporated into free-debris space during

600   the dry season, and then possibly transferred to occluded-debris and/or mineral associated

601   fractions during the subsequent wet season, along with C from new litter.

602

603   **5. Conclusion**

604   This study illustrates that the physical and biochemical nature of soil C stocks will be

605   sensitive to changes in tropical forest NPP with global change. Most notably, the relatively stable

606   mineral-associated SOC fraction showed large changes with a decade of litter manipulation.

607   Litter addition promoted the accumulation of C into organo-mineral associations that were

608   relatively stable (i.e. not leachable as DOC), suggesting that strongly weathered tropical soils

609   have the capacity to store more C if tropical forest NPP increases. Litter removal demonstrated

610   that the most stable portion of mineral-associated SOC had elevated dominance of lipids like

611   waxes (alkyl C), and microbial biomass products like proteins and cell walls (*N*-alkyl and *O*-

612   alkyl C). In contrast, plant-derived compounds, characterized by aromatic and phenolic C,

613   formed a more dynamic portion of mineral-associated SOC, demonstrating that these may have

614   limited importance for long-term soil C storage in strongly weathered tropical soils. Seasonal

615   patterns indicated an accumulation of free-debris SOC during the dry season, followed by

616   occluded-debris and mineral-associated SOC increases during the wet season, promoting an

617   overall increased in bulk soil C during the wet season. Thus, a change in the duration or severity

618   of the dry season may interact with changes in tropical forest NPP to alter soil C storage in

619   tropical forests. For example, NPP-driven increases in mineral-associated SOC could diminish

620   under drying scenarios. Overall, our findings show that changes in tropical forest NPP are likely

621   to cause large changes in the quantity, stability, and biochemical character of soil C stocks.

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853 **Figure Legends**

854 **Figure 1.** (**A.**) Bulk soil C concentrations, made up of three SOC fractions, are shown for litter

855 addition (L+), control (C), and litter removal (L-) plots during a wet and dry season (0 − 5 cm

856 depths). Bulk soil C concentrations were significantly greater in L+ versus C and L-, driven

857 primarily by greater mineral-associated SOC. (**B.**) Free-debris SOC was greater in L+ versus C

858 and L-, with the largest treatment effect during the dry season. (**C.**) Occluded-debris SOC was

859 greater in L+ versus L-, with the largest treatment effect during the wet season. ^ indicates

860 significantly greater free-debris SOC during the dry season; # indicates significantly greater

861 mineral-associated and occluded-debris SOC fractions during the wet season. Tukey HSD means

862 separation results are shown in capital letters for bulk soil C concentrations, and lower case

863 letters for SOC fractions, showing differences across both seasons. Averages are shown for each

864 fraction ± one SE for that fraction (n = 5).

865

866 **Figure 2.** Average radiocarbon (Δ ^{14}C) values for mineral-associated SOC are shown for litter

867 addition (L+), control (C), and litter removal (L-) plots. All Δ ^{14}C values reflect ^{14}C released

868 during bomb testing in the 1960s (i.e., "modern" values), so lesser Δ ^{14}C values in L+ versus L-

869 indicate more retention of recent C inputs into mineral-associated SOC with litter addition.

870 Letters shown in the bars indicate Tukey HSD differences in the mean among the treatments.

871 There were no seasonal effects so values were pooled. Averages are shown ± one SE (n = 5).

872

873 **Figure 3.** Representative ^{13}C NMR spectra are shown for mineral-associated SOC in a litter

874 removal plot (black line, L-), and a litter addition plot (gray line, L+). Chemical shift regions

875 shown on a unitless δ-scale represent seven SOC functional groups: alkyl (0-45 ppm), *N-*

876 alkyl+methoxyl (45-60 ppm), O-alkyl (60-95 ppm), di-O-alkyl (95-110 ppm), aromatic (110-145

877 ppm), phenolic (145-165 ppm), and amide+carboxyl (165 – 215 ppm). The proportion of total

878 SOC in the alkyl, N-alkyl and O-alkyl regions was significantly after a decade of litter removal

879 versus addition (Table 2).

880

881 **Figure 4.** The proportion of C leached as DOC is shown for (**A.**) free-debris SOC, and (**B.**)

882 mineral-associated SOC, comparing litter addition (L+), control (C), and litter removal (L-)

883 plots. (**A.**) A greater proportion of free-debris SOC was leachable as DOC in L- versus L+. (**B.**)

884 In contrast, a greater proportion of the mineral-associated SOC was leachable as DOC in L+

885 versus L-, suggesting greater C desorption. Letters indicate Tukey HSD differences in the mean

886 among the treatments. There were no seasonal effects so values were pooled. Averages are

887 shown ± one SE (n = 5).

888 **Tables**

889 **Table 1:** Effects of litter manipulation on bulk soil chemistry and soil characteristics are shown for a wet and a dry season in Panama

890 (0 – 5 cm depth). Averages are given ± one SE (n = 5).

891

Season	Treatment	Bulk Soil %C [a]	Bulk Soil %N [a]	Bulk Soil C:N ratio [a]	Soil Moisture g-water/ g-soil [a, b, c]	Bulk Density (g/cm^3) to 5 cm depth [a]	C Stock (mg/cm^2) to 5cm depth [a]	Leachable DOC mg-C/g-soil [a]	Leachable TDN μg-N/g-soil [a]
Dry	Addition	6.7 ± 0.5	0.63 ± 0.02	10.7 ± 0.8	0.43 ± 0.03	0.82 ± 0.08	269 ± 20	1.1 ± 0.3	254 ± 38
	Control	5.2 ± 0.3	0.52 ± 0.02	9.9 ± 0.3	0.42 ± 0.01	0.78 ± 0.03	200 ± 14	0.9 ± 0.1	213 ± 40
	Removal	3.6 ± 0.2	0.38 ± 0.01	9.4 ± 0.4	0.35 ± 0.01	0.99 ± 0.04	180 ± 15	0.5 ± 0.04	128 ± 5
Wet	Addition	7.3 ± 0.6	0.61 ± 0.04	12.0 ± 0.8	0.72 ± 0.02	0.76 ± 0.02	278 ± 25	1.6 ± 0.3	227 ± 35
	Control	5.4 ± 0.3	0.51 ± 0.02	10.5 ± 0.3	0.66 ± 0.02	0.68 ± 0.03	184 ± 15	0.9 ± 0.1	150 ± 15
	Removal	3.7 ± 0.2	0.38 ± 0.02	9.6 ± 0.4	0.52 ± 0.01	0.95 ± 0.06	175 ± 16	0.6 ± 0.1	111 ± 17

892

a significant effect of treatment

b significant effect of season

c interacting effect of treatment and season

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896

Table 2. The percentages of SOC belonging to seven C functional groups as detected using ^{13}C NMR are shown for the mineral-associated SOC fraction in litter addition, control, and litter removal plots. Examples of common environmental organic compounds containing each C group are listed in parentheses, and chemical shift regions of the spectra are given for each C group. There were no seasonal effects so data are pooled for two seasons. Averages are given ± one SE (n = 5).

Plant Litter Treatment	Alkyl C (waxes, other lipids) 0-45 ppm [a]	N-Alkyl + Methoxyl C (proteins, peptides) 45-60 ppm [a]	O-Alkyl C (cellulose, other carbohydrates) 60-95 ppm [a]	Di-O-Alkyl C (hemicellulose) 95-110 ppm [a,b]	Aromatic C (lignin, tannin) 110-145 ppm [a]	Phenolic C (acids, tannin) 145-165 ppm [a]	Amide + Carboxyl C (chitin + hemicellulose) 165-215 ppm [b]	Ratio of (Alkyl+O-alkyl +N-alkyl):(phenolic + aromatic) C [a]
Addition	25.3 ± 0.5	9.2 ± 0.2	18.2 ± 0.4	7.3 ± 0.3	17.4 ± 0.4	7.9 ± 0.2	14.7 ± 0.5	2.1 ± 0.08
Control	25.4 ± 0.3	9.7 ± 0.2	19.5 ± 0.3	7.0 ± 0.2	16.4 ± 0.3	7.2 ± 0.1	14.8 ± 0.4	2.3 ± 0.06
Removal	28.4 ± 0.5	10.3 ± 0.2	21.1 ± 0.5	6.4 ± 0.2	14.3 ± 0.4	5.9 ± 0.3	13.6 ± 0.4	3.0 ± 0.1

a Significant effect of litter manipulation.

b While cellulose and hemicellulose are both examples of carbohydrates, which are built from O-Alkyl C, hemicellulose contains carboxyl C and can thus be distinguished in NMR spectra. Glucoronic acid is a major constituent of hemicelluloses in gymnosperms

906   and angiosperms, and glucoronic acid contains carboxyl C. The *N*-Acetyl glucosamine polymer (chitin) is the primary cell wall

907   component of fungi, and as the name indicates, chitin contains acetyl groups. Acetyl C is a type of carboxyl C. Therefore, both

908   hemicellulose and chitin contain carboxyl C in measurable quantities. Hemicellulose and chitin are both carbohydrates and both cell

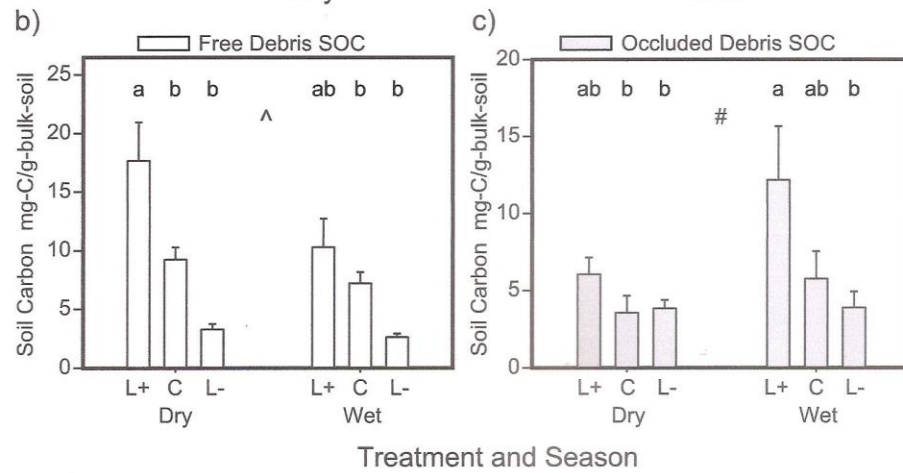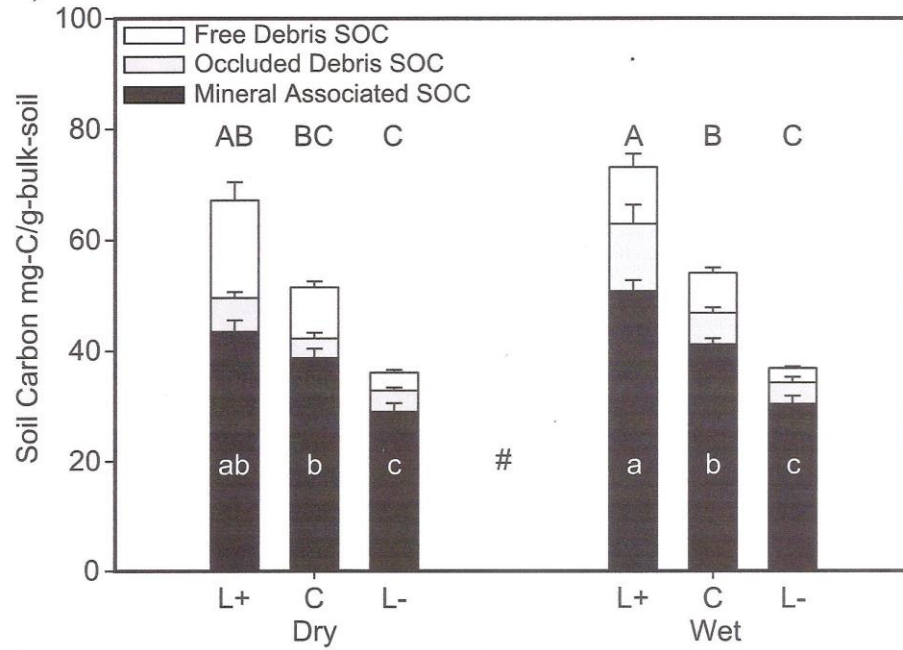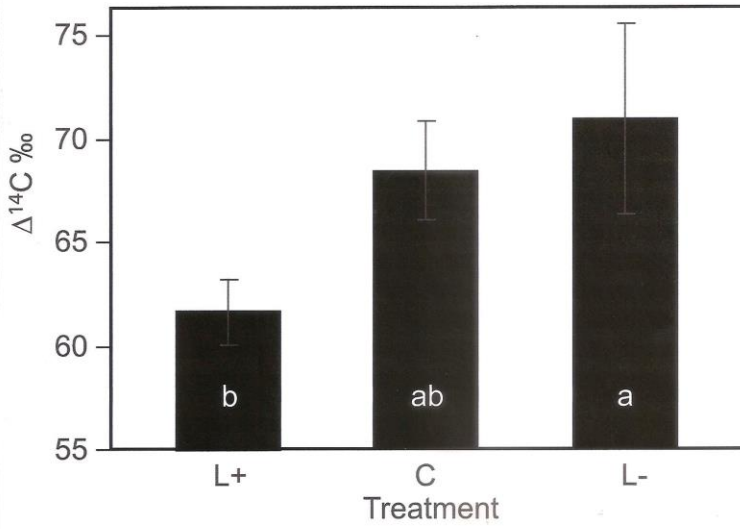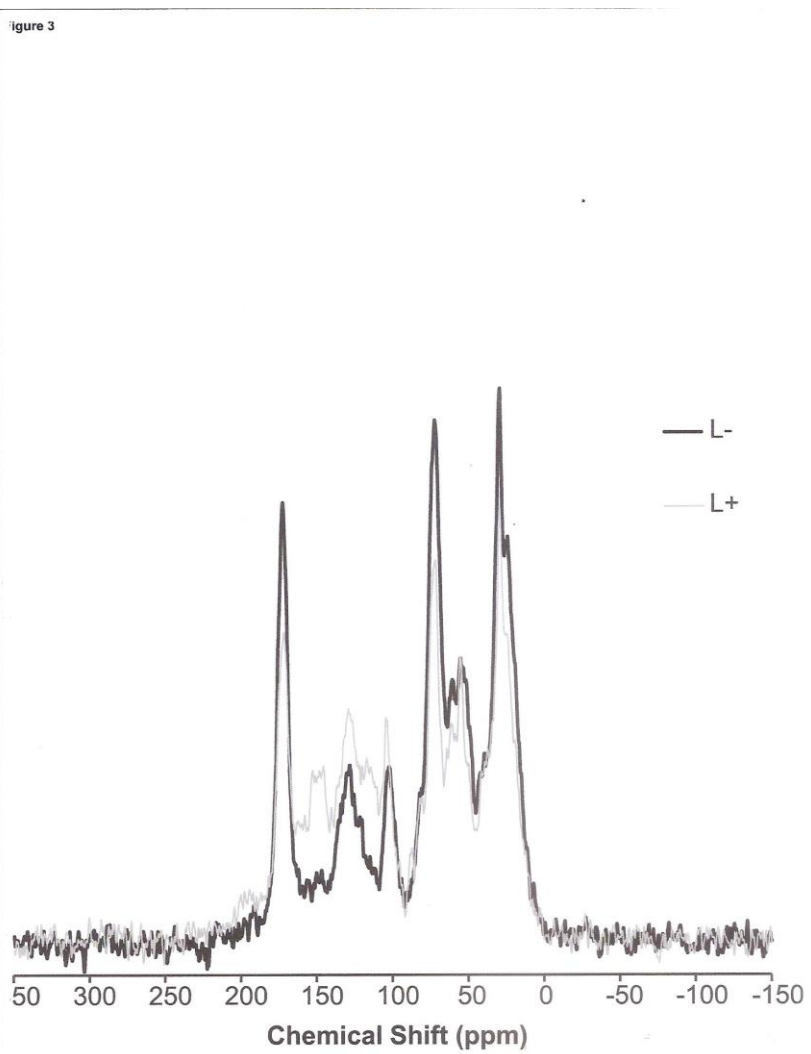909   wall components of abundant organisms in the ecosystem.

910

Figure 1

a)

b) Free Debris SOC

c) Occluded Debris SOC

Treatment and Season

911

Figure 2

912

Figure 3

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Figure 4

a)

b)

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Figure S2

a)

b)

c)

Treatment and Season

916

Figure S4

a)

b)

Free Debris Fraction
Occluded Debris Fraction
Mineral Associated Fraction

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